REVIEW OF INGREDIENTS ADDED TO CIGARETTES

PHASE ONE: THE FEASIBILITY OF TESTING INGREDIENTS ADDED TO CIGARETTES

LSRO

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Editor
FOREWORD

The Life Sciences Research Office, Inc. (LSRO) provides scientific assessments of topics in the biomedical sciences. Reports are based on comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in relevant areas of science and medicine. This LSRO report was developed for and supported by Philip Morris, USA, Inc., P.O. Box 26583, Richmond, VA 23261 (Philip Morris) in accordance with a contract between Philip Morris and LSRO.

An Expert Panel provided scientific oversight and direction for all aspects of this project. The LSRO independently appointed members of the Panel based on their qualifications, experience, judgment, and freedom from conflict of interest, with due considerations for balance and breadth in the appropriate professional disciplines. The Panel was selected with the concurrence of the LSRO Board of Directors. The Expert Panel convened eight times (seven full meetings and one conference call) to assess the available data. LSRO invited submission of data, information, and views bearing on the topic under study, held a widely advertised Open Meeting on August 26, 2002, and accepted written submissions. Information about the process, including the critical literature and presentations upon which the Expert Panel based their deliberations, was made publicly available by posting on the LSRO web site.

The LSRO staff and special consultants considering all available information and the deliberations of the Expert Panel drafted the report. The LSRO report was edited by Daniel M. Byrd III, Deputy Director, LSRO who, with the assistance of LSRO staff, submitted the report for review by an independent reviewer, incorporated the reviewer’s comments, and provided additional documentation and viewpoints for incorporation into the final report. The Expert Panel and the LSRO Board of Directors reviewed and approved the final report. On completion of these reviews, the report was transmitted to the Sponsor for technical comments by the Executive Director, LSRO.

The listing of members of the Expert Panel, others who assisted in preparation of this report, and the LSRO Board of Directors, does not imply their endorsement of all statements in the report. The report was developed independently of Philip Morris and conclusions drawn therein do not necessarily represent the view of Philip Morris or any of its employees. The LSRO accepts full responsibility for the study conclusions and accuracy of the report.

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Executive Director
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January 9, 2004

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EXECUTIVE SUMMARY

REVIEW OF INGREDIENTS ADDED TO CIGARETTES

Governments throughout the world do not regulate ingredients added to cigarettes. A U.S. National Academy of Sciences (NAS) report, *Clearing the Smoke*, recommended testing of ingredients added to cigarettes as a public health and scientific research objective (Institute of Medicine, 2001). *Clearing the Smoke* also posed this testing as a manufacturer’s responsibility. Philip Morris U.S.A., a manufacturer of cigarettes, engaged the Life Sciences Research Office (LSRO) to evaluate independently the additional health risks associated with the nontobacco ingredients of cigarettes. LSRO gave the overall project the following organization:

*Phase One. Determination of the feasibility of testing*
*Phase Two. Establishing scientific criteria for the review*
*Phase Three. Reviews of specific ingredients*

By added ingredients, LSRO means chemical substances directly added during manufacturing, not indirect additives like pesticides applied to tobacco and thus, present in cigarettes indirectly. In this report, LSRO also has focused attention on the existing, not new, ingredients typified by the substances listed in Appendix D. LSRO defines “feasibility” in the sense of practicality, not in the sense of measuring cost-benefit. This report of the first phase examines two questions about the feasibility of testing ingredients added to cigarettes: (A) What objectives would tests of ingredients added to cigarettes achieve? (B) If testing is feasible, is it also worthwhile?

Like the NAS panel in *Clearing the Smoke*, LSRO believes that the primary, if not the only objective of testing ingredients added to cigarettes should be to assure, to the extent practical, that ingredients do not increase the premature mortality and morbidity known to be associated with cigarette smoking. Prospective epidemiological studies established this association with cigarette smoking. However, epidemiological associations alone do not prove causation. Understanding causation requires diverse kinds of data. For LSRO, the descriptive standard for adverse health effects of cigarettes remains the long-term observation of human smokers.
Thus, the best comparison would be between smokers of cigarettes containing an added ingredient with smokers of nearly identical cigarettes lacking this ingredient. However, observational studies such as these present many challenges. For the foreseeable future, the optimal public health goal will remain prevention of smoking initiation and encouragement of smoking cessation.

Manufacturers make cigarettes from tobacco, a natural product. Burning a cigarette produces smoke. Normally a combustion aerosol, like cigarette smoke, consists of both particles and gases and also contains a mixture of many thousands of chemical substances, some short-lived and reactive. Cigarette smoke is dynamic in nature, changing rapidly with time, airflow, puff frequency, and type of cigarette. Aerosol particles exchange substances with the gas phase, and their chemical composition(s) vary over time. When humans inhale cigarette smoke, their mouths and respiratory tract surfaces exposed to the smoke react with, and absorb, many of the chemical substances, sometimes producing effects at the site of deposition. Once deposited, these chemicals and/or their reactive products may be transported to systemic tissues by circulation. Biological effects depend in part on complex interactions between the substances and/or their components and the target tissues.

Epidemiological studies have shown that certain major causes of death, cancer, cardiovascular diseases, and chronic obstructive pulmonary disease (COPD), contribute more than half of the premature mortality associated with cigarette smoking. All of these diseases increase in incidence as unexposed nonsmokers age, as smokers age, as smokers consume more cigarettes, and as smokers consume cigarettes for longer periods of time. Among smokers, latency and reversibility vary by disease. For example, the induction of lung cancer has a long period of latency, and lung cancer incidence eventually decreases to nearly background rate after cessation of smoking. In contrast, COPD does not revert to background rate after cessation.

The testing of the adverse effects of ingredients added to cigarettes should evaluate adverse effects associated with (A) inhalation of the ingredient or its pyrolysis products within the hot smoke matrix, (B) inhalation of cigarette smoke constituents altered by the ingredient, and/or (C) changes in smoking behavior, so that smoke inhalation pathways change. Tests to predict these effects must have both internal validity (reliability, reproducibility, data quality, and positive signal-to-noise characteristics) and external validity. An invalid test cannot eliminate a false hypothesis. Useful tests should generate data that also have external validity consistent with observed human outcomes.

In addition to observational studies of human smokers, scientists artificially generate cigarette smoke using machines. Test data reveal many interesting characteristics of the cigarette smoke matrix and the chemical substances in it, but the results of these tests do not yield satisfactory explanations of the diseases seen in
human smokers. In addition, few test data have addressed the effects of ingredients added to cigarettes. Thus, testing these ingredients will reveal significant research needs.

Data about the pyrolytic fate of an ingredient under cigarette smoking conditions would be uniformly desirable for all ingredients, whether the ingredient transfers to smoke or, pyrolyzes in whole or in part. Research strategies will be necessary to support the testing of potential ingredient pyrolysis in burning cigarettes. In determining the feasibility of testing ingredients, the key is preparation of comparative test cigarettes, nearly identical to a commercial product but lacking or containing lesser, defined amounts of an ingredient. Within the precision of standard methods, test data about the effects of combustion within the cigarette also will reveal whether the ingredient shifts the chemical composition of other substances in the smoke matrix.

In evaluating the feasibility of testing ingredients added to cigarettes, LSRO has concluded that the application of every conceivable test is neither necessary nor desirable. While research proceeds into methods of pyrolysis and transfer during human smoking and while a scientific consensus arises about new methods, LSRO recommends that investigators continue to include reference cigarettes, such as those developed at the University of Kentucky for comparison, and to use the reference smoke generation conditions specified by the Federal Trade Commission or International Standards Organization, unless a compelling scientific rationale dictates otherwise (Davies & Vaught, 1990; Federal Trade Commission, 1967a; International Organization for Standardization, 2000). Extensive comparative data are available for these reference cigarettes, providing internal standards.

Research needs exist on the pyrolysis and transfer of ingredients under common cigarette smoking conditions. There is a need to know, for example, whether transfer in smoke is concentration dependent. Consensus methods for data acquisition about pyrolysis and transfer do not exist. Research needs also exist about observation studies of changes in relative mortality and morbidity, particularly for studies to achieve pre-specified limits of statistical power. Consensus methods for data acquisition are not available when conducting studies of comparative mortality, although smoking is an everyday activity. In addition, LSRO has not yet specified tests that would constitute a balanced (or optimal) set. More research about general testing approaches would aid this task.

Because manufacturers already use many ingredients, setting priorities on the evaluations of these ingredients makes good sense. The need to set priorities explains why LSRO regards an incremental approach as beneficial. A checklist of specific tests to apply to all added ingredients is neither feasible, nor desirable. A program of testing ingredients added to cigarettes will reduce the possibility of an additive-related adverse human health effect, beyond the premature mortality and morbidity
already seen with cigarette smoking. In this regard, manufacturers should retest their proprietary mixtures of added ingredients, in the proportions used in their brands of cigarettes, to self-assure that their mixtures lack effects which were not seen with individual ingredients, generated through some interaction between ingredients.

Scientists can evaluate data from balanced, diverse tests of an ingredient and identify a maximum level of the ingredient per cigarette, based on these data, or specify what additional testing would be required to identify the level.

LSRO approaches the testing by estimating relative risk, defined as the risk of exposure to a cigarette with the ingredient, relative to the risk of exposure to a cigarette without the ingredient. An “unchanged relative risk” does not imply that the underlying activity, cigarette smoking, is safer or that addition of the ingredient has changed the risk of cigarette smoking. LSRO is not attempting to test ingredients in an effort to achieve safer cigarettes. Relative risk depends on testing paired cigarettes that differ only in the presence or absence of an ingredient or mixture of ingredients. The endpoint here is no change in risk.

Although the addition of ingredients to tobacco is unlikely to change significantly the adverse health effects of cigarettes based on the magnitude of the health effects of cigarettes and the incremental mass of pyrolyzed materials contributed by the added ingredients; guidelines for cigarette labeling and specification of excursion limits for ingredients added to cigarettes developed by an independent, outside group of scientists will insure an objective and uniform interpretation of data and further minimize the possibility of a contribution to adverse health effects. Overall, LSRO concludes that testing of ingredients added to cigarettes with this objective is both feasible and worthwhile.
INTRODUCTION

OUTLINE

2.1 INTENT & PURPOSE

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2.5 SUMMARY
INTRODUCTION

2.1 INTENT & PURPOSE

The goal of this report is to assess whether non-tobacco ingredients added to cigarettes can feasibly be tested for their toxicological effects within the appropriate context of their intended delivery. If such testing can be accomplished, the human health risks associated with smoking cigarettes containing specific added ingredients can be compared to the risks associated with smoking cigarettes that lack those ingredients. The ability to perform such a comparison could lead to significant advances in the current scientific knowledge regarding the ability of non-tobacco ingredients to alter the risks of human cigarette smoking. Therefore, a process has been initiated to answer unresolved questions about the risks of added ingredients in cigarettes and, if possible, remove doubts surrounding the safety of these substances. Specifically, this report seeks to address two important issues:

1. If the testing of ingredients added to cigarettes is scientifically possible, what specific objectives do these tests achieve?

2. If testing is feasible, is it worthwhile?

The Life Sciences Research Office (LSRO), its staff and their advisors, the Added Ingredients Review Ad Hoc Expert Panel (referred to collectively as “LSRO” in the remainder of this report), first addressed the feasibility of testing non-tobacco ingredients added to cigarettes in October of 2002. Given its positive determination of feasibility, the purpose of this Phase One report is to explain the scientific rationale for LSRO’s conclusion. LSRO will ultimately continue this process by undertaking two subsequent steps. In Phase Two, the scientific criteria for specific tests as well as the optimal types of tests to employ will be presented. State-of-the-art information about test data for each commonly used additive will also be reviewed. In Phase Three, preferred additive testing strategies along with their scientific rationale will be described. (See Appendix H)

This LSRO review will not address the safety of the use of tobacco products in general, nor will it weigh the risk of human exposure to any specific commercial tobacco product, since the aim is not to conduct a review of existing tobacco testing programs or products. In addition, this report will not address directly which substances in cigarette smoke cause the most harm to human health.
A potential method to address the health effects of added ingredients is to determine their relative risk. To calculate relative risk, the absolute risk of one substance is divided by the absolute risk of another. Absolute risk is defined as an increased probability of an adverse outcome due to exposure to a defined substance. Therefore, if no difference exists between two substances, the relative risk must equal 1.0. For example, if two groups of smokers consume identical cigarettes, the relative risk will be 1.0, since the risk of one cigarette relative to the risk of the exact same cigarette is equivalent. Alternatively, if the absolute risk to smokers of cigarettes containing a specific ingredient (the experimental group) is divided by the absolute risk to smokers of cigarettes without that ingredient (the control group), with the assumption that the cigarettes are otherwise identical, the relative risk will be 1.0, unless the ingredient induces a change in the absolute risk of smokers in the experimental group. Therefore, risk common to both the control and experimental cigarette are removed from consideration. Only altered risk contributed by the added ingredient is assessed.

During its deliberations of feasibility, LSRO adopted an approach based on relative risk. LSRO argues that a relative risk approach most effectively addresses the health consequences of added ingredients and can be supported experimentally, based on the ability of manufacturers to make paired experimental cigarettes nearly identical in every aspect except for the presence and absence of a particular ingredient. If the health effects from inhaling the smoke of a cigarette containing a specific ingredient compared to the same cigarette without the ingredient are adequately tested, any direct and indirect effects of the ingredient on the inhaled smoke toxicity should be evident and scientifically defensible.

The development of reliable testing methods able to detect both direct and indirect adverse health effects attributable to added cigarette ingredients is critically important. Direct health effects result from exposure to an ingredient, or its pyrolysis products as a result of cigarette smoking. Indirect health effects differ from direct effects in that they do not result from an individual’s cigarette use, but instead from exposure to environmental tobacco smoke. However, the general applicability of scientific results obtained from a particular brand of cigarettes will need to be considered carefully, since changes in the kind of tobacco, smoker behavior, cigarette structure, or composition also may alter the health effects of the added ingredient under investigation.

2.2 PUBLIC HEALTH CONSIDERATIONS

Cigarette smoking is associated with adverse human health effects. Whether most non-tobacco ingredients added to cigarettes augment, diminish, or do not change these health effects is unknown (Institute of Medicine, 2001). Scientific advances associating cigarette smoking with increased morbidity and mortality
have been attributed predominantly to early research conducted within the Framingham Heart Study sponsored by the National Heart Institute, now known as the National Heart, Lung and Blood Institute. In 1948, this study began investigations into the major risk factors contributing to near epidemic rates of cardiovascular disease associated mortality. Notably, the investigators identified a significant relationship between cigarette smoking and the average annual death rate of a cohort of 5,209 individuals (Freund et al., 1993). Subsequent epidemiologic studies supported an association between smoking and premature death from cardiovascular disease, pulmonary disease, cancer, and other disorders (National Institutes of Health, 1997).

Available data appears to underestimate global smoking-related mortality (Forey et al., 2002). Death rates are calculated from death certificates – the raw material for tabulation of lists of causes of death (Kircher & Anderson, 1987). To be useful, these certificates need to be accurate, complete, and consistent. However, different countries, and even different regions within those countries, have different systems of assigning causes of death. In addition, autopsies are not always performed, and in some areas of the world they are extremely rare. Although primary care practitioners, specialists, urban and rural doctors, and other physicians, fill out death certificates, their technical levels of expertise vary widely. Studies of the accuracy of death certificates reported significant discrepancies between autopsy diagnoses and entries on death certificates (Nielsen et al., 1991). Adjustments for factors such as age make the data more useful, as do formulas that include so-called “risk factors.” These formulas attempt to control for confounding because many diseases have multi-factorial causes. The use of these formulas, however, is controversial (Lee, 1996; Malarcher et al., 2000). One complicating factor in assessing worldwide disease incidence with respect to added ingredients in cigarettes is that certain added ingredients are used in some parts of the world that are not used elsewhere. Therefore, global comparisons may prove difficult.

Animal models have been developed to analyze the effects of added cigarette ingredients. Measurements of general toxicity, expressed as mortality, inhibition of weight gain, or other biological indicators, following chronic use or inhalation, appear to translate to human exposure levels, as recalculated by investigators (Carmines, 2002; Heck et al., 2002; Vanscheeuwijck et al., 2002). However, the results of animal bioassays cannot be extrapolated to human health endpoints with confidence, thus necessitating comparison to human studies (Elcombe et al., 2002; Goodman & Wilson, 1991).

Despite the declining prevalence of cigarette smoking over the last fifty years in the developed world, smoking still causes substantial morbidity and contributes to a variety of diseases. Smoking increases the risk of all leading causes of disease among Americans and also takes an indirect toll on society in the form of subsidized

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and unsubsidized health care costs, lost productivity in the workplace, and reduction of personal wealth, as well as other human costs, which are not easily calculated. The principle of harm reduction assumes continuation of a behavior (i.e., cigarette smoking) and aims to lower the total adverse consequences associated with that behavior (Institute of Medicine, 2001). Therefore, in the context of cigarette smoking, harm reduction would seek to achieve a low enough level of a specific substance so that no ill health effects result from the addition of the substance to cigarettes. In this context, an ingredient-by-ingredient review will contribute to determining the ability of added substances to change the relative risk of cigarette smoking.

2.3 FEASIBILITY

Smoking cessation is the most feasible approach to the problem of cigarette-related premature mortality and morbidity. Despite the well-publicized health risks caused by tobacco, a large portion of the U.S. public continues to smoke cigarettes. Many efforts have been made to persuade smokers to quit with varying degrees of success (Institute of Medicine, 2001). The smoking public, however, has the right to know whether steps have been taken to minimize their health risks, given their predilection, and whether these steps have any scientific validity.

Cigarette smoking poses chronic health risks, but some scientific hypotheses oversimplify the association between cigarette smoke and its adverse health effects. Thousands of chemical substances have been identified in smoke and many systems have been developed to measure them. However, certain experimental methods, such as the fractionation of cigarette smoke to identify potential carcinogens, have yielded unconvincing results, and no single component can explain the carcinogenicity of cigarette smoke (Institute of Medicine, 2001). Adding further complication is the fact that many measured biological activities do not equal the total activity of the original mixture nor do they account directly for human disease, since many biomarkers are not themselves primarily responsible for the observed increases in morbidity and premature mortality.

Many scientific studies have investigated the association between cigarette smoking and cancer, particularly the association between cigarette smoking and lung cancer. The relationship between human lung cancer risk and cigarette smoking is, therefore, well defined. However, substantial disagreement still exists over whether certain categories of substances, such as polyaromatic hydrocarbons or nitrosamines, are responsible for the carcinogenic effects of smoking. In order to consider additives scientifically, LSRO determined that it must examine the impact of an additive or some measured effect of it. This effect may arise from the amount of a specific chemical substance in the smoke or from a biological activity of the smoke in total. Some ingredients are expected to produce no effect on human health. A “no
detectable change” standard, therefore, was defined to accommodate these situations.

This review distinguishes between premature mortality, causes-of-death, morbidity, and other indicators of product safety. As outlined above, premature mortality and causes-of-death are available from death certificate data. From this information epidemiologists should be able to reconcile estimates of risk between different studies. Such an approach makes the evaluation of scientific test data straightforward, if difficult. Based on this information, scientists may evaluate the ability of a test to predict overall premature mortality, or a cause-of-death, observed as a specific increased mortality in the population. Without this evaluation, test data are not useful for purposes of risk estimation. One caveat to the use of epidemiological data needs to be addressed. If a single cause-of-death is exclusively tabulated, it is impossible to determine whether all other causes-of-death change in proportion to it. As an illustration, suppose that LSRO discovers that the addition of a particular ingredient to cigarettes causes a small decrease in lung cancer incidence. The result, no doubt, would be worldwide addition of this ingredient to cigarettes. A later discovery that the same ingredient doubles cardiovascular deaths would promptly reverse such attitudes.

LSRO is disinclined to recommend alterations in the levels of ingredients in a product, in an effort to provide assurances, when the effects of these substances, if any, are poorly understood. Therefore, in preparing this review, LSRO has evaluated industrial cigarette production methods, identified the ingredients added in the manufacture of cigarettes, evaluated biological endpoints and current methodologies for the assessment of cigarette smoke and its risks, and considered potential future endpoints and methodologies. LSRO has also addressed methods to evaluate total body burden, subpopulations at risk (i.e., gender or ethnic groups), and interactions between ingredients.

2.4 THE SCIENTIFIC REVIEW PROCESS

2.4.1 The Committee to Assess the Science Base for Tobacco Harm Reduction of the Institute of Medicine (IOM)

In 2001, the Institute of Medicine’s (IOM’s) Committee to Assess the Science Base for Tobacco Harm Reduction published *Clearing the Smoke: The Science Base for Tobacco Harm Reduction* (Institute of Medicine, 2001). The IOM report emphasized policy and regulatory considerations. It established a comprehensive framework, including a set of eleven principles for the regulation of tobacco products. Some principles in *Clearing the Smoke* underscore the value of reviewing added ingredients. In referring to “potential reduced-exposure products” (PREPs), the IOM panel wrote:

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“Regulatory Principle 3. Manufacturers of PREPs should be required to conduct appropriate toxicological testing in preclinical laboratory and animal models as well as appropriate clinical testing in humans to support the health-related claims associated with each product and to disclose the results of such testing to the regulatory agency.”

“Regulatory Principle 8. All added ingredients in tobacco products, including those already on the market, should be reported to the agency and subject to a comprehensive toxicological review.”

The IOM report explains these two Principles in greater detail in Chapter 7 (Institute of Medicine, 2001). The two Principles also interrelate in that they both would: (1) require that government list ingredients or additives in tobacco products and (2) establish the need for a structured process to assess the safety of such additives. The Principles extend naturally to ingredients intended not to change exposure or cigarette-related health effects. Results of the safety assessment would be reported to the public in an organized framework. In this way the public would have a way to verify claims about the risk of additives in tobacco products. Thus, the process would be valuable, even if no regulatory agency exists.

The precedent for this kind of regulatory requirement would be the regulation of food additives by the U.S. Food and Drug Administration (FDA), which began addressing the safety of food additives with the passage of the Pure Food and Drugs Act of 1906 (U.S. Congress, 1906). However, testing an added ingredient in the matrix produced by the products that consumers use, is not a usual procedure in product safety. Instead, food additives, pesticides and industrial chemicals undergo regulatory review in relative independence from the matrix of their intended use. For example, FDA seldom requires testing of a food additive in the matrix of a food, although consumers may primarily experience exposure in this way. In 1938, the Federal Food, Drug and Cosmetic Act required drug manufacturers to provide scientific proof that new products could be safely used before putting them on the market (U.S. Congress, 2000). As a result of 1949 hearings chaired by Rep. James Delaney, three amendments passed, the Pesticide Amendment (1954), the Food Additives Amendment (1958), and the Color Additive Amendments (1960) (Janssen, 1981; National Research Council, 1987). The Delaney clause in the Food Additives Amendment provided that FDA could not approve an additive, if it caused cancer in man or experimental animals (Janssen, 1981). The Delaney clause led to the establishment of compliance protocols as new regulations ensued (Janssen, 1981).

The Center for Food Safety and Applied Nutrition (CFSAN) in FDA enforces food additive regulations. CFSAN derives its authority from the 1958 Food Additives Amendment. CFSAN provided guidance for regulatory compliance in 1982 by publishing “Redbook I,” which contained guidelines for testing direct and indirect
food and color additives (U.S. Food and Drug Administration et al., 1982). As changes occurred in safety assessment procedures, and as the body of literature on metabolism and pharmacokinetics of food and color additives grew, CFSAN made a revised edition of these guidelines available for public comment in 1993 in the Draft Redbook II (U.S. Food and Drug Administration et al., 1993). Since its publication, FDA has refined its thinking about test end points and methods. The latest version of Redbook is now available in an electronic form as Redbook 2000: Toxicological Principles for the Safety of Food Ingredients (U.S. Food and Drug Administration et al., 2001), which provides guidance for a broad range of substances added to food. It covers information for safety assessment of food ingredients including direct food additives, color additives, Generally Recognized as Safe (GRAS) substances, food contact substances, and constituents or impurities of any of these (Collins et al., 1999). These guidelines describe a safety assessment paradigm that emphasizes the identification of adverse effects and no observed adverse effect levels (NOAEL) that are used to establish acceptable exposure levels (Gaylor et al., 1998).

Most of the ingredients added to cigarettes have “Generally Regarded As Safe” (GRAS) status. This means that a reasonable certainty exists that the substance is not harmful under the intended conditions of use. GRAS status does not mean that a substance is safe when used for any purpose in any amount. It applies to the safety of substances directly or indirectly added to food. LSRO previously published a report evaluating the safety assessment procedure for food ingredients (Raiten, 1999). Assurances that ingredients added to cigarettes have GRAS status are irrelevant to the purpose of this report, as the conditions of use involve burning in a cigarette and inhaling combustion products.

Literally interpreted, IOM’s Regulatory Principle 3 envisions that manufacturers of tobacco product additives would conduct the same kinds of tests required of manufacturers of food additives (Institute of Medicine, 2001). Redbook 2000 provides requirements and recommendations for safety testing. Historically, manufacturers performed these tests on a wide variety of food additives, including sweeteners and flavorings (Goldsmith, 2000; Munro & Kennepohl, 2001; Soni et al., 2001; Takayama et al., 2000). Revolutions in molecular biology and transgenic science have generated even newer methods of testing (Elespuru, 1996; MacGregor et al., 1995; Pauluhn & Mohr, 2000; Sills et al., 2001).

LSRO views clearly differ from a literal interpretation of the two IOM passages (above). Testing each ingredient in a standard set of Redbook tests would make no sense, when most of the ingredients pyrolyze. Even if the pyrolysis products of ingredients underwent testing, the data would have no use when an ingredient undergoes complete combustion. Yet, such testing might leave crucial questions unanswered. The key to the IOM’s advice in Regulatory Principle 3 is the term “appropriate.” Guidelines are ideal approaches for ideal circumstances. No
regulatory agency ever intended a guideline to override case-by-case needs or scientific judgment. Instead, an approach tailored to the circumstances of cigarette smoking is in order.

The burning of a cigarette usually changes the nature of the added ingredient, and the route of exposure will differ. The collection of smoke for testing purposes may actually interfere with the chemical species relevant to the human health effects, if it is evanescent. FDA acknowledges the possibility of this effect in Guidance for Industry - Preparation of Premarket Notifications for Food Contact Substances: Toxicology Recommendations (U.S. Food and Drug Administration et al., 2002).

Section 309 of the FDA Modernization Act of 1997 amended Section 409 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 348), and established a procedure for the FDA to regulate food additives that are classed as food contact substances - substances used in the manufacturing, packaging, transporting or holding of food. FDA states: “Some food contact substances decompose to other substances that exert technical effects during the manufacture of the food contact substance. There are other food contact substances that are known to decompose during processing, in storage, and in food or food-simulating solvents. In such cases, decomposition products of the food contact substances may be appropriate test substances for toxicity studies” (U.S. Food and Drug Administration et al., 2002).

LSRO’s report presents a scientific rationale for a strategy to test ingredients added to cigarettes that implements Principles 3 and 8 of the IOM report. In part, the IOM Committee anticipated the establishment of a regulatory body within the U.S. government that has not, subsequently, come to pass. However, many statements in Clearing the Smoke make equally good sense without a regulatory agency. To achieve this interpretation of the text, the reader has to parse the document, while omitting phrases such as, “to the regulatory agency,” or “to the agency.” Similarly, the IOM report concerned claims of harm reduction, but many of its conclusions extend to claims of no change in effect from the addition of ingredients.

Cigarette smoking induces premature mortality and morbidity. The IOM Committee appeared willing to accept data other than direct observation of mortality and morbidity, but only to the extent that other data, such as bioassays, predicted changes in human mortality and morbidity. LSRO agrees with this interpretation. In the context of this report, test data, other than direct observations of human smokers, are useful to the extent that such data are prognostic of premature human mortality and morbidity, or allow a better understanding of related disease mechanisms.
2.4.2 The Added Ingredients Review Ad Hoc Expert Panel of the Life Sciences Research Office (LSRO)

This Phase One report, which is a scientific review, follows on, and closely agrees, with the primary objective of the IOM report, *Clearing the Smoke* (Institute of Medicine, 2001). To avoid potential misunderstandings, LSRO reiterates several key statements from that document.

An ingredient added to a cigarette does not change the risk of adverse health effects of smoking, if cigarette-related mortality and morbidity do not change.

Manufacturers of cigarettes with added ingredients should conduct appropriate testing to determine whether expected cigarette-related mortality and morbidity changes after addition of the ingredient.

Manufacturers should report all ingredients added to cigarettes, including those already on the market, as a published list. Manufacturers should report these data to an appropriate agency, if one exists.

All ingredients added to cigarettes, including those already on the market, should be subject to comprehensive review of relative risk.

A structured process needs to be established to assess the relative risks of cigarettes containing added ingredients.

To conduct an independent, third party review, LSRO selected an expert panel of qualified scientists and developed specific review standards, criteria, and procedures. It conducted the review in such a manner so as to prevent the influence of factors other than scientific principles and scientific data. LSRO invited public comments. By publishing the current and future reports of its activities, LSRO will make the data, decisions, and deliberations of the panel available to the public.

2.5 SUMMARY

The plan of this report is first to review cigarette smoke, including its physics and chemistry in Chapter 3, because testing the smoke matrix for changes will be particularly feasible. Physical, chemical, and biological analysis of the smoke matrix is intrinsically more accurate and more precise than tests of smoke exposed recipients. If the smoke does not change, and if the ingredient does not transfer, understanding how an ingredient could cause an effect, other than a change in smoking behavior, will be difficult.

Chapter 4 reviews human exposure to cigarette smoke, to enable dosimetric analysis of ingredients added to cigarettes or their pyrolysis products that transfer in smoke.
Only in this way can investigators understand whether the exposure to the substance or its pyrolysis products would merit additional testing. If the ingredient, or a pyrolysis product of the ingredient, transfers in smoke, estimation of exposure and dose will become crucial to considerations of potential human health effects. If neither the ingredient, nor any pyrolysis product of the ingredient, transfers in smoke, but the ingredient does induce a change in the composition of the smoke matrix, estimation of exposure and dose from the changed components may similarly become crucial to considerations of potential human health effects, if only to reinforce that an excursion limit can be estimated below which change in the matrix cannot be detected.

Chapter 5 briefly reviews the adverse human health effects of cigarette smoking. For changes induced in the smoke matrix, these outcomes are the gold standard that other tests attempt to predict. With testing in mind, the report moves on to Chapter 6, which reviews some principles of testing. Compliance with these principles is a key element in making a decision about the feasibility of testing. Chapter 6 also reviews the possible linkage of test methods and test data in part to provide some insight into how LSRO might proceed under different circumstances and briefly reviews existing testing procedures and includes a case study of menthol, which transfers in smoke.

Since LSRO noted some areas where additional scientific data would remove uncertainty from the question of feasibility, Chapter 7 reviews research needs associated with the feasibility of testing ingredients added to cigarettes, as distinct from testing the ingredients themselves.

The penultimate chapter, Chapter 8, summarizes LSRO’s conclusions for the convenience of the reader. The Appendices, A-H provide supporting information to assist readers’ in obtaining more detailed and referenced information quickly.
CIGARETTES AND CIGARETTE SMOKE

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3.5 SUMMARY
CIGARETTES AND CIGARETTE SMOKE

3.1 INTENT & PURPOSE

Historically, an initial pharmacological approach to complex mixtures with important biological properties has been the isolation of a substance or a family of related substances responsible for the biological effect. Identification of these pharmacologically active substances represents significant research achievements. Often overlooked by non-scientists however, but just as valuable to the research community, have been demonstrations of the absence of explanatory substances; that is, demonstrations that neither a single substance nor a family of related substances confers the important biological properties of a complex mixture. To scientists, ruling out hypotheses is as important as substantiating them and is necessary for scientific progress. Non-scientists, however, tend to view these explorations as failures and undervalue them.

The presence of a single chemical substance, nicotine, explains many of the attractive properties of cigarette smoking. However, chemical and biological analyses of cigarette smoke have not shown that any single chemical substance or family of chemical substances explains the variety and extent of adverse human health effects of cigarette smoking, as observed in epidemiological studies. Chemical analyses of cigarette smoke have documented a variable, complex mixture of many thousands of substances. LSRO emphasizes that the investigators who established these facts should be viewed as succeeding in their research enterprise. Besides overall properties, such as the physical properties outlined in this chapter, quantitative measurements of the chemical fractions or individual chemical substances characterize cigarette smoke. These physical and chemical properties will prove useful in testing ingredients added to cigarettes.

3.2 CIGARETTES

3.2.1 Cigarette Definition

By definition, a cigarette consists of tobacco and a paper wrapper. (See Appendix E and Fig. 3.1, which illustrates the components of a cigarette). Although manufacturers attempt to control the qualitative and quantitative composition of cigarettes, even within a specific brand, cigarettes vary inherently, because both major components of cigarettes arise from biological sources (CORESTA, 1991c).
A typical U.S. cigarette is approximately 84 mm long with a cellulose acetate filter approximately 21 mm long. It has a tobacco rod of approximately 63 mm and a circumference of approximately 25 mm (diameter of 8 mm). It contains tobacco weighing approximately 0.8 grams (Norman, 1999). Despite high variation in smoke yields from smoker behavior and cigarette properties, an average cigarette yields ten puffs of smoke during ten minutes of smoking, approximately one, fifty milliliter puff each minute (Djordjevic et al., 2000).

At least four physical characteristics of the tobacco rod influence smoke yields: length, circumference, fineness of the tobacco cut, and packing density (Federal Trade Commission, 2000b; Hoffmann & Hoffmann, 1997). Changing the length and/or circumference (keeping the packing density of tobacco constant) will alter the amount of tobacco burned and the amounts of smoke constituents generated (Hoffmann & Hoffmann, 1997; Terrell & Schmeltz, 1970). Changing the resistance-to-draw will make drawing a puff of mainstream smoke more difficult. Resistance-to-draw is the amount of negative pressure applied through suction by the mouth and lungs to the butt end of a cigarette, causing mainstream smoke to flow from the butt end (International Organization for Standardization, 2000).

Engineering changes in cigarette structure to control airflow, such as changes in paper permeability (higher porosity) and alterations in the number of air holes in the filter, can modify the chemical composition of cigarette smoke.
Engineering changes modify the dilution and the concentration of the substrate for pyrosynthesis (Baker, 1999). Engineering changes modify delivered smoke constituents to a greater degree than changes in the composition of the tobacco rod and its added ingredients (Norman, 1999).

### 3.2.2 Cigarette Tobaccos

Tobacco is the principal ingredient of cigarettes. The tobacco plant belongs to the family Solanaceae, genus Nicotiana. Tobacco grows indigenously in the Americas as 64 different species (Tso, 1999). The properties of any single kind of tobacco can vary with the quality of the seed, soil, fertilizer, geographical area, weather conditions, growing season, harvesting time, and other variables (Fisher, 1999). U.S. cigarettes use the leaves of *N. tabacum*, which are classified according to curing method and/or by geographic region where grown. Tobacco leaves are complex biological substances that differ in content of nicotine, sugar and nitrogen, depending not only on the growing conditions mentioned above, but also on the type of tobacco plant, the position of the leaf on the stalk [e.g., higher leaves produce less acidic smoke (Brunnemann & Hoffmann, 1974)], the portion of the leaf sampled (Jenkins & McRae, 1996) and the method of curing. Differences in curing conditions, even with the same tobacco lot, contribute to variation in the tobacco constituents found within the cigarette. Stems, reconstituted sheet and other manufactured products of tobacco plants also may contribute to the nicotine content of cigarettes.

Three traditional curing methods, flue, air, and sun, yield different tobaccos (Institute of Medicine, 2001). The curing process preserves the inherent qualities of tobacco leaves and imparts flavor. Flue curing, which uses tightly constructed barns to apply artificial heat to freshly harvested tobacco, best controls temperature and humidity (Peedin, 1999). Barn temperatures begin at approximately 35°C and end at approximately 75°C, during a five- to seven-day curing period. The main chemical change in the first part of the curing process involves degradation of complex carbohydrate (starch) to sugar. High dry heat applied in the second part of the drying process stops cellular respiration and retains simple sugar content. Such chemical changes increase aromas and decrease bitter tastes in flue cured tobacco, relative to air cured tobacco. The nicotine content of flue cured tobacco (Virginia tobacco) ranges from 2% to 4.5% (Fisher, 1999).

Air curing places tobacco in well-ventilated barns with little or no artificial heat and uses ambient environmental conditions for a duration of four to eight weeks (Jenkins, Jr. *et al.*, 1971). During slow air-drying, cellular respiration in the leaf continues, resulting in decreased natural starch and sugar. The main air cured tobaccos are Burley and Maryland. Burley tobacco was originated in southern and northern Kentucky in 1864. Today, Burley is still grown in Kentucky and surrounding states, setting a standard for full flavor (Palmer & Pearce, 1999).
Burley is less likely to extinguish while smoldering between puffs (Institute of Medicine, 2001). The range of nicotine content of Burley is from 2.5% to 5.0% (Fisher, 1999).

Water loss and shrinkage occur during the curing process. The moisture content of tobacco affects the yield of nicotine and phenols in its smoke. Both yields decrease as the moisture content increases (DeBardeleben et al., 1978). Manufacturers control the moisture content of the tobacco leaves during processing, primarily to control leaf pliability prior to the final cut.

In addition to the field grown tobacco leaf, manufacturers of U.S. cigarettes use two processed tobaccos: reconstituted and expanded tobaccos. Processed tobaccos serve as fillers to reduce tar yields (Fisher, 1999; Norman, 1999). The Federal Trade Commission (1967a) refers to tar as the weight in grams of total particulate matter collected on a Cambridge filter minus the weight of alkaloids, as nicotine, and water. Reconstituted tobacco also delivers lower yields of phenols, carbon monoxide, and benzo[a]pyrene than standard tobacco, because the methods of manufacturing reconstituted tobacco result in a loss of a portion of the tobacco components which apparently generate more of these substances (Fisher, 1999; National Institutes of Health, 1996). Processed filler tobaccos (reconstituted and expanded) add bulk to a cigarette while maintaining desirable characteristics (e.g., ease of draw). Their effects on the smoke depend on the type of reconstituted tobacco and the relative amount in the blend in a cigarette.

Expanding tobacco increases filling capacity (bulk). Today, the most widely used method of manufacturing expanded (“puffed”) tobacco uses carbon dioxide. Each type of tobacco in a blend is separately expanded, rather than expanding the whole blend at one time. More than 150 patents concern the tobacco expansion process. Expanded tobacco has a lower density than other types of tobacco, allowing more aeration, enhancing combustion (Hoffmann & Hoffmann, 2001). Norman (1999) gives specific details of different methods of expanding tobacco.

### 3.2.3 Blends of Different Tobaccos

Manufacturers blend tobaccos to achieve specific tastes (Fisher, 1999; Hoffmann & Hoffmann, 2001). Changes in the proportions of different tobaccos in the blend contribute to variations from season to season in the final product. According to Hoffmann and Hoffmann (2001) and Fisher (1999), the U.S. blend of tobacco is widely recognized as the preferred cigarette by Americans and many world markets.
3.2.4 Cigarette Papers

Cigarette papers are constructed of an inorganic filler and cellulose fiber from either wood pulp or flax fiber (Norman, 1999). Cigarette papers also typically contain added calcium carbonate. Among 26 American brands, permeability to air varied (Taylor, Jr. et al., 2000). The type of paper wrapping, its weight, chemical composition, bulk density, and porosity (air permeability of the paper) will influence the burn rate of a cigarette and will contribute to the concentration of cigarette smoke by modulating the inward transport of air and the outward diffusion of the smoke during and between puffs (Hoffmann & Hoffmann, 2001). By altering airflow, cigarette papers affect burn temperature and concentrations of mainstream and sidestream smoke constituents (See Appendix B and Section 3.2.5, below). Thus, the composition and engineering of cigarette paper strongly contribute to cigarette smoke.

Highly porous papers reduce nitrogen oxides in cigarette smoke and reduce volatile and tobacco specific N-nitrosamines in mainstream smoke (Brunnemann et al., 1994; Brunnemann et al., 1996; Hoffmann & Hoffmann, 2001). Highly porous cigarette paper only minimally changes the yields of tar, nicotine, and benz(a)anthracene in the particulate phase (Hoffmann & Hoffmann, 2001). Phosphate and citrate impregnated into paper reduce the burn rate and can reduce smoke yields of tar and carbon monoxide (Norman, 1999; Owens, 1978).

3.2.5 Cigarette Filters

In 1995, cigarettes with filters comprised 97% of the U.S. domestic market (Federal Trade Commission, 1997). Cellulose acetate filters presently account for nearly the entire market in the U.S. (Norman, 1999). Mainstream smoke is the smoke drawn through the butt end of the cigarette into the mouth as a smoker puffs on a cigarette, while sidestream smoke is the smoke emitted directly into the air from the burning end of the cigarette, largely during the smolder interval between puffs. In general, filtered cigarettes have reduced mainstream smoke yields relative to unfiltered counterparts (wt/wt) but have similar sidestream yields (Baker, 1999; Browne et al., 1980; Chortyk & Schlotzhauer, 1989; Hoegg, 1972). According to Hoffmann and Hoffmann (2001), cellulose acetate filters remove up to 80 percent of the phenols in cigarette smoke and up to 75 percent of the volatile nitrosamines. The presence of phenols (section 3.4.5.2) and nitrosamines (section 3.4.6.3) in tobacco smoke is discussed later in this chapter.

Filter ventilation (air dilution of the mainstream draw) represents the major engineering innovation associated with the low-tar cigarettes (Kozlowski et al., 2001b). The vents usually consist of one or more rings of small holes along the circumference of the filter (Burns & Benowitz, 2001; Hoffmann & Hoffmann, 2001). These perforations allow for a variable amount of air dilution of the smoke during a puff. This dilution slows the velocity of smoke through the burning cone...
of the cigarette as well as slows the flow of smoke through the tobacco rod. Ventilation holes can become blocked with smokers’ lips, fingers or tape which may increase the delivered levels of tar and nicotine (Kozlowski & O’Connor, 2002). When not occluded, the result of filter ventilation can be a more complete combustion and higher retention of particulate matter in the cigarette filter. Presently, more than 50 percent of all cigarettes marketed in the U.S. have perforated filter tips (Benowitz, 2001; Burns et al., 2001).

3.2.6 Ingredients

Literally, a cigarette ingredient is any substance used in making a cigarette. Cigarette ingredients vary by type and brand of cigarette but typically include tobacco, paper wrapping, adhesives, monogram ink and filter, as well as ingredients inherent to tobacco processing and manufacturing, and substances within or added to each of these components. For example, filters are treated with glycerol triacetate, to remove some of the volatile and semi-volatile compounds in mainstream smoke (Hoffmann et al., 1995).

Legal and common definitions of cigarettes encompass tobacco and paper. With this report LSRO is reviewing added ingredients that are any chemical substance, other than tobacco or paper, used to make a cigarette. Paper, inks and filters as well as chemicals used in their manufacture have been excluded from this review and these will be addressed at a later date. Although design and engineering features such as filter ventilation can alter the chemistry of cigarette smoke, this report focuses on the effect of added ingredients that are chemical substances. The term often used to describe such a chemical substance is an “additive.”

For purposes of this report, LSRO restricted considerations of added ingredients to chemical substances currently used as ingredients in cigarettes, excluding engineering controls, such as paper porosity. For testing purposes, an added ingredient can be a single added chemical compound or substance, or a specific recipe of compounds or substances, e.g., a proprietary flavor formula, or a complex mixture, e.g., cocoa. In theory, testing of engineering changes in cigarette design, such as paper porosity, should be amenable to the same strategy as the one LSRO outlines in this report. However, changes in paper porosity are not familiar to LSRO and might not change cigarette smoke in increments as expected in response to changes in amounts of an ingredient. LSRO will defer application of the strategy outlined here until experience accumulates with direct, existing chemical substances added to cigarettes.

No agency of the federal government directly regulates chemical substances added to cigarettes. In 1994, six cigarette manufacturers published a list of 599 substances (now commonly referred to as the “599 list”) that might be added to some cigarettes (Appendix D) (Doull et al., 1994; Select Committee on Health, 2000; Tobacco
In addition, cigarette manufacturers disclose a list of current tobacco additives to the Department of Health and Human Services, as indicated by the industry’s different websites (Philip Morris USA, 2002), (R.J.Reynolds Tobacco Company, 2000), (Brown & Williamson Tobacco Corporation, 2000). LSRO distinguishes these substances as “existing additives,” distinct from newly arising chemical substances that a manufacturer might want to add to a particular brand of cigarette. Most product safety laws and regulations similarly differentiate new from existing substances (Viscusi et al., 2000). The World Health Organization, the Province of British Columbia in Canada and the United Kingdom have issued inquires about ingredients added to cigarettes (Government of British Columbia, 1998; United Kingdom Department of Health, 2003; World Health Organization, 2003).

Connolly and coworkers (2000) summarized the added ingredients that are used to reduce the odor, irritation, visibility and emission of sidestream smoke. For example, vanillin may reduce odors, aluminum sulfate may reduce irritation, calcium carbonate may reduce the visibility of smoke, and magnesium oxide may reduce the quantity of sidestream emissions (Connolly et al., 2000). Three major groups of ingredients added to tobacco include: casings, humectants and flavorings.

A casing refers to a solution that is sprayed onto the whole tobacco leaf or into which the tobacco leaf is dipped. Some examples of casings are water, sugars, licorice, cocoa, and fruit extracts (Fisher, 1999). Manufacturers add casings to improve “processability” of the tobacco in that the tobacco leaf is more pliable than if the casings had not been applied. This provides more consistency in the size and shape of the cut tobacco (Fisher, 1999). Casings also improve smoke quality by retarding the burn rate (Fowles, 2001) and by moderating or masking the harshness and irritation of the smoke, particularly for Burley tobacco (Fisher, 1999). For these reasons, Burley tobaccos receive the greatest application of casings compared to the other tobacco types in the blend of U.S. cigarettes.

Humectants retain moisture and plasticity of tobacco (Hoffmann & Hoffmann, 2001). Presently, the principal humectants added in cigarette manufacturing are glycerol and propylene glycol. Less frequently, sorbitol and diethylene glycol are added (Hoffmann & Hoffmann, 2001). Humectants preserve flavor compounds and produce a less harsh tasting cigarette smoke.

Flavorings add an aroma to packaged cigarettes and improve cigarette quality by enhancing the taste of tobacco without masking it (Fisher, 1999). Flavorings are added near the end of cigarette processing and applied to cut tobacco in relatively small amounts (concentrations in parts per million by weight) (Fisher, 1999). Therefore, flavorings are not ordinarily measured with quantitative analytic methods because the concentrations in smoke fall below detection levels and because the
complex matrices add to the difficulty of obtaining precise and accurate data (Clinton et al., 2000; International Organization for Standardization, 2000).

Flavoring substances number in the hundreds (Fisher, 1999). Ingredients added to cigarettes are essentially unregulated by governments in the U.S. and throughout the world, although the World Health Organization, the Province of British Columbia in Canada, and the United Kingdom have published inquires about the health effects of ingredients added to cigarettes (Government of British Columbia, 1998; United Kingdom Department of Health, 2003; World Health Organization, 2003). See Appendix D for examples of flavorings added to tobacco in the U.S. Manufacturers usually dissolve flavorings in ethanol, which evaporates near the end of the manufacturing process and leaves the flavorings adhered to the tobacco (Fisher, 1999). Menthol, a common flavoring agent can be added up to approximately 2% by weight in some mentholated cigarettes (Select Committee on Health, 2000; Tobacco Institute, 1994). Other flavoring substances include sucrose up to 4% (by weight), glycerol (7%) propylene glycol (2.5%), and cocoa and cocoa products (1.5%). It is possible that added ingredients could increase the level of exposure to combustion products compared to cigarettes without these additives.

### 3.3 CIGARETTE SMOKE

#### 3.3.1 Smoking

LSRO is primarily interested in the health effects of smoking cigarettes and the potential influence of nontobacco ingredients added to cigarettes on these health effects. The behavior of individual smokers is highly variable, and the smoke they inhale is relatively inaccessible to investigators (Adams, 1976; Creighton et al., 1978; Höfer et al., 1992). Thus, the study of the toxic material to which smokers are exposed is a relatively difficult scientific subject.

#### 3.3.2 Types of Smoke

Most of a cigarette will burn to smoke and ash (Baker, 1999). Cigarette smoke is a complex mixture of heterogeneous inorganic and organic compounds consisting of vaporized chemicals and particulate matter suspended as an aerosol formed by the combustion of tobacco (Sethi & Rochester, 2000).

Cigarette smoke occurs in three relatively independent forms: mainstream smoke, sidestream smoke, and exhaled smoke. A smoker draws mainstream smoke through the non-burning, butt end of the cigarette into the mouth while puffing on a cigarette (Institute of Medicine, 2001). Frequent reference also is made to environmental tobacco smoke (ETS), which is found in the air around smokers or air carried away from smokers. ETS is composed of sidestream smoke and exhaled smoke.
in varying proportions, greatly diluted by ambient air. The terms “passive” and “second hand” smoke are often used interchangeably for ETS.

Smoke emitted during the interval between puffs is sidestream smoke, including the smolder stream emitted from the butt end. The amount of sidestream smoke produced will fluctuate during the puff interval, and the total amount of sidestream smoke produced during the puff interval correlates with the weight of tobacco burned during this interval (Baker, 1984). Smokers inhale all types of smoke including mainstream smoke while puffing, sidestream smoke from the immediate breathing zone, air mixed with some previously exhaled mainstream smoke, and even some environmental tobacco smoke (Institute of Medicine, 2001; Sethi & Rochester, 2000).

![Diagram of the major processes generating cigarette smoke during a puff.](image)

**3.3.3 Processes in the Formation of Cigarette Smoke**

The formation of cigarette smoke is a dynamic process. Many processes contribute to the generation of smoke, including combustion, pyrolysis, pyrosynthesis, direct transfer, distillation, filtration and dilution (Fig. 3.2). Jenkins and McRae (1996) reviewed the last half-century of research on the formation of smoke.
### 3.3.3.1 Combustion

After ignition of the end of the tobacco rod, a process of combustion ensues, actively driven by heat and the oxygen from the ambient air the smoker draws through the cigarette. A burning cone forms within the cylinder of a lit cigarette. Atmospheric conditions and suction applied by the smoker influence the burning. Cigarette structure and ingredients, such as sodium citrate added to the cigarette paper, also affect the burn rate.

The burn region lacks oxygen, temperatures range up to $950^\circ C$, and heating rates go as high as 500 degrees per second (Baker, 1975; Baker, 1999). Maximum heat production occurs at the tip (ash) side of the paper burn line while the smoker puffs (Fig. 3.3). Using a thermocouple inserted to a depth of approximately 4 mm into the cigarette at 1.5 cm from the tip end before lighting, Langer and coworkers (1989) measured burn temperatures until the combustion zone passed the thermocouple. The peak temperature of burn ranged from $520^\circ C$ to $620^\circ C$ and averaged $575 \pm 30^\circ C$ for 17 different U.S. cigarettes.

![Figure 3.3 Variation in temperatures (°C) inside the combustion zone of a cigarette following one second of a 2-second puff. The estimated direction of vapor flow pattern is shown. Reprinted with permission from (Baker, 1975). Reproduced with permission of Humana Press.](image)

The region of combustion continually advances toward its source of fuel, unburned tobacco. Complete combustion generates carbon dioxide, carbon monoxide, nitric oxide, moisture (water), and ash. Ash is primarily an inorganic mineral residue and amounts to an average of $116 \pm 16$ mg/cigarette (average of 17 U.S. brands) or
approximately 14-15% of the original weight of the tobacco, when burned at 550°C (Langer et al., 1989). An invisible plume of vapor rises from the combustion zone, 4 to 8 mm from the tip side of the paper burn line (Baker, 1999), which can mix with the visible aerosol plume arising from the pyrolysis-distillation zone. (See below.) The rest of the vapor generated in the combustion zone is drawn deeper into the tobacco rod.

### 3.3.3.2 Change of state

As a cigarette burns, many chemical substances present in the solid state in tobacco liquefy and/or turn to gas, as the temperature climbs. As these gases pass down the column of tobacco and cool, they re-liquefy and re-solidify on the surface of the tobacco. Changes of state advance ahead of the heat generating combustion zone, moving down the burning tobacco rod. Thus, the materials undergoing combustion change, as the region of combustion advances.

### 3.3.3.3 Distillation and chromatography

Distillation is a physical process. Application, then removal, of heat separates a liquid mixture of two or more chemical compounds. The application of heat selectively vaporizes the compounds according to their boiling points. Subsequent removal of heat condenses the vapor back into a liquid.

Distillation in a smoked cigarette occurs as the heated smoke moves down the tobacco column. When highly volatile, low-boiling point substances enter the vapor phase, they move ahead of the burning zone. Ambient air enters the cigarette adjacent to the butt side of the paper burn line, cooling the internal temperature of the cigarette. The gas containing suspension of fine liquid and solid particles cools, condensing into an aerosol. Some vaporized compounds become trapped in aerosol droplets. Examples of substances distilled out of tobacco while cigarettes undergo smoking include saturated and unsaturated aliphatic and terpenoid hydrocarbons, lactones, esters, carboxyls, alcohols, sterols, alkaloids, amino acids and aliphatic amines (Green, 1977; Hecht et al., 1977; Stedman, 1968).

Chromatography is a related physical process through which one chemical compound passes down the tobacco rod with a different velocity than a second, different chemical compound. In either gas or liquid state, the two compounds absorb to tobacco differentially and the hot gas passing along the column acts as a carrier, moving the two compounds along. Progression of the pyrolysis-distillation zone and paper burn line will eventually destroy the two substances by combustion or pyrolysis, or displace them through volatilization.

Distillation and chromatography play roles in changing the chemical composition of cigarette smoke as rod length decreases during the smoking of a cigarette.
3.3.3.4 Pyrolysis

A pyrolysis-distillation zone forms just behind the combustion area, on the butt side of the paper burn line (Baker, 1975; Baker, 1981; Baker, 1999). The majority of organic smoke products form in the pyrolysis-distillation zone (Dube & Green, 1982; Jenkins & McRae, 1996). High temperatures in the pyrolysis-distillation zone thermally decompose tobacco. However, pyrolysis is not a synonym for combustion, as it includes nonoxidative processes. Nonvolatile substances in tobacco which decompose through pyrolytic degradation include sugars, polysaccharides, polyphenols, and proteins (Baker, 1999).

Pyrolysis and combustion will completely eliminate some substances found in cigarettes, whereas other highly volatile and thermally stable substances (e.g., menthol or n-dotriacontane) can transfer from the tobacco into smoke (Baker, 1999; Stedman, 1968; Wakeman, 1972). Small amounts of substances of poor volatility and/or high molecular weight in hot, fine particles of tobacco and ash, may propel into the smoke stream without pyrolysis (Baker, 1999). Such entrainment of solids can include inorganic salts, metals, sterols, carbohydrates, and/or pigments (Jenkins & McRae, 1996; Stedman, 1968).

Dube and Green (1982) estimated that analytical chemists have identified 1135 compounds which could transfer unchanged from tobacco to smoke. That is, approximately one-third of the chemically identified substances in mechanically generated cigarette smoke might escape pyrolysis and transfer directly from tobacco into smoke (Green, 1977). A recent pyrolysis study of 291 added ingredients predicted that almost two thirds of these ingredients would transfer into the mainstream smoke 95% intact (Baker & Bishop, 2004)).

3.3.3.5 Pyrosynthesis

Pyrosynthesis involves the formation of new chemical substances in smoke through recombination of pyrolysis fragments. Pyrolysis and pyrosynthesis occur simultaneously. Chemically reactive fragments, including free radicals, subsequently recombine, particularly in the hot gas phase within the burning cigarette. These reactions create new chemical substances, which include, pyridines, indoles, nitriles, aromatic amines, furans, phenols and carbonyls (Baker, 1987; Brunnemann & Hoffmann, 1982; Chortyk & Scholtzhauer, 1973; Hecht et al., 1977; Jenkins, Jr. et al., 1975; Jenkins, Jr., 1990; Scholtzhauer & Chortyk, 1987). If these new substances have sufficiently high boiling points, they may liquefy or even solidify onto tobacco between the pyrolysis-distillation region and the butt end. Few secondary oxidation products form in the migrating pyrolysis-distillation region, because concentrations of oxygen are low.

The chemical reactions that occur during pyrolysis and pyrosynthesis do not produce random chemical fragmentation. Instead, the patterns of bond breaking and bond
formation involve distinctive patterns dictated by thermodynamic considerations, analogous to the uniform patterns obtained when chemical substances undergo fragmentation during mass spectrometry. Some of these reactions and the associated, overall considerations are theoretically predictable. The Rice-Ramsperger-Kassel-Marcus (RRKM) theory describes chemical energy transfer and activation under the steady state and non-steady state conditions important to the unimolecular bond breaking and formation events, such as those involved in pyrolysis and pyrosynthesis (Davis et al., 1999; Kiefer et al., 1997; Schranz & Sewell, 1996).

3.3.3.6 Transfer

The physical process of transfer of a chemical substance in smoke remains poorly characterized. In principle, a volatile substance that resists pyrolysis will have the optimal thermodynamic properties to transfer in cigarette smoke. Thus, transferability seems highly predictable. As the heat generating combustion zone moves down the burning tobacco rod, a substance that resists pyrolysis will undergo changes of state, become a gas, and move with the plume of smoke. Eventually, such substances will enrich mainstream smoke in higher concentrations as it exits the end of the cigarette.

3.3.3.7 Filtration, agglomeration, and dilution

Liquid smoke particles (~20 per cent water) vary in size from < 0.1 to 1.0 µm. Particles less than 0.1 µm in diameter attach either to larger particles in the aerosol or to the surface of tobacco (Institute of Medicine, 2001). Tobacco also filters out particles greater than approximately 1 µm in diameter from the smoke stream (Institute of Medicine, 2001). Thus, the length of unburned cigarette will change smoke characteristics. Fibers in the filter at the butt end of the cigarette also remove aerosol particles from the smoke stream. Impaction removes particles, when a particle is too large to follow the air flow, as it bends around the fibers in the filter, by diffusion. The filter fibers capture smaller particles, by interception as the particles in the smoke stream rub against the filter fibers (Jenkins & McRae, 1996). Gaseous components of smoke may exchange into the particulate phase as it migrates through the rod. Air diffusing into the filter, before the smoker inhales it, dilutes the smoke.

Smoke yields vary with the length of the remaining, unburned cigarette. As the tobacco burns, and as cigarette length decreases, fewer smoke constituents escape through the paper wrapper, and fewer constituents filter from the smoke into the tobacco rod. Thus, a cigarette delivers relatively more of its components, such as nicotine, in mainstream smoke per cm, near the butt end, as the burning tobacco rod shortens (Baker, 1999).
3.3.4 Smoke Characteristics

Most of the knowledge of cigarette smoke comes from studies of mechanically generated cigarette smoke that reflect the composition and properties of the smoke as it is exposed to human tissues during smoking. Thus, scientists interpret the database about cigarette smoke with caution. Whole cigarette smoke is an aerosol, which scientists usually partition into two phases, a vapor (gas) phase and a particulate phase (Pankow, 2001). Molecules in the smoke distribute between the vapor and particulate phases. Each substance seeks a state of vapor/particulate equilibrium (Pankow, 2001).

A portion of the aerosol rises out of the tobacco rod, cooling from ~ 600°C to nearly ambient temperature in a few milliseconds (Baker, 1999; Institute of Medicine, 2001). A visible plume forms from the butt side of the burn line in areas where the temperature has cooled to less than 150°C (Baker & Robinson, 1990). Less volatile components quickly condense into airborne droplets or particles. Another fraction of the aerosol is drawn through the tobacco rod. Some particles in this portion of the aerosol condense on the surface of tobacco. These particles re-volatilize, pyrolyse or combust during the next puff, as the burning zone reaches the zone of condensation. A smoker’s inhalation draws the remaining aerosol through the cigarette, away from the burning zone.

The conditions that generate sidestream smoke differ from those producing mainstream smoke in at least two ways: (1) natural convection drives the air flow that results in sidestream smoke, and (2) the burning zone temperature is nearly 800°C as the cigarette smolders during the puff interval, compared with the ~ 950°C of the burning zone during puffs (Baker, 1999).

The total volume of mainstream smoke generated from approximately 10 puffs (50 mL each) of a typical cigarette, is approximately 500 mL; of which about 350 mL (~70%) is vapor (Djordjevic et al., 2000). The vapor phase consists mostly of common atmospheric gases (e.g., oxygen, nitrogen, carbon dioxide) that constitute 90 to 96% of its total weight (Pankow, 2001).

The particulate phase of smoke consists largely of non-aqueous (~10% water) liquid droplets containing a heterogeneous mixture of condensed organic compounds (e.g., nicotine or benzo furans). These particulate droplets suspend in the vapor phase. Together the two phases form an aerosol (Pankow, 2001). The molecular weights of the chemical compounds in the particulate phase usually exceed 200 (Institute of Medicine, 2001). The concentrations of particles in a smoker’s puff range from 0.3 to 3.0 x 10^9 per cm^3 (Martonen, 1992; Sethi & Rochester, 2000). The estimated densities of particles in mainstream and sidestream smokes are approximately 1.0 g/cm^3 (McRae, 1990). The amount of total particulate matter in smoke varies. Rustemeier and coworkers (2002) reported that added ingredients
commonly used in cigarette manufacturing increase total particulate matter by 13 to 28 percent.

Ogg (1964) reported data from an early, collaborative study of twelve laboratories concerning the determination of particulate matter collected from smoke of commercial cigarettes with and without filters. Smoke generated under conditions similar to those recommended by the Federal Trade Commission (FTC) generated an average of 32 ± 1.1 mg of particulate matter for non-filtered cigarettes and 24.6 ± 0.8 mg for filtered cigarettes.

### 3.3.5 Aging of Smoke

The aerosol of cigarette smoke changes both chemically and physically over time as it migrates from the combustion zone, through the tobacco rod, and eventually into the ambient air. Baker (1999) has reviewed changes in the composition of smoke during aging. Cigarette smoke contains many highly reactive chemical fragments, such as beta-carbolines and free radicals (Totsuka et al., 1999). Synthesis of substances with decreased chemical reactivity continues, as time and distance from tobacco combustion increases. Thus, the age of cigarette smoke is a crucial parameter in experimental studies.

In an early effort to measure the chemical changes of mainstream smoke, Vilcins and Lephardt (1975) scanned undiluted mainstream smoke immediately after it exited a mechanically smoked cigarette with infrared spectroscopy. They scanned every 3.5 seconds for three minutes. An initial and rapid decrease in the concentration of nitric oxide (NO) occurred, accompanied by a rapid build up in nitrogen dioxide (NO₂), which reached a maximum after approximately one minute and slowly decreased. Oxygen in ambient air mixed with the smoke and converted nitric oxide to nitrogen dioxide. Similar kinetic changes in smoke chemistry were reported by Cueto and Pryor (1994).

As smoke forms, components of the smoke randomly collide, at all ranges of physical observation, from molecular to macroscopic. Larger components coalesce into droplets ranging in size from <0.1 to 1.0 µm in diameter, with ~45% of the droplet particles in the range of 0.15 to 0.20 µm (Baker, 1999). These droplets further coalesce. Aerosols, including cigarette smoke, generally do not attain a stable equilibrium of particle size and mass concentration. Volatile and semi-volatile substances evaporate out of smoke particles as cigarette smoke ages (Baker, 1999). Further decay and elimination of the smoke aerosol is caused by eddy, Brownian diffusion and sedimentation. With additional aging (catabolic chemical transformations) and continuing dilution, the smoke constituents disperse and eventually dissipate into (or precipitate out of) the atmosphere (Hollander & Stober, 1986).
3.3.6 Sensory Evaluation of Smoke

Judging from the published literature, the perception of smoke by human smokers remains poorly understood. If smokers decline cigarettes, the effects of changes induced by alterations in ingredients or structure become irrelevant. Cigarettes made only with Virginia tobacco (flue cured; high natural sugar content of 15.5\% by weight) may be more acidic than those made with blended tobaccos. Smokers of American blended cigarettes perceived them as having an “unbalanced” flavor (Leffingwell, 1999). Schievelbein (1982) demonstrated that cigarettes made with Virginia tobacco yielded higher amounts of nicotine and produced higher blood nicotine concentrations in intubated dogs than cigarettes made with black type tobacco or a German blend of tobacco, the latter thought more alkaline.

Based on available evidence, nicotine yield influences the sensation of impact perceived by the smoker. Kochhar and Warburton (1990) determined that cigarettes with higher nicotine yields (1.7 mg) associated with greater mouth, throat and chest sensations than lower yielding cigarettes (0.8 mg). For each type of cigarette, impact increased puff-by-puff over the duration of smoking. Bevan (1991) determined that the addition of 5 mg of naturally occurring S-isomer of nicotine to denicotinized cigarettes resulted in a sensation of “impact” for two of three smokers compared to cigarettes spiked with other alkaloids. Leffingwell (1999) and Pankow (2001) suggested that the unprotonated (freebase) form of nicotine associated with sensory and physiological perceptions of harshness and intensity. However, under experimental conditions, Burch and coworkers (1993) found no statistical difference in symptoms of cough, throat burning or salivation among ten volunteers who inhaled aerosols varying in pH from 5.6 to 11 and containing 0.484 mg of nicotine per puff, delivered every 30 seconds over a five minute period (10 puffs total). The particle size of the aerosol in this study exceeded that in cigarette smoke.

3.3.7 Reference Cigarettes

In 1968, the Scientific Advisory Board of the Council for Tobacco Research asked the Industry Technical Committee to arrange for the development of a reference cigarette (Davies & Vaught, 1990). In response, scientists at the Tobacco and Health Research Institute (THRI) of the University of Kentucky developed several reference cigarettes, beginning with 1R1 in 1969 (Table 3-1) (Coggins, 2000; Davies & Vaught, 1990). The latest reference cigarette that THRI designed, the 2R4F, yields tar and nicotine near the sales-weighted average of cigarettes currently sold in the U.S. (Tobacco and Health Research Institute, 2002). Table 3-1 provides the yields of tar, nicotine and carbon monoxide of various reference cigarettes. Other reference cigarettes have been developed (e.g., low alkaloid yield) (Davies & Vaught, 1990; World Health Organization, 1986).
Table 3-1. Component levels of mainstream smoke from Kentucky research cigarettes (85 mm) as measured under standard laboratory conditions.\(^a\)

<table>
<thead>
<tr>
<th>Kentucky series</th>
<th>Year introduced</th>
<th>Year introduced</th>
<th>Butt length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23 mm</td>
<td>30 mm</td>
<td>23 mm</td>
</tr>
<tr>
<td>1R1(^b)</td>
<td>1969</td>
<td>34.3</td>
<td>30.1</td>
</tr>
<tr>
<td>2R1(^b)</td>
<td>1974</td>
<td>36.8</td>
<td>32.9</td>
</tr>
<tr>
<td>2R1F(^b)</td>
<td>1974</td>
<td>-</td>
<td>23.4</td>
</tr>
<tr>
<td>2A1(^c)</td>
<td>1974</td>
<td>36.4</td>
<td>31.8</td>
</tr>
<tr>
<td>3A1</td>
<td>1975</td>
<td>31.1</td>
<td>26.8</td>
</tr>
<tr>
<td>1A2 (^b)</td>
<td>1969</td>
<td>34.</td>
<td>29.5</td>
</tr>
<tr>
<td>1A3 (^b)</td>
<td>1969</td>
<td>29.0</td>
<td>25.8</td>
</tr>
<tr>
<td>1A4 (^b)</td>
<td>1969</td>
<td>35.5</td>
<td>29.8</td>
</tr>
<tr>
<td>1R4F</td>
<td>1983</td>
<td>-</td>
<td>9.2(^d)</td>
</tr>
<tr>
<td>1R5F</td>
<td>1989</td>
<td>-</td>
<td>1.67(^d)</td>
</tr>
<tr>
<td>2R4F</td>
<td>2002</td>
<td>-</td>
<td>9.9(^d)</td>
</tr>
</tbody>
</table>

\(^a\) Data from Davies and Vaught (1990), International Agency for Research in Cancer, Table 9 (World Health Organization, 1986) and Tobacco and Health Research Institute (2002).

\(^b\) No longer available; codes designate the run, series, blend and whether or not the cigarette had a filter (F) (e.g., 1R1 indicates first run, R series, blend 1, without a filter).

\(^c\) Equivalent to the original 1A1, produced in 1969 and no longer available; 4A1 was the fourth run in this low alkaloid series, produced in 1990.

\(^d\) 35-mm butt length.
While the sales-weighted tar was 12 mg in 1998, the range for 1294 domestic brands analyzed was less than 0.5 to 27 mg per cigarette and 1 to 26 mg per cigarette for 26 brands of U.S. cigarettes analyzed one year later (Federal Trade Commission, 2000a) (Gray & Boyle, 2000) (Taylor, Jr. et al., 2000). Tar yields from cigarettes vary widely, and exposures should vary commensurately.

The scientific community uses standardized cigarettes to generate smoke for research, to obtain standardized materials for inter-laboratory comparisons, and to validate analytical methods. Analysis of the smoke from a reference cigarette in parallel with an experimental cigarette, which is the object of study, serves as an internal standard to the study. The FTC monitors cigarettes of known yields for tar, nicotine and carbon monoxide in four or more of twenty ports of their automated smoking machine to verify its proper function (National Institutes of Health, 1996). Inclusion of reference cigarette data would shed light on the precision and accuracy of the methods used, when chemical composition of cigarette smoke is tested.

Descriptions of smoke composition in the literature typically refer to “plain, unfiltered cigarettes of various types” or “high tar” or “low tar” cigarettes, without accompanying indications that the investigators used reference cigarettes (Baker, 1999; World Health Organization, 1986). Quantitative data exist for the tar, nicotine and carbon monoxide contents of mainstream smokes generated with reference cigarettes under standard U.S. Federal Trade Commission (FTC) procedures (1967a). However, U.S. consumers smoke many brands of cigarettes (e.g., 1294 in 1998)(Federal Trade Commission, 2000a). Care should be taken to select an appropriate reference cigarette. Reference cigarettes made decades ago do not necessarily represent cigarettes manufactured today.

3.3.8 Mechanical Methods of Smoke Generation

Automated smoking machines usefully improve consistency in generating smokes from cigarettes and make the smokes accessible to study. One early standard, promulgated by the FTC, acquired regulatory status. LSRO recommends a single smoke generation protocol be used throughout a study for consistency between experiments. The protocol should deliver smoke components in sufficient quantity, not deliver smoke so diluted as to be below the limits of detection of current assays.

One crucial matter of interpretation is that machines generate FTC protocol cigarette smoke that differs in composition from the smoke inhaled by a typical smoker. Many experimental studies use mainstream smoke obtained by drawing air through cigarettes, either in a pulsatile or a continuous manner, although human smokers inhale a mixture of mostly mainstream smoke and variable amounts of sidestream smoke (Benowitz et al., 1983; Benowitz, 2001; Guerin, 1996). Special chambers to generate sidestream smoke were developed by Brunnemann and Hoffmann (1974; World Health Organization, 1986). Klus (1990) also described devices to
collect sidestream cigarette smoke. Witschi and coworkers (1997b) used an experimental mixture of sidestream and mainstream smoke in a mouse lung adenoma model.

Private standard setting organizations also have developed specifications for cigarette smoking machines (CORESTA, 1991a; International Organization for Standardization, 2000). Baumgartner and Coggins (1980) described older models of smoking machines. Jaeger (2002) reviewed the functioning of a modern smoking machine. Computers now control the functions of smoking machines. A puff profile graphically represents the rate of mainstream smoke flow, plotted as a function of time during the puff (International Organization for Standardization, 2000). Puff recorders have been designed to measure the smoking patterns of human smokers (Bentovato et al., 1995; Djordjevic et al., 1995; Puustinen et al., 1987). This approach allows the drawing of each puff and smoking of each cigarette in a manner similar to human smoking (Bentovato et al., 1995; Creighton et al., 1978; Puustinen et al., 1987). (See Chapter 4.)

3.4 COMPONENTS OF CIGARETTE SMOKE

3.4.1 Intent of Analysis of Cigarette Smoke

Throughout this report, LSRO refers to the “chemical matrix,” or the “smoke matrix,” as a shorthand way of communicating the concept of cigarette smoke as a complex mixture of many chemical substances, which changes with time. Scientists collect smoke from cigarettes (mainstream smokes and/or various smoke fractions) to assure consistency in manufacturing, to compare brands, especially for tar, nicotine and carbon monoxide, and to understand the effects of the cigarettes (Dube & Green, 1982; Kozlowski et al., 2001b). Many research studies test the effects of smoke and smoke constituents on parameters of health outcomes. Standardized methods of testing cigarette smoke are also important for the accuracy and reproducibility of industrial and research studies.

3.4.2 Measurement of Substances in Cigarette Smoke

Various factors alter the chemical composition of mainstream smoke. Bright tobacco (flue-cured) produces less nicotine and more benzo[a]pyrene in smoke than Burley (air-cured) tobacco (Gori, 1980). Cigarettes with filters have lower mainstream smoke yields than unfiltered cigarettes but may have similar sidestream yields (Baker, 1999; Browne et al., 1980; Chortyk & Schlotzhauer, 1989; Hoegg, 1972). Therefore, most authors include ranges, instead of median or mean values, in surveys of smoke constituents (Baker, 1999; Davies & Vaught, 1990; Hoffmann & Hoffmann, 2001). Concentrations of various substances in cigarette smoke can vary widely (reportedly threefold to twenty-fold) between brands (Gray & Boyle, 2002). In addition, the yields of constituents will vary with the method of smoking the cigarette.
Reviewers frequently refer to the smoke composition data as “approximate” or “typical” (Institute of Medicine, 2001; World Health Organization, 1986). Most of the data in this section came from model studies, not direct measurements of human smoking, which adds uncertainty to interpretations that attempt to relate the composition of cigarette smoke to the delivery of chemical constituents to the human respiratory tract.

The chemical characteristics of smoke differ from the chemical characteristics of unburned tobacco. Roberts (1988) identified more than 25 classes of chemical substances in unburned tobacco and cigarette smoke, which he categorized by functional groups. Hoffmann and coworkers (2001) added N-nitrosamines to Robert’s list (1988). Table 3-2 identifies classes of compounds detected in tobacco leaf, cigarette smoke, or both. Gudzinowicz and Gudzinowicz (1980) also listed chemical substances detected in tobacco leaf and cigarette smoke.

The number of chemical substances identified in tobacco and cigarette smoke has increased rapidly over the last 30 years (Fig 3.4) (Green & Rodgman, 1996). Dube and Green (1982) found 1414 substances in the tobacco leaf pyrolysed during smoking and not transferred to smoke. They also found that cigarette smoke contained 3875 substances. Of these substances, an estimated 1135 transferred directly with the smoke. These substances did not pyrolyse during smoking. They estimated that 2740 substances occurred uniquely in smoke that they did not detect in tobacco. These components apparently formed through pyrolytic degradation of tobacco and/or subsequent pyrosynthesis. They did not report the types of cigarettes smoked and the methods used to generate and analyze the smoke. Later, Green and Rodgman (1996) estimated approximately 4800 chemical substances in cigarette smoke. (Fig. 3.4)

Figure 3.4 Cumulative number of chemical substances identified in tobacco and cigarette smoke by year. Reprinted with permission and modified from (Green & Rodgman, 1996).
### Table 3-2. Numbers of substances in different chemical classes identified in tobacco leaf and/or cigarette smoke.\(^a\)

<table>
<thead>
<tr>
<th>Chemical category</th>
<th># in both leaf and smoke</th>
<th>Additional # in leaf</th>
<th>Additional # in smoke</th>
<th>Total # detected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Functional Groups</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carboxylic acids</td>
<td>140</td>
<td>450</td>
<td>69</td>
<td>659</td>
</tr>
<tr>
<td>Amino acids</td>
<td>16</td>
<td>95</td>
<td>18</td>
<td>129</td>
</tr>
<tr>
<td>Lactones</td>
<td>39</td>
<td>129</td>
<td>135</td>
<td>303</td>
</tr>
<tr>
<td>Esters</td>
<td>314</td>
<td>529</td>
<td>456</td>
<td>1299</td>
</tr>
<tr>
<td>Amides and imides</td>
<td>32</td>
<td>205</td>
<td>227</td>
<td>464</td>
</tr>
<tr>
<td>Anhydrides</td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>48</td>
<td>111</td>
<td>106</td>
<td>265</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>12</td>
<td>138</td>
<td>30</td>
<td>174</td>
</tr>
<tr>
<td>Nitriles</td>
<td>4</td>
<td>4</td>
<td>101</td>
<td>109</td>
</tr>
<tr>
<td>Ketones</td>
<td>122</td>
<td>348</td>
<td>461</td>
<td>931</td>
</tr>
<tr>
<td>Alcohols</td>
<td>69</td>
<td>334</td>
<td>157</td>
<td>560</td>
</tr>
<tr>
<td>Phenols</td>
<td>40</td>
<td>58</td>
<td>188</td>
<td>286</td>
</tr>
<tr>
<td>Amines</td>
<td>37</td>
<td>65</td>
<td>150</td>
<td>252</td>
</tr>
<tr>
<td>N-nitrosamines(^b)</td>
<td>19</td>
<td>23</td>
<td>18</td>
<td>60</td>
</tr>
<tr>
<td>Sulfur compounds</td>
<td>2</td>
<td>3</td>
<td>37</td>
<td>42</td>
</tr>
<tr>
<td><strong>N-Heterocyclics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyridines</td>
<td>46</td>
<td>63</td>
<td>324</td>
<td>433</td>
</tr>
<tr>
<td>Pyroles and indoles</td>
<td>3</td>
<td>9</td>
<td>88</td>
<td>100</td>
</tr>
<tr>
<td>Pyrazines</td>
<td>18</td>
<td>21</td>
<td>55</td>
<td>94</td>
</tr>
<tr>
<td>Non-aromatics</td>
<td>7</td>
<td>13</td>
<td>43</td>
<td>63</td>
</tr>
<tr>
<td>Polycyclic aromatics</td>
<td>0</td>
<td>1</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>Other heterocyclics</td>
<td>2</td>
<td>4</td>
<td>50</td>
<td>56</td>
</tr>
<tr>
<td>Ethers</td>
<td>15</td>
<td>53</td>
<td>88</td>
<td>156</td>
</tr>
<tr>
<td><strong>Hydrocarbons</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saturated aliphatics</td>
<td>44</td>
<td>58</td>
<td>113</td>
<td>215</td>
</tr>
<tr>
<td>Unsaturated aliphatics</td>
<td>10</td>
<td>38</td>
<td>178</td>
<td>226</td>
</tr>
<tr>
<td>Monocyclic aromatics</td>
<td>25</td>
<td>33</td>
<td>138</td>
<td>196</td>
</tr>
<tr>
<td>Polycyclic aromatics</td>
<td>35</td>
<td>55</td>
<td>317</td>
<td>407</td>
</tr>
<tr>
<td><strong>Pesticides</strong>(^b)</td>
<td>25</td>
<td>28</td>
<td>25</td>
<td>78</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td>19</td>
<td>112</td>
<td>110</td>
<td>241</td>
</tr>
<tr>
<td><strong>Inorganic and metallic</strong></td>
<td>69</td>
<td>105</td>
<td>111</td>
<td>285</td>
</tr>
</tbody>
</table>

\(^a\) Modified from Roberts (1988).

\(^b\) Two additional groups, N-nitrosamines and pesticides, added by Hoffmann et al. (2001).
Hoffmann and coworkers (2001) identified 48 major groups of chemical compounds in the vapor phase of mainstream smoke from cigarettes without filters. The data in Table 3-3 provide ranges that illustrate the variation in chemical composition of the vapor phase, assuming accurate analytical methods and absolute separation of the two phases by filtration. Table 3-4 lists the major constituents in the particulate matter phase of mainstream smoke of unfiltered cigarettes, as originally reported by Hoffmann and coworkers (2001). Undiluted sidestream smoke contains 30-to-90% more particulate matter, 2-to-4 times more nicotine, 20-to-30 fold more aromatic amines, and more trace metals than mainstream smoke (Klus, 1990; US Consumer Product Safety Commission, 1993).

3.4.2.1 Tar
The FTC (1967a) refers to tar as the weight in grams of the total particulate matter collected on a Cambridge filter minus the weight of alkaloids, as nicotine, and water. Cigarette smoke aerosols typically contain particulate matter which is approximately 7% nicotine, 10% water, and 83% tar (Pankow, 2001). Tar is a heterogenous condensate of myriad substances filtered from smoke. Under FTC definitions, a cigarette will generate 10 puffs of 35 ml, which in turn typically yields 40,000 to 60,000 mg/m³ of tar.

3.4.2.2 Nicotine
Analysts estimate nicotine in the particulate phase from the measured amount of extracted particulate matter injected into a gas chromatograph. The resulting nicotine peak is compared against a standard curve. The gas chromatographic method for nicotine was modified from the method of Wagner and Thaggard (Federal Trade Commission, 1979; Federal Trade Commission, 1980). Earlier data, such as the widely quoted review by Dube and Green (1982), reflected the composition of smoke from typical cigarettes used in the early 1980’s, which had higher levels of nicotine (Institute of Medicine, 2001). Baker (1999) cited the yields of mainstream smoke from 17 reports published between 1966 and 1990. His compilation of data reflected the chemical compositions of unfiltered cigarettes. Baker (1999) found a threefold range of values for nicotine. Nicotine accounts for 90% or more of the alkaloid fractions of cigarette smoke (Gudzinowicz & Gudzinowicz, 1980).

Manufacturers control the tobacco blend and cigarette design (porosity of papers and filters to yield target concentrations of tar and nicotine (Fisher, 1999; Fowles, 2001). Cigarette filters alter the amount of nicotine in smoke. Ogg (1964) reported data from an early collaborative study of 12 laboratories concerning the determination of nicotine collected from smoke of commercial cigarettes with and without filters. Smoke, generated under conditions similar to those of the FTC, produced an average of 1.57 ± 0.06 mg nicotine for unfiltered cigarettes and 1.26 ± 0.54 mg for filtered cigarettes. Perforation of the filter with ventilation holes further alters nicotine yields. Browne and coworkers (1980) determined that nicotine
Table 3-3. Chemical substances and chemical classes in the vapor phase of mainstream smoke of cigarettes without filters.\(^a\)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Yield/cigarette smoked (% weight of mainstream smoke)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>280-320 mg (56 - 64%)</td>
</tr>
<tr>
<td>Oxygen</td>
<td>50-70 mg (11-14%)</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>45-65 mg (9-13%)</td>
</tr>
<tr>
<td>Carbon Monoxide</td>
<td>14-23 mg (2.8 – 4.6%)</td>
</tr>
<tr>
<td>Water</td>
<td>7-12 mg (1.4-2.4%)</td>
</tr>
<tr>
<td>Argon</td>
<td>5 mg (1.0%)</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>0.5-1.0 mg</td>
</tr>
<tr>
<td>Ammonia</td>
<td>10-130 µg</td>
</tr>
<tr>
<td>Nitrogen oxides (NO(_x))</td>
<td>100-600 µg</td>
</tr>
<tr>
<td>Hydrogen cyanide</td>
<td>400-500 µg</td>
</tr>
<tr>
<td>Hydrogen sulfide</td>
<td>20-90 µg</td>
</tr>
<tr>
<td>Methane</td>
<td>1.0-2.0 mg</td>
</tr>
<tr>
<td>Other volatile alkanes (20)</td>
<td>1.0-1.6 mg*</td>
</tr>
<tr>
<td>Volatile alkenes (16)</td>
<td>0.4-0.5 mg</td>
</tr>
<tr>
<td>Isoprene</td>
<td>0.2-0.4 mg</td>
</tr>
<tr>
<td>Butadiene</td>
<td>25-40 µg</td>
</tr>
<tr>
<td>Acetylene</td>
<td>20-35 µg</td>
</tr>
<tr>
<td>Benzene</td>
<td>12-50 µg</td>
</tr>
<tr>
<td>Toluene</td>
<td>20-60 µg</td>
</tr>
<tr>
<td>Styrene</td>
<td>10 µg</td>
</tr>
<tr>
<td>Other volatile aromatic hydrocarbons (29)</td>
<td>15-30 µg</td>
</tr>
<tr>
<td>Formic acid</td>
<td>200-600 µg</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>300-1700 µg</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>100-300 µg</td>
</tr>
<tr>
<td>Methyl formate</td>
<td>20-30 µg</td>
</tr>
<tr>
<td>Other volatile acids (6)</td>
<td>5-10 µg(^c)</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>20-100 µg</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>400-1400 µg</td>
</tr>
<tr>
<td>Acrolein</td>
<td>60-140 µg</td>
</tr>
<tr>
<td>Other volatile aldehydes (6)</td>
<td>80-140 µg</td>
</tr>
<tr>
<td>Acetone</td>
<td>100-650 µg</td>
</tr>
<tr>
<td>Other volatile ketones (3)</td>
<td>50-100 µg</td>
</tr>
<tr>
<td>Methanol</td>
<td>80-180 µg</td>
</tr>
<tr>
<td>Other volatile alcohols (7)</td>
<td>10-30 µg</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>100-150 µg</td>
</tr>
<tr>
<td>Other volatile nitriles (10)</td>
<td>50-80 µg(^c)</td>
</tr>
<tr>
<td>Furan</td>
<td>20-40 µg</td>
</tr>
<tr>
<td>Other volatile furans (4)</td>
<td>45-125 µg(^c)</td>
</tr>
<tr>
<td>Pyridine</td>
<td>20-200 µg</td>
</tr>
<tr>
<td>Picolines (3)</td>
<td>15-80 µg</td>
</tr>
<tr>
<td>3-vinylpyridine</td>
<td>10-30 µg</td>
</tr>
<tr>
<td>Other volatile pyridines (25)</td>
<td>20-50 µg(^c)</td>
</tr>
<tr>
<td>Pyrrole</td>
<td>0.1-10 µg</td>
</tr>
<tr>
<td>Pyrrolidine</td>
<td>10-18 µg</td>
</tr>
<tr>
<td>N-methylpyrrolidine</td>
<td>2.0-3.0 µg</td>
</tr>
<tr>
<td>Volatile pyrazines (18)</td>
<td>3.0-8.0 µg</td>
</tr>
<tr>
<td>Methylamine</td>
<td>4-10 µg</td>
</tr>
<tr>
<td>Other aliphatic amines (23)</td>
<td>3-10 µg</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent individual compounds in a given group.

\(^a\) Modified from Hoffmann and Hoffmann (2001).

\(^b\) Quantity that passes through a Cambridge glass fiber filter.

\(^c\) Estimate.
Table 3-4. Chemical substances and chemical classes in the particulate matter of mainstream smoke of cigarettes without filters.a

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Yield (µg/cigarette smoked)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>1.000-3.000</td>
</tr>
<tr>
<td>Nor nicotine</td>
<td>50-150</td>
</tr>
<tr>
<td>Anatabine</td>
<td>5-15</td>
</tr>
<tr>
<td>Anabasine</td>
<td>5-12</td>
</tr>
<tr>
<td>Other tobacco alkaloids</td>
<td>NA</td>
</tr>
<tr>
<td>Bipyridyls (4)</td>
<td>10-30</td>
</tr>
<tr>
<td>n-hentriacontaine (n-C_{31}H_{64})</td>
<td>100</td>
</tr>
<tr>
<td>Total nonvolatile hydrocarbons (45)</td>
<td>300-400^c</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>2-4</td>
</tr>
<tr>
<td>Naphthalenes (23)</td>
<td>3.6^c</td>
</tr>
<tr>
<td>Phenanthrenes (7)</td>
<td>0.2-0.4^c</td>
</tr>
<tr>
<td>Anthracenes (5)</td>
<td>0.05-0.1c</td>
</tr>
<tr>
<td>Fluorenes (7)</td>
<td>0.6-1.0^c</td>
</tr>
<tr>
<td>Pyrenes (6)</td>
<td>0.3-0.5^c</td>
</tr>
<tr>
<td>Fluoranthenes (5)</td>
<td>0.3-0.45</td>
</tr>
<tr>
<td>Carcinogenic polynuclear aromatic hydrocarbons (11)</td>
<td>0.1-0.25</td>
</tr>
<tr>
<td>Phenol</td>
<td>80-160</td>
</tr>
<tr>
<td>Other phenols (45)^c</td>
<td>60-180^c</td>
</tr>
<tr>
<td>Catechol</td>
<td>200-400</td>
</tr>
<tr>
<td>Other catechols (4)</td>
<td>100-200^c</td>
</tr>
<tr>
<td>Other dihydroxybenzenes (10)</td>
<td>200-400^c</td>
</tr>
<tr>
<td>Scopoletin</td>
<td>15-30</td>
</tr>
<tr>
<td>Other polyphenols (8)^c</td>
<td>NA</td>
</tr>
<tr>
<td>Cyclotenes (10)^o</td>
<td>40-70^o</td>
</tr>
<tr>
<td>Quinones (7)</td>
<td>0.5</td>
</tr>
<tr>
<td>Solanesol</td>
<td>600-1000</td>
</tr>
<tr>
<td>Neophytadienes (94)</td>
<td>200-350</td>
</tr>
<tr>
<td>Limonene</td>
<td>30-60</td>
</tr>
<tr>
<td>Other terpenes (200-500)^c</td>
<td>NA</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>100-150</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>50-75</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>40-110</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>150-250</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>150-250</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>60-80</td>
</tr>
<tr>
<td>Indole</td>
<td>10-15</td>
</tr>
<tr>
<td>Skatole</td>
<td>12-16</td>
</tr>
<tr>
<td>Other indoles (13)</td>
<td>NA</td>
</tr>
<tr>
<td>Quinolines (7)</td>
<td>2-4</td>
</tr>
<tr>
<td>Other aza-arenes (55)</td>
<td>NA</td>
</tr>
<tr>
<td>Benzo furans (4)</td>
<td>200-300</td>
</tr>
<tr>
<td>Other O-heterocyclic compounds (42)</td>
<td>NA</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>40-70</td>
</tr>
<tr>
<td>Sitosterol</td>
<td>30-40</td>
</tr>
<tr>
<td>Campesterol</td>
<td>20-30</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>10-20</td>
</tr>
<tr>
<td>Aniline</td>
<td>0.36</td>
</tr>
<tr>
<td>Toluclidines</td>
<td>0.23</td>
</tr>
<tr>
<td>Other aromatic amines (12)</td>
<td>0.25</td>
</tr>
<tr>
<td>Tobacco-specific N-nitrosamines (6)</td>
<td>0.34-2.7</td>
</tr>
<tr>
<td>Glycerol</td>
<td>120</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent individual compounds identified.

\(^a\) Modified from Hoffmann and Hoffmann (2001).

\(^b\) Portion trapped on Cambridge glass fiber filter.

\(^c\) Estimate. NA= Not available.
yields decreased in the mainstream particulate phase as filter ventilation increased, from 1.69 mg nicotine/cigarette, in ventilated cigarettes to 0.55 mg/cigarette with 83% dilution (measured by pressure drop).

Nicotine yields in mainstream smoke correlate better with tar ($R^2 = 0.99$) than carbon monoxide yields ($R^2 = 0.81$) using FTC methods (Taylor, Jr. et al., 2000). The correlation between tar and carbon monoxide is weaker ($R^2 = 0.64$) using the Massachusetts method (Taylor, Jr. et al., 2000). Chemists in the Massachusetts Benchmark Study suggested that vapor phase constituents, such as aromatic hydrocarbons (e.g., benzene), correlated best with carbon monoxide, whereas particulate phase constituents, such as aromatic amines (e.g., 1-aminonaphthalene) correlated best with nicotine or tar, constituents of total particulate matter (Taylor, Jr. et al., 2000).

### 3.4.2.3 Carbon monoxide

Carbon monoxide in the vapor phase of smoke makes up 2.8 to 4.6% of the weight of mainstream smoke (Table 3-3). In 1980, the FTC adopted a new method for determining carbon monoxide yields using an infrared spectrophotometer to analyze the post filter gas collected in a plastic sampling bag (Federal Trade Commission, 1980).

### 3.4.2.4 Water

As the standard benchmark of tar is nicotine-free dry particulate matter, the amount of water in the TPM should be determined. A measured amount of extracted particulate matter is injected into a gas chromatograph, and the resulting peak is compared against a standard curve to estimate moisture in the particulate phase. The FTC measured particulate matter on a dry basis using the gas chromatographic methods published by Sloan and Sublett (1965) as modified by Schultz and Spears (1966) to determine moisture content (Federal Trade Commission, 1967a; Federal Trade Commission, 1967b).

### 3.4.2.5 Free radicals

Pryor (1992a) analyzed cigarette smoke using electron spin resonance (ESR) analysis. The half-lives of vapor-phase free radicals differ from those in tar. The free radicals in the vapor phase of smoke are small reactive radicals with short half-lives (Pryor, 1992a). Pryor and Stone (1993) estimated that vapor-phase cigarette smoke contained approximately $1 \times 10^{15}$ free radicals per puff, mainly reactive carbon and oxygen centered radicals that typically have a lifetime of less than 1 second and include nitric oxide and alkyl, alkoxy, and peroxyl chemical species. Radicals identified in the particulate phase are more stable, for example semiquinone. Kodama and coworkers (1997) identified hydrogen peroxide and
superoxide free radical in the particulate phase of cigarette smoke which they ascribed mainly to polyphenols e.g., hydroquinone.

Using electron spin resonance technique, Anderson and coworkers (2002) reported approximately $1 \times 10^{16}$ free radicals per cigarette in the vapor phase and approximately $7 \times 10^{14}$ free radicals per cigarette in the particulate phase. The free radical yield also varied by type of cigarette. Cigarettes made from dark, air-cured tobacco produced $2.0 \times 10^{16}$ free radicals per cigarette in the vapor phase and $1.3 \times 10^{15}$ free radicals per cigarette in the particulate phase. Cigarettes with charcoal filters produced $9.9 \times 10^{15}$ free radicals per cigarette in the vapor phase and $5.4 \times 10^{14}$ free radicals per cigarette in the particulate phase (Anderson et al., 2002).

Mainstream smoke exiting the butt of a cigarette contains nitric oxides, primarily in the form of nitric oxide, which is a free radical species (Table 3-3) (Brunnemann & Hoffmann, 1982; Cueto & Pryor, 1994; Hoffmann & Hoffmann, 2001; Sloan & Kiefer, 1969). Sloan and Kiefer (1969) measured 44 $\mu$g of nitric oxide and 1.5 $\mu$g of nitrogen dioxide per 35 mL puff of smoke from a commercial cigarette. The amount of nitric oxide per puff increased from the second puff to the tenth puff, whereas the amount of nitrogen dioxide did not change significantly. The nitric oxide yield, obtained by summing individual puff yields, was approximately double the 275 $\mu$g obtained when the total smoke vapor was trapped and analyzed, implying that these chemical species have short half-lives.

Pyrolysis of nitrates is the main source of nitric oxide in mainstream smoke (Brunnemann & Hoffmann, 1982; U.S. Department of Health and Human Services, 1988; Williams, 1980). Cigarettes made from bright leaf tobacco (flue cured) containing only 0.1% nitrate yield an average of 4 $\mu$g nitric oxide per puff, whereas cigarettes made from nitrate-treated tobacco containing 5.8% nitrate will yield in excess of 200 $\mu$g of nitric oxide per puff (Sloan & Kiefer, 1969). A linear relationship ($r = 0.99$) exists between the percentage of nitrate in the tobacco in the range typically smoked and the yield of nitric oxide in the vapor phase of mainstream smoke, with an extrapolated residual amount of 3 to 10 $\mu$g of nitric oxide in mainstream smoke per puff that could not be attributed to the nitrate’s nitrogen (Norman et al., 1983; Unemura et al., 1986; Williams, 1980). For sidestream smoke the percentage of nitrate in the tobacco blends also positively correlates ($r = 0.98$) with nitric oxide yields (Norman et al., 1983; Unemura et al., 1986).

Baker (1999) cited the studies of Norman and coworkers (1983) and Unemura and coworkers (1986) to support his idea that, in addition to the tobacco nitrate, “there must be other sources of nitric oxide.” Use of nitrogen-free cellulose cigarettes demonstrated that oxidation of atmospheric nitrogen generated some nitric oxide in mainstream smoke (Norman et al., 1983; Unemura et al., 1986). However, for sidestream smoke, nitrogen oxidation only accounted for approximately
15% of residual nitric oxide. Most of the nitric oxide in sidestream smoke comes from the oxidation of organic nitrogen compounds, such as amino acids and proteins (Unemura et al., 1986).

### 3.4.2.6 Metals

To avoid artefactual contamination, Kalcher and coworkers (1993) preferred electrostatic precipitators instead of Cambridge filters for metals analysis. Electrostatic precipitators charge the particles, usually negatively, and deposit them on a counter electrode, enclosed in a quartz tube thus avoiding contact with metallic electrodes. Once collected, voltametry is used to analyze the collected particles for individual metals.

Chortyk and Schlotzhauer (1984) detected the transfer of selenium to smoke either by spiking the cigarettes with aqueous solutions of selenium or by spraying selenium onto growing tobacco plants. For high tar reference cigarettes (Kentucky 2R1), spiked with 0 to 25 µg Se/cigarette, an average of 10% of the added selenium transferred into the mainstream smoke condensate. Similarly, the average amount transferred to the mainstream smoke condensate from tobacco (sprayed with selenium in the field) was 8.9%.

Kalcher and coworkers (1993) measured the distribution of cadmium and lead in the ash, butt and mainstream smoke of French filtered cigarettes, 70 mm in length, using a smoking machine modified for trace element analysis. They calculated the amounts of metals in sidestream smoke by subtraction. Butt or ash retained one-half of the cadmium. Sidestream smoke lost 40%, and particulate matter of mainstream smoke transferred 10%. Butt or ash retained 84% of lead. Sidestream smoke lost 10%, and mainstream smoke transferred 6%, equally divided between gas and particulate phases.

### 3.4.3 Separation of Smoke

#### 3.4.3.1 Filtration (particulate and vapor phases)

Many studies partition cigarette smoke into two phases, usually designated particulate and vapor (gas), using filtration and trap (collection) devices. McRae (1990) reviewed both historically important and recently used chambers. Dube and Green (1982) reviewed the traps used to collect mainstream smoke, including electrostatic precipitators, jet impactors, cold traps, solid adsorbents, solvent traps, and Cambridge filters.

The Cambridge filter, once produced by the Cambridge Filter Corporation of Syracuse, New York, served as the standard method of separation in studies of cigarette smoke (Ogg, 1964; Pillsbury, 1996; Wartman et al., 1959). The FTC used the original Cambridge Filter Method to separate vapor phase and particulate
phase constituents (Wartman et al., 1959) (Pillsbury, 1996). Ogg (1964) specified, and Pillsbury and coworkers (1969) reiterated that the Cambridge filter or other filters of equivalent construction must collect at least 99.9% of all particles more than 0.3 µm in diameter at a flow rate of 28 linear feet per minute, having a maximum pressure drop not exceeding 93 mm water at 28 feet per minute and containing not more than 5% acrylic type binder.

Dube and Green (1982) described the Cambridge filter as a glass fiber pad stabilized by an organic binder. The filter was positioned just after the cigarette with no dead space. The pump was positioned after the filter, suctioning smoke through the filter and forcing vapor into a plastic collection bag (Guerin, 1996; Pillsbury, 1996).

Investigators define total particulate matter or condensate of cigarette smoke as the portion of smoke collected on a conventional Cambridge filter (Baker, 1999; Dube & Green, 1982). The filter pad traps the aerosol particles or liquid phase, and the vapor or gas phase passes through. Tar per cigarette is obtained by subtracting the final weight of the filter from its initial weight and dividing this difference by the number of cigarettes smoked and filtered. The FTC (1967a) refers to tar as the weight in grams of the total particulate matter collected minus the weight of alkaloids, as nicotine, and water. In other words, FTC’s tar is nicotine-free dry particulate matter filtered from mainstream smoke (CORESTA, 1991c).

Investigators often use Cambridge filters to define particulate and vapor phases of cigarette smoke empirically. Most studies do not attempt to characterize the proportion of the vapor phase trapped on a filter or the proportion of particles that a filter fails to trap. Particulate and vapor phases separated by Cambridge filters differ from one another in chemical composition and other physicochemical properties (Dube & Green, 1982; Hoffmann & Hoffmann, 2001). Baker (1999) summarized the evidence of the distributions of exogenously radioisotopically labeled nicotine and n-dotriacontane between particulate and vapor phases in both mainstream and sidestream smoke, based on nine studies conducted between 1958

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of Studies</th>
<th>Mainstream Smoke</th>
<th></th>
<th>Sidestream Smoke</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Particulate phase</td>
<td>Vapor phase</td>
<td>Particulate phase</td>
<td>Vapor phase</td>
</tr>
<tr>
<td>¹⁴C-nicotine</td>
<td>5</td>
<td>19.6 – 23.5</td>
<td>2.2 – 5.3</td>
<td>52.6 – 61.9</td>
<td>18.5 – 24.3</td>
</tr>
<tr>
<td>¹⁴C-dotriacontane</td>
<td>4</td>
<td>24.7 – 42.9</td>
<td>0.4 – 13.5</td>
<td>46.0 – 60.6</td>
<td>9.8 – 13.5</td>
</tr>
</tbody>
</table>

*Modified from Baker (1999). n-Dotriacontane is a C₃₂ straight chain alkane found in tobacco and other plants.*
and 1978. Table 3-5 shows that distribution of some substances, including nicotine, varies between mainstream and sidestream smoke, as well as between the particulate matter and vapor phases of these types of smoke.

Reliance on Cambridge filters assumes that filtration effectively separates the two phases and that continued transfer of substances between vapor and the particulate phase is either very slow or unimportant (Pankow, 2001). Williamson and Allman (1966) described a third phase of smoke, which indicates that the separation is not absolute. They defined a semi-volatile phase as the fraction retained on a Cambridge filter pad at an ambient temperature, which becomes a volatile gas at 100-200 °C. Hoffmann and coworkers (2001) classified phenol, N-nitrosoamines, hydrogen cyanide and low boiling aldehydes in the semi-volatile phase.

Kalcher and coworkers (1993) reported that the Cambridge glass fiber filters were not ideal for the collection of particulate matter, because they clogged. Also, filters having a large surface area generate contamination. Caldwell and Conner (1990) reported an artifact in measuring N-nitrosamines. They showed that some N-nitrosamines formed on the Cambridge filter pad under FTC conditions, as described by Hoffmann and coworkers (1983) and Hecht and coworkers (1983), when smoke passed through the filter. This led to significant overestimates of N-nitrosamine concentrations in cigarette smoke (Caldwell & Conner, 1990). Treating the filter with vitamin C before smoke collection inhibited the formation of nitrosamines (Caldwell & Conner, 1990).

Baker (1999) reported the creation of artifacts during the collection and storage of smoke from reactions occurring in the aging of smoke. Dube and Green (1982) reported finding cyanohydrins in mainstream smoke collected in water but not when collected in a cold trap (-79°C). Cyanohydrins might also form as artifacts through reactions between hydrogen cyanide and volatile carbonyl components of the smoke (Baker, 1999; Dube & Green, 1982).

While characterization of a brand or kind of cigarette by tar content may provide useful chemical and regulatory characterizations, and while analysts have established the accuracy of tar content as a measure, the biological implications of particulate or tar content remain unclear. LSRO does not understand from the available information whether the epidemiologically described adverse human health effects of cigarette smoking relate to the particulate, vapor, or both fractions of cigarette smoke.
3.4.3.2 Chromatographic separation

Developments in solvent partition, column chromatography, flame ionization detection, electron capture detectors and isotope dilution techniques enabled quantitative identification of cigarette smoke constituents (Gudzinowicz & Gudzinowicz, 1980). For a review of the analysis of cigarette smoke constituents in smoke and human tissues (urine, blood, and exhaled breath), see the book by Gudzinowicz & Gudzinowicz (1980).

Measurements of substances in smoke are not made simultaneously; investigators generally do not collect and run a single chromatography column to estimate several components at once. Instead, they measure different substances with different methods, applied in parallel or in succession (CORESTA, 1991c; Rustemeier et al., 2002). Problems with the interpretation of data for whole animal studies and chemical studies can arise, if investigators obtained and analyzed samples with different devices and methods.

3.4.4 Manufacturers’ Data

In addition to reporting the numbers of cigarettes sold for tax purposes, manufacturers report the quantities of substances, or the yields from mainstream smoke, collected and measured using standard procedures (Federal Trade Commission, 2000b; Government of British Columbia, 2001; Hoffmann &

Sales Weighted, Tar and Nicotine Yields:
1968-1998

Based on volume of sales of different cigarette brands, nicotine and tar yields per cigarette smoked have decreased since the early 1950’s (Fig. 3.5) (Burns & Benowitz, 2001; Federal Trade Commission, 2000a). The sales-weighted tar yields reported by the FTC (2000a) represented a 45% decrease from 21.6 mg per cigarette in 1968 to 12.0 mg per cigarette in 1998. The FTC (2000a) reported sales-weighted nicotine yields of 1.35 mg/cigarette in 1968 and 0.88 mg/cigarette in 1998.

The FTC closed its analytical laboratory in 1987, reporting that the Commission could obtain data from other sources and by other means to verify the accuracy of tests for tar, nicotine, and carbon monoxide (Federal Trade Commission, 2000a). The FTC did not reference a quality control program in this notice but did list the tar, nicotine and carbon monoxide yields of 1294 varieties of cigarettes sold in the U.S. in 1998. Five of the largest cigarette manufacturing companies in the U.S. carried out the 1998 testing. The Tobacco Institute Testing Laboratory, a private laboratory operated by the cigarette industry, conducted most of the tar, nicotine, and carbon monoxide analyses (Federal Trade Commission, 2000a). A gas chromatographic method for tar and nicotine was modified to use the method of Wagner and Thaggard (1979).

### 3.4.5 Classes of Chemical Substances

The subsections below briefly describe a few categories of chemical substances found in cigarette smoke, hydrogen ion, phenols, pyridines, and aldehydes. This list is not intended to be comprehensive but rather to illustrate the chemical complexity of cigarette smoke. When classified by functional groups, cigarette smoke contained more than 25 categories of chemical substances (Roberts, 1988).

#### 3.4.5.1 Hydrogen ion

Rodgman (2000), Pankow (2001) and Bevan (1991) reviewed the pH of cigarette smoke, including analytical methods and interpretation of results. The major hydrogen ion donors remain uncharacterized. Kruszynski (1982) prepared a list of reports discussing methods of pH determination in smoke published between 1930 and 1981. Battig and coworkers (1982) reported that the pH of smoke did not correlate with the number of puffs, puff interval, puff duration, peak pressure or puff volume. The pH of cigarette smoke interested investigators concerned that substances with pKₐ’s in the physiological range or substances with zwitterionic structures might absorb differentially, given changes in hydrogen ion concentration.
3.4.5.2 Phenols

In 1982, Dube and Green estimated that mainstream smoke contained 282 phenols (Dube & Green, 1982). Phenols mostly partitioned to the particulate phase. Smith and coworkers (2002) reported the chemical structures of 253 substituted phenols in cigarette smoke. The estimated yield of phenol was 60-140 µg/cigarette in mainstream smoke from unfiltered cigarettes with a sidestream/mainstream ratio of 1.6-3.0. Other major substituted phenols were estimated at mainstream levels per cigarette of 11-37 µg for cresols and 100-360 µg for hydroquinone (Baker, 1999).

The Massachusetts Benchmark Study showed that the average phenol level of mainstream smoke from 26 brands was 37 µg/cigarette with a range of 10-64 µg/cigarette (Taylor, Jr. et al., 2000). The range was greater than any of the other of the 43 mainstream smoke constituents measured (Gray & Boyle, 2002; Taylor, Jr. et al., 2000). A further study of smoke constituents from 25 brands representing 58% of the UK market (Tobacco Manufacturers Association [London], 2003) also observed a wide range of 0.91-46.2 µg/cigarette, mean level of phenol in mainstream smoke.

3.4.5.3 Pyridines

The four major pyridine alkaloids of tobacco are nicotine, nicotinic acid, nicotinamide and nornicotine (Bush, 1999). Tobacco pyridines have organoleptic properties and influence the flavor of cigarette smoke. Pyridine is found primarily in the particulate phase of cigarette smoke (Taylor, Jr. et al., 2000). However, Brunnemann and coworkers (1978) detected 21 volatile pyridines in mainstream smoke, including 32.4 µg/cigarette of pyridine and 12.3 µg/cigarette of α-picoline. In their study, sidestream smoke contained up to 28 times greater concentrations of pyridines than mainstream smoke. They identified 11 individual pyridine compounds in mainstream smoke and 12 in sidestream smoke with inhibitory properties.

In 1999, the Massachusetts Benchmark study produced estimates of pyridines in mainstream and sidestream cigarette smoke from 26 commercial brands of cigarettes. The mainstream smoke yields of pyridine averaged 19 µg/cigarette with a range of 13-24 µg/cigarette. For sidestream smoke, the yield was 268 µg/cigarette with a range of 196-340 µg/cigarette, confirming earlier data that pyridine concentrations in sidestream smoke were higher than mainstream smoke. Filters selectively remove volatile pyridines, reducing concentrations by 63% with cellulose acetate filters and by 69% with charcoal filters (Brunnemann et al., 1978). Comparisons of volatile pyridines in tobacco to those in cigarette smoke indicated that most pyridines in smoke arose from pyrolysis and pyrosynthesis (Brunnemann et al., 1978).
Using a new GC-MS technique, Kulshreshtha and Moldoveanu (2003) reported that the levels of pyridines in mainstream smoke depended on the type of cigarette. For full flavored cigarettes, they found pyridine levels as high as 18 µg/cigarette, compared to 3 µg/cigarette for an ultralight cigarette. Substituted pyridine yields varied between 0.1 and 5.0 µg/cigarette for full flavored cigarettes to between a few and 0.2 µg/cigarette for ultralight cigarettes.

### 3.4.5.4 Aldehydes

The most assayed chemical constituents in mainstream smoke, nicotine, tar and carbon monoxide occur in milligram quantities per cigarette (Taylor, Jr. et al., 2000). Of 34 minor mainstream smoke components measured by the Massachusetts Benchmark Study, the acetaldehyde level of 1.618 mg exceeded the other 33 substances, including the other aldehydes that occur in microgram quantities per cigarette (Gray & Boyle, 2002; Taylor, Jr. et al., 2000). In 1982, Dube and Green (1982) estimated that tobacco smoke contained 108 aldehydes. However, the present literature usually reports data for less than ten (<10) aldehydes, almost entirely partitioned into the vapor phase of mainstream smoke (Dube & Green, 1982).

Formaldehyde is a chemically reactive substance, which can cross-link proteins with other proteins or with nucleic acids (Yu, 1998). Cross-linking may be a mechanism of toxicity (Boor & Hysmith, 1987) (Boor, 1983). Formaldehyde forms after incomplete combustion of organic materials, including tobacco (Budavari, 1989; Taylor, Jr. et al., 2000). Hypothetically, metabolism of nicotine increases formaldehyde formation. Animal model information supporting this hypothesis has been reported (Wisborg et al., 2000). Although generally considered a gas, the Massachusetts Benchmark Study assigned formaldehyde to the particulate phase, where many other aldehydes partition (Taylor, Jr. et al., 2000). In contrast, Hoffmann and Hoffmann (2001) classified formaldehyde and other volatile aldehydes as part of the vapor phase.

The Massachusetts Benchmark Study reported an average yield of formaldehyde of 69 µg/cigarette (Taylor, Jr. et al., 2000). The estimated yield for sidestream smoke was more than tenfold higher, 787 µg/cigarette. The range of the mean estimated yields of formaldehyde in mainstream smoke exceeded eightfold, from 12 µg/cigarette for a light-filtered cigarette to 105 µg/cigarette for a full-flavored cigarette (Taylor, Jr. et al., 2000). Seventeen of the 26 brands of produced average formaldehyde yields below the 60 µg/cigarette reported for Kentucky Reference cigarettes (1R4F).

Recent studies by Baker et al. (2004a; 2004b), looked at the effect of different groups of added ingredients (482 total) on 44 smoke constituents. Formaldehyde levels in smoke increased in nearly all cigarettes that contained a form of sugar in...
the casing material. In one case an increase of 73% by weight was observed, compared to smoke from a cigarette without casing. Rustemeier et al. (2002), also observed increases in smoke formaldehyde from cigarettes containing corn syrup sugar among other added ingredients.

Acetaldehyde also is a chemically reactive aldehyde. Acetaldehyde is the most prevalent aldehyde in cigarette smoke and is primarily found in the vapor phase (Hoffmann & Hoffmann, 2001; Taylor, Jr. et al., 2000). At sufficiently high concentrations, acetaldehyde is a general narcotic (Budavari, 1989). For many cigarette brands, yields of acetaldehyde correlate with yields of tar and of carbon monoxide. The primary region of mainstream smoke acetaldehyde deposition in humans is the upper respiratory tract including the mouth (Seeman et al., 2002).

Seeman and coworkers (2002) reported that natural tobacco polysaccharides, including cellulose, are the primary precursors of acetaldehyde in mainstream smoke. Sugars added as ingredients, including D-glucose, D-fructose and sucrose, did not produce higher yields of acetaldehyde in mainstream smoke than tobacco on a weight-for-weight basis (Seeman et al., 2002). Cigarette design apparently influenced the acetaldehyde content of smoke more than native or added sugars. Seeman and coworkers suggested that the design factors potentially affecting acetaldehyde yields included filtration, ventilation and paper porosity (Seeman et al., 2002).

A third aldehyde, acrolein, also partitions mostly into the vapor phase of cigarette smoke (Taylor, Jr. et al., 2000). Acrolein can penetrate the upper respiratory passages and forms a highly reactive zwitterion (Leikauf et al., 2002). Mainstream smoke yielded approximately 80 µg/cigarette (Baker, 1999). The sidestream to mainstream ratio was in the range of 8-15. The Massachusetts Benchmark Study reported 184 µg/cigarette (range 150-218 µg/cigarette) in mainstream smoke (Taylor, Jr. et al., 2000), and in sidestream smoke the average was higher, 450 µg/cigarette (Taylor, Jr. et al., 2000). The range of acrolein yields from mainstream smoke appeared unrelated to cigarette length but related to the type of tobacco. Analysis of cigarette smoke from puffed, expanded, and freeze-dried tobaccos yielded 105.0, 87.7, and 92.4 µg/cigarette of acrolein, respectively (Gori, 1977; National Institutes of Health, 1996). Leikauf and coworkers (2002) suggested that aldehydes, such as acrolein are enriched in sidestream smoke, because of lower combustion temperatures.

Acetaldehyde, formaldehyde, and acrolein are, in order, the prevalent aldehydes on a molar basis in cigarette smoke and are found mostly in the vapor phase. Their reactive properties anticipate biological effects and raise interest in the toxicity of the vapor phase.
3.4.6 Carcinogens

Scientists working on the chemistry and physics of tobacco smoke searched intensively for carcinogens in cigarette smoke. A lack of rapid bioassays and a lack of bioassays with external validity in predicting human carcinogenic effects of cigarette smoke have hampered overall progress. The sections below briefly summarize some of the analytical work.

3.4.6.1 Mutagenic activity

Many experts subscribe to a theory of cancer causation called somatic mutation. Instead of mutations passing heritable changes from a person to progeny, under somatic mutation heritable changes pass from cell to the cell’s progeny (Herrero-Jimenez et al., 2000). If more than one mutation alters both growth control and location control of a cell, uncontrolled growth of cells at abnormal locations could occur, which is the very definition of a malignancy. If a permanent mutation were to develop in a critical region of DNA, close to or within the code for an oncogene or tumor suppressor gene, activation of the oncogene or inactivation of the tumor suppressor gene might occur (Brambilla & Brambilla, 1997).

Other nongenetic mechanisms of cancer have been proposed. Inhibition of methylation of regulatory regions of DNA or other mechanisms might lead to genomic instability (Hoeijmakers, 2001). Abnormal formation of gap junctions also could cause mutations or serve as a nongenetic mechanism of carcinogenesis. Substances in cigarette smoke could affect any of these processes. In addition, all mutagens, and even all DNA adduct forming substances, are not necessarily carcinogens (Ashby et al., 1989; Elcombe et al., 2002).

The most frequently applied test for assessing ability of cigarette smoke and its components to induce point mutations is the mutation reversion assay in *Salmonella typhimurium* (the Ames test) developed by Ames and coworkers (1973). *Salmonella typhimurium* test strains carry a mutant gene, which renders the bacteria unable to synthesize histidine. Reversion refers to the ability of a chemical or its metabolite to reverse this mutation and is measured by the ability of the bacteria to grow in the absence of histidine. Mutation reversion is therefore a measure of the mutagenic potential of a chemical or substance. With bacterial strains for this assay investigators can assay cigarette smoke itself as well as the urine of human smokers for reversion potency. Moreover the assay may be utilized to assay fractionates of smoke and urine to identify the mutagenic substances.

In 1977, Yamasaki and Ames (1977) reported that urine from cigarette smokers exhibited mutagenic activity, using the Ames assay. Curvall and coworkers (1987) reported nearly tenfold higher mutagenic activity in 24 hour samples of urine of smokers compared to the activity in similar samples from non-smokers. Chepiga
and coworkers (2000) reported a correlation between mutagenic activity and tar delivery in mainstream smoke.

Curvall and coworkers (1984) used the Ames test to estimate mutagens in total cigarette smoke condensate and its fractions. They reported that the total and the nonvolatile fraction contained direct-acting base-pair mutagens and indirect-acting frame-shift mutagens. Although the semi-volatile (SV) fraction was not mutagenic, two subfractions, (acids and phenols), had base pair mutagens that required no metabolic activation. The contribution of these subfractions to the total activity of the SV fraction must be considered low since they contribute only a small portion of the SV fraction. After removal of protein and peptides from flue-cured cigarettes by water extraction and protease digestion, Clapp and coworkers (1999) reported significant reductions in *Salmonella* reversion activity in smoke condensates. Based on these results, the authors concluded that protein pyrolysis products contribute to the mutagenic properties of cigarette smoke condensates. However, in these condensates they did not examine cell proliferation, regenerative hyperplasia or chronic irritation.

Other assays estimate potential clastogenic DNA damage or chromosomal aberrations from cigarette smoke, including sister chromatic exchange (Institute of Medicine, 2001), micronucleated, polychromatic erythrocytes (Hayashi et al., 2000), micronucleated hamster lung cells (Massey et al., 1998), and DNA strand breaks. The comet assay is a popular way of measuring strand breaks (Tice et al., 1994; Tice et al., 2000). A recent study compared cigarette smoke condensates from several products with a battery of genotoxicity assays (Eclipse Expert Panel, 2000).

Roemer and coworkers (2002) evaluated the potential effects of three different groups of added ingredients commonly used in cigarette manufacturing. Each group was added at low and high levels to test cigarettes, and *Salmonella* reversion potency of the particulate phase of the cigarette smoke was measured, using five Ames test strains. Control cigarettes with the same construction and blend, but without the addition of the added ingredients, and Kentucky Reference Cigarette IR4F, served as controls. Within the limitations of the sensitivity and specificity of the Ames test, the *in vitro* mutagenicity of cigarette smoke did not appear to be increased by the added ingredients (Roemer et al., 2002). However, due to the experimental design of testing cigarettes, using only large numbers of added ingredients grouped together, the effect of single ingredients could not be assessed.

### 3.4.6.2 Polycyclic aromatic hydrocarbons

More than 500 polycyclic aromatic hydrocarbons have been identified in cigarette smoke and some are classified as carcinogens by regulatory organizations (Rodgman & Green, 2002). Hoffmann and Hoffmann (2001) list ten carcinogenic polycyclic
aromatic hydrocarbons found in cigarette smoke, with benzo[a]pyrene B[a]P classified as carcinogenic on human evidence and the remaining nine on laboratory animal studies (Hoffmann & Hoffmann, 2001). B[a]P is found in the particulate phase of mainstream smoke (Taylor, Jr. et al., 2000). The average yield of B[a]P in the mainstream smoke of 26 U.S. brands was 22.2 ng/cigarette with a range from 5.6 to 41.5 ng/cigarette (Gray & Boyle, 2002; Taylor, Jr. et al., 2000). The type of tobacco may relate to the B[a]P level. The lowest levels of B[a]P generally were obtained with light or ultra light brands and the highest levels were obtained with traditional, full-flavored cigarettes (Gray & Boyle, 2002; Taylor, Jr. et al., 2000). Rustemeier and coworkers (2002) report that added ingredients incorporated into U.S. blend test cigarettes resulted in a 50% decrease in polycyclic aromatic hydrocarbons in mainstream smoke.

3.4.6.3 Nitrosamines

Nitrosamines occur in green tobacco leaves (Adams et al., 1983; Taylor, Jr. et al., 2000). A portion of the nitrosamines in mainstream smoke directly transfers from tobacco to the particulate phase. However, the largest portion of nitrosamines forms during the smoking process (Adams et al., 1983; Taylor, Jr. et al., 2000). Nicotine, a tertiary amine, is the major alkaloid in tobacco. Some minor alkaloids are formed by the demethylation of nicotine and structural rearrangement: nornicotine, anatabine, and anabasine (Brunnemann et al., 1996; Hecht et al., 1994). Seven major tobacco specific nitrosamines (TSNAs) have been identified (Hecht & Hoffmann, 1989). Studies on four of these have generated more data: N’Nitrosonornicotine (NNN), 4-(N’-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), N’Nitrosoanabasine (NAB), and N’-Nitrosoanatabine (NAT). Interest in the TSNAs relates to their biological properties. Two of the TSNAs, NNN and NNK, are hypothetically causes of human cancer in smokers (Hecht et al., 1994). Hecht and coworkers (1994) developed biomarkers for NNN and NNK.

TSNA levels markedly increase during the curing of tobacco (Djordjevic et al., 1989; Hecht & Hoffmann, 1989; Sasson et al., 1985; World Health Organization, 1986). TSNAs are the most prevalent of all nitrosamines in cigarette smoke (Hecht et al., 1994). During curing, microbes can convert nitrate to nitrites, which can combine with alkaloids in the tobacco to form TSNAs. During cigarette combustion pyrosynthetic nitrosation of the tobacco alkaloids generates some, but not all, TSNAs (Brunnemann et al., 1996; Fischer et al., 1990; Hecht & Hoffmann, 1989). Adams and coworkers (1984) added nitrate to cigarette tobaccos and obtained significant increases in TSNA levels in the mainstream smoke. In contrast, after the addition of endogenous nitrate to tobacco, Fischer and coworkers (1990) obtained inconclusive results for NNN and NNK. Thus, there are data conflicts about the source of nitrate for the formation of TSNAs during smoking (Rodgman & Green, 2002). Microwave treatment reduces TSNA levels in cured tobacco leaf, apparently by inhibiting microbes (Blackwell, 2000; Institute of Medicine, 2001).
However, Djordjevic and coworkers (1989) could not establish whether the increase in TSNAs during the curing process related to biochemical processes within the leaf or to microflora activity. In addition, atmospheric nitrogen can apparently serve as a source of nitrogen to produce nitrosamines and TSNAs through oxidation of NO to NO₂. Norman and coworkers (1983) supported this pathway by reporting that nitrogen free, cellulose cigarettes produced approximately 50 µg/cigarette of NO.

### 3.4.6.4 Interpretation of carcinogenicity and mutagenicity data

For some investigators, the presence of animal carcinogens in cigarette smoke, particularly lung carcinogens, is sufficient evidence to explain the biological effects of cigarette smoke, at least the carcinogenic effects of cigarette smoke (Hecht, 1999). Stemming from extensive investigations, the suggestion has been that polycyclic aromatic hydrocarbons and TSNAs explain the cancer observed in epidemiological studies of cigarette smokers (Hecht et al., 1983). For others, however, the carcinogenic substances in cigarette smoke need to provide a quantitative explanation of observed biological effects (Rodgman & Green, 2002; Tricker, 2001). General skepticism exists about the relevance of data from animal models of carcinogenesis to human cancer (Freedman & Zeisel, 1988). In addition, Fowles and Bates (2000) in their ranking of all carcinogenic substances in cigarette smoke, using regulatory estimates which were essentially upper bounds on potency, found that the most potent substance, 1,3-butadiene, which is found in the vapor phase, had a cancer risk approximately equivalent to 0.001 cigarettes smoked per day over a lifetime. Other substances would contribute little to the overall estimate, and the modeling assumptions force additivity onto the calculation, eliminating the possibility of synergy. Similar calculations with polycyclic aromatic hydrocarbons and TSNAs imply that their quantitative potencies do not account for the cancer observed in epidemiological studies of cigarette smokers.

Extensive literature is available about the toxicity and tumorigenic properties of cigarette smoke condensate components using experimental test systems (Table 3-6) (Institute of Medicine, 2001; World Health Organization, 1986). Classes of chemical substances found in the particulate (tar) phase of mainstream smoke have been targeted as primary causes of human cigarette-induced lung cancer. These substances include B[a]P, other polycyclic aromatic hydrocarbons, NNK, and other TSNAs, and they have been assayed in cell culture and animal models. Using these test systems as surrogates for humans, the substances have been classified as carcinogens (D’Agostini et al., 2001; Rodgman & Green, 2002). However, negative or quantitatively inconclusive results over the last fifty years, particularly with whole cigarette smoke, illustrate the difficulties of inducing an animal disease like human lung cancer (D’Agostini et al., 2001; Hoffmann & Hoffmann, 2001; Roberts, 1988; Witschi et al., 1997b).
Table 3-6. Carcinogens in cigarette smoke

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration in smoke</th>
<th>1986 IARC category</th>
<th>Laboratory animal</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PAH</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Benz (a) anthracene</td>
<td>20-70 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo (b) fluoranthene</td>
<td>4 - 22 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo (j) floranthene</td>
<td>6-21 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo (k) floranthene</td>
<td>6-12 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo (a) pyrene</td>
<td>20-40 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dibenz (a,h) anthracene</td>
<td>4 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dibeno (a,l) pyrene</td>
<td>1.7-3.2 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dibenzo (a,e) pyrene</td>
<td>Present</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indeno (1,2,3-cd) pyrene</td>
<td>4 - 20 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Methylchrysene</td>
<td>0.6 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
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<tr>
<td><strong>Heterocyclic hydrocarbons</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furan</td>
<td>18-37 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinoline</td>
<td>1-2 ng</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dibenz (a,h) acridine</td>
<td>0.1 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dibenz (a,i) acridine</td>
<td>3-10 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dibeno (c,g) carbazole</td>
<td>0.7 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo (b) furan</td>
<td>Present</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>N-nitrosamines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-nitrosodimethylamine</td>
<td>2-180 ng</td>
<td>Sufficient</td>
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<tr>
<td>N-nitrosoethyldimethylamine</td>
<td>3-13 ng</td>
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<tr>
<td>N-nitrosodimethyamine</td>
<td>ND-2.8 ng</td>
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<tr>
<td>N-nitrosodi-n-propylamine</td>
<td>ND-1.0 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-nitrosodi-n-butylamine</td>
<td>ND-30 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-nitrosodihydrazine</td>
<td>3-110 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-nitrosonitrilactone</td>
<td>ND-9 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-nitrosodihydrazine</td>
<td>ND-68 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-nitrosodihydrazine</td>
<td>120-3700 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-(methylnitrosamino)-1- (3-pyridyl)-1-butanone</td>
<td>80-770 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aromatic amines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Toluidine</td>
<td>30-337 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,6-Dimethylaniline</td>
<td>4 - 50 µg</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Naphthylaniline</td>
<td>1-334 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Aminobiphenyl</td>
<td>2 - 5.6 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>N-Heterocyclic amines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AaC</td>
<td>25-260 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>0.3 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trp-P-1</td>
<td>0.3-0.5 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trp-P-2</td>
<td>0.8-1.1 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu-P-1</td>
<td>0.37-0.89 ng</td>
<td>Sufficient</td>
<td></td>
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</tr>
<tr>
<td>Glu-P-2</td>
<td>0.25-0.88 ng</td>
<td>Sufficient</td>
<td></td>
<td></td>
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<tr>
<td>PhIP</td>
<td>11-23 ng</td>
<td>Sufficient</td>
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<td></td>
</tr>
<tr>
<td><strong>Adehydes</strong></td>
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<tr>
<td>Formaldehyde</td>
<td>70-100 µg</td>
<td>Sufficient</td>
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<td>Limited</td>
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<tr>
<td>Acetaldehyde</td>
<td>500-1400 µg</td>
<td>Sufficient</td>
<td></td>
<td>Insufficient</td>
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Table 3-6. Carcinogens in Smoke (continued)\(^{a}\)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration in smoke</th>
<th>Laboratory Animal</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volatile hydrocarbons</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,3-Butadiene</td>
<td>20-75 µg</td>
<td>Sufficient</td>
<td>Insufficient</td>
</tr>
<tr>
<td>Isoprene</td>
<td>450-1000 µg</td>
<td>Sufficient</td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>20-70 µg</td>
<td>Sufficient</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Styrene</td>
<td>10 µg</td>
<td>Limited</td>
<td></td>
</tr>
<tr>
<td><strong>Misc. Organic compounds</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetamide</td>
<td>35-56 µg</td>
<td>Sufficient</td>
<td></td>
</tr>
<tr>
<td>Acrylamide</td>
<td>Present</td>
<td>Sufficient</td>
<td></td>
</tr>
<tr>
<td>Acrylonitrile</td>
<td>3-15 µg</td>
<td>Sufficient</td>
<td>Limited</td>
</tr>
<tr>
<td>Vinyl chloride</td>
<td>11-15 ng</td>
<td>Sufficient</td>
<td>Sufficient</td>
</tr>
<tr>
<td>DDT</td>
<td>800-1200 µg</td>
<td>Sufficient</td>
<td>Probable</td>
</tr>
<tr>
<td>DDE</td>
<td>200-370 µg</td>
<td>Sufficient</td>
<td></td>
</tr>
<tr>
<td>Catechol</td>
<td>100-360 µg</td>
<td>Sufficient</td>
<td></td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>&lt;3 µg</td>
<td>Sufficient</td>
<td></td>
</tr>
<tr>
<td>Methyleugenol</td>
<td>20 ng</td>
<td>Sufficient</td>
<td></td>
</tr>
<tr>
<td>Nitromethane</td>
<td>0.3-0.6 µg</td>
<td>Sufficient</td>
<td></td>
</tr>
<tr>
<td>2-nitropropane</td>
<td>0.7-1.2 µg</td>
<td>Sufficient</td>
<td></td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>25 µg</td>
<td>Sufficient</td>
<td></td>
</tr>
<tr>
<td>Ethyl caramate</td>
<td>20-38 µg</td>
<td>Sufficient</td>
<td></td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>7 µg</td>
<td>Sufficient</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Propylene oxide</td>
<td>12-100 ng</td>
<td>Sufficient</td>
<td></td>
</tr>
<tr>
<td><strong>Inorganic compounds</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrazine</td>
<td>24-43 ng</td>
<td>Sufficient</td>
<td>Inadequate</td>
</tr>
<tr>
<td>Arsenic</td>
<td>40-120 µg</td>
<td>Inadequate</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Beryllium</td>
<td>0.5 ng</td>
<td>Sufficient</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Nickel</td>
<td>ND-600 ng</td>
<td>Sufficient</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Chromium (only hexavalent)</td>
<td>4-70 ng</td>
<td>Sufficient</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Cadmium</td>
<td>7-350 ng</td>
<td>Sufficient</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Cobalt</td>
<td>0.13-0.2 ng</td>
<td>Sufficient</td>
<td>Inadequate</td>
</tr>
<tr>
<td>Lead</td>
<td>34-85 ng</td>
<td>Sufficient</td>
<td>Inadequate</td>
</tr>
<tr>
<td>Polonium-210</td>
<td>0.03-1.0 pCi</td>
<td>Sufficient</td>
<td>Sufficient</td>
</tr>
</tbody>
</table>

\(^{a}\) Modified from Hoffmann et al. (2001).

\(^{b}\) Abbreviations: AaC, 2-amino-9H-pyridol [2,3-b] indole; DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane; DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene; Glu-P-1, 2-amino-6-methyl[1,2-a:3',2''-d]imidazole; Glu-P-2, 2-aminopyrido[1,2-a:3',2''-d]imidazole; IQ, 2-amino-3-methylimidazo [4,5-b]quinoine; ND, not detected; PAH, polynuclear aromatic hydrocarbons; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; Trp-P-1, 3-amino-1,4-dimethyl-5H-pyrido [4,3-b]indole; Trp-2, 3-amino-1methyl-5H-pyrido [4,3-b]indole.

\(^{c}\) Not reviewed for carcinogenicity.
Carcinogenic substances are not limited to the particulate phase. The vapor phase of mainstream smoke includes chemical substances classified as carcinogenic, including acetaldehyde, isoprene, benzene, styrene and 1,3-butadiene (Hoffmann & Hoffmann, 2001; World Health Organization, 1986). Indeed, some scientists question whether the active substances in cigarette smoke are limited to the particulate phase and have developed as alternative hypotheses, either whole smoke or vapor (Witschi et al., 1997a). To test these hypotheses, both \textit{in vitro} and \textit{in vivo} studies have been designed to test the toxicity of whole smoke or vapor phase, instead of particulate (tar) phase.

Using a strain of mouse that develops lung adenomas, Witschi and colleagues published a series of studies that test the inhalation of a combination of mainstream and sidestream smoke regarding its potency to induce lung carcinomas (Witschi et al., 1995; Witschi et al., 1997b; Witschi, 1998; Witschi et al., 1999; Witschi et al., 2000; Witschi, 2000b).

Witschi’s protocol is novel. It involves a five-month exposure to cigarette smoke followed by a four-month recovery period (Institute of Medicine, 2001). It also involves a mixture of mainstream and sidestream smokes. The lung tumors seen in this mouse model, adenomas, are thought to resemble human lung tumors which have become more prominent, as smokers have switched to filtered and light cigarette brands. Witschi and coworkers reported that exposure of the mice to the gas phase of this experimental smoke produced lung tumor multiplicity at a similar rate to whole smoke (Witschi et al., 1997a; Witschi, 1998). Witschi and coworkers (1999; 2000) also showed that chemopreventive agents known to protect against tumors induced by substances found in the particulate phase did not protect against their experimental cigarette smoke. Other laboratories recently replicated Witschi’s A/J mouse model experiments (D’Agostini et al., 2001). The relevance of the system to human lung cancer must be confirmed experimentally.

\section{3.4.7 Other Biological Properties}

No particular scientific reason exists to focus attention on the carcinogenic effects of cigarette smoke, to the exclusion of other health effects. The rationale appears to lie in public attention and policy objectives. Other endpoints, such as ciliatoxic effects, which seem a useful model for COPD, appear equally useful candidates for research (Riveles et al., 2003). Therefore, the search for an animal model of emphysema has absorbed attention (Wright & Churg, 2002b).
3.5 SUMMARY

In studying the potential health effects of any substance, an early and essential step is the definition of what toxicologists call “the study substance” or “the test material.” If an ingredient added to a cigarette changed the chemical and physical composition of the resulting smoke, then the ingredient might contribute to an adverse health impact already caused by smoking the cigarette. In this circumstance, cigarette smoke becomes the test material, and the added ingredient augments or modifies the test properties of the cigarette smoke.

Cigarette smoke is a complex and variable mixture. LSRO has explored the chemical and physical composition of smoke from cigarettes and thus, how scientists could measure the influence of added ingredients on smoke. This chapter also lays a foundation to understand what manufacturers might practically accomplish by testing the effect of added ingredients on the chemistry and physical composition of smoke.

Most of the data about cigarette smoke come from mechanically generated smoke, often intended to mimic at least in some average way, human smoking patterns, but not from studies of inhaled human cigarette smoke. Because humans draw mainstream smoke immediately into their pulmonary trees, an inaccessible location, scientists have to infer the properties of inhaled mainstream smoke from the study of these mechanically generated smokes. Mechanically generated smoke may have a different chemical composition from the smoke inhaled by humans, depending on cooling, dilution, and other processing. Thus, scientists need to exercise caution in interpreting these studies. In addition, cigarette smoke inhaled by humans contains variable proportions of sidestream smoke.

Even when the mechanical generators better mimic human smoking patterns with respect to number of puffs, puff intervals, inspiration rates, and other properties, which they generally cannot do, these machines will still need to duplicate other physiological factors such as impaction within the branching pulmonary tree, hydration from fluid lining of the pulmonary cavity, and so forth. In addition there is high variation within the human smoking population in the way cigarettes are smoked. Should mechanically generated smoke ever improve on its ability to duplicate human smoking and the variation between individuals, it would be not be unexpected to still find high variation in the physical characteristics and analytical yields of chemical substances in cigarette-to-cigarette comparisons. Yields change as smoking proceeds down the tobacco rod and paper, from which the smoke is generated.

Many confounding factors also contribute to variation in the content of cigarette smoke. Tobacco growing, cigarette engineering, and control of cigarette manufacturing, will affect the compositions of brands of cigarettes and the
constituents of their smokes. Added nontobacco ingredients, such as menthol, can affect cigarette composition and to a measurable degree, alter smoke composition. The scientific literature does not document the effects on smoke composition of many added ingredients.

Cigarette smoke is a variable, heterogeneous mixture of several thousand organic and inorganic chemical constituents. The overall high variance in the chemistry and physics of cigarette smoke does not mean that the human health effects of cigarette smoking (or the testing of nontobacco ingredients added to the cigarettes) are not subjects amenable to scientific study. It does mean that scientific methods that depend on a high precision of data cannot be employed.

Some substances in cigarette tobacco undergo pyrolysis, whereas other substances undergo complex chemical reactions in the smoke. The chemical substances added to tobacco will undergo the same processes. Because of the amounts of ingredients added to cigarettes, a relatively high level of exposure to the combustion products of added ingredients is possible. Therefore, LSRO needs to understand how added ingredients change smoke chemistry and composition. Such testing is readily performed but quantitative measurement of components of cigarette smoke will require robust experimental controls. The use of reference cigarettes and reference smoking conditions is required for purposes of reproducibility and internal comparison.
CIGARETTE SMOKE: EXPOSURE AND DOSIMETRY

OUTLINE

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   4.2.1 Cigarette Consumption
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   4.3.1 Structure of the Human Respiratory Tract
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4.6 SUMMARY

For internal use of Philip Morris personnel only.
CIGARETTE SMOKE: EXPOSURE AND DOSIMETRY

4.1 INTENT & PURPOSE

The terms exposure and dose express different but related concepts. An environmental exposure is a potential dose because not all of the exposed material is absorbed or otherwise made available for potential biological activity (Byrd & Cothern, 2000). For cigarette smoking, pulmonary exposure is the relevant route. For inhalation, exposure at any moment in time is the quantity of a chemical substance just outside of the membranes of the pulmonary system, and dose is the quantity of the same substance that has moved inside the membranes. From this perspective, the absorbed amount would be the same as the dose. Transit through the pulmonary membrane is not a simple process. Some portion of a chemical substance may not be available to the circulation but may remain adhered to, or within, the membranes of the pulmonary system.

Biological measures of the absorbed amount of a substance in biological fluids, such as integrated measures of metabolites in urine, contrast usefully with simultaneous measures of exposure to the same substance, either in individuals or in populations. As explained in the previous chapter, cigarette smoke is a complex mixture of many thousands of chemical substances. Thus, relating the inhalation exposure to cigarette smoke to biological measures of dose is scientifically difficult.

The purpose of understanding exposure in this context is ultimately to allow the subsequent establishment of an exposure-response relationship for external observers to measure. The driving forces in human health effects are dose and time (Rozman, 1998). Dose bears a complex, often nonlinear relationship to exposure. Time also has complex relationships to exposure, as time has several component relationships bearing on chronic health effects. In epidemiology, these relationships relate to variables recorded for each subject, including chronological year of birth, age of initiation of exposure, duration of exposure, and age at observation or death. In toxicology, each substance will have at least three time scales: pharmacokinetic, pharmacodynamic, and frequency of administration. All three scales interact with dose and species to produce a complex situation for interpretation in chronic health effects (Rozman & Doull, 2000). For inhalation studies dose is not easily observed or measured.
Cigarettes contain a psychoactive drug, nicotine (U.S. Department of Health and Human Services, 1988). Smoking of cigarettes permits self-administration of nicotine. Cigarette smoking also exposes smokers to chemical substances besides nicotine. Because nicotine contributes to increasing smokers’ exposure to cigarette smoke, this chapter describes the pharmacokinetics of nicotine. To understand the feasibility of testing ingredients added to cigarettes, scientists need to understand the factors contributing to smoke exposure and dosimetry, of the mixture of all substances together, as well as the individual substances in the smoke.

4.2 HUMAN SMOKING

4.2.1 Cigarette Consumption

Human smoking and smoke exposure are complex processes, which include the decision to smoke, puffing behavior, inspiration into the lung, coating of pulmonary surfaces, and the absorption, metabolism, distribution, and elimination of substances in the smoke. Human consumption of a cigarette involves ignition of the cigarette and inhalation of smoke as the cigarette decreases in length, through a series of oral inspirations (puffs). Smokers usually inhale a breath containing cigarette smoke more deeply and more rapidly than a normal inhaled breath (Pearson et al., 1985). Cigarette smoke is held briefly in the mouth and throat during inspiration of a puff (Hinds et al., 1983). Subsequently, the smoker inhales the smoke into the respiratory tract, mixed with ambient inhaled gases after the puff is completed. The residual constituents are gradually exhaled, and the breathing pattern quickly returns to baseline.

Smoking machine yields of cigarette constituents, such as carbon monoxide, generated under standard FTC conditions, do not accurately predict human biomarker measurements (Benowitz, 2001; Gori & Lynch, 1985; Woodward & Tunstall-Pedoe, 1993). Wide variation in human smoking behaviors may explain some of this difference. Inhaled volumes of cigarette smoke vary between 5 mL and 75 mL per puff.

4.2.2 Time-Weighted Average, Haber’s rule, and Pack-Years

The usual epidemiological measure of smoke exposure, the pack-year, implies that exposure to substances in smoke does not change over the smoker’s lifetime. However, the smoker changes, even if the brand of cigarette and the smoker’s smoking behavior do not change. The structures of the body tissues, including the respiratory tract, change with age and change even more with chronic exposure to smoke. Chronic cigarette consumption compromises ciliated cells in the respiratory tract. Mucus producing cells and metaplastic, squamous-like cells replace ciliated cells (Dalhamn & Rylander, 1971; Kensler & Battista, 1966). Removal of airway secretions becomes compromised, while simultaneously hyperplasia of the mucus
producing cells and glands increases. Chronic exposure to cigarette smoke activates inflammatory and immune cells, contributing to progressive destruction of lung tissues.

Duration of exposure and intensity of exposure to cigarette smoke both contribute to the development of disease (Doll & Peto, 1978). Measures such as the number of cigarettes smoked per day, the depth of smoke inhalation and the tar and nicotine content of the cigarette help to reveal the intensity of cigarette exposure. Epidemiologists usually elicit patterns of cigarette consumption from questionnaires or interviews, with the usual uncertainty surrounding retrospective self-assessments (Kuper et al., 2002). Biological markers can provide more accurate estimates of the smoking intensity.

Haber’s rule is identical to the standard measure of smoke exposure, pack-years, both mathematically and biologically (smoking intensity in packs/day multiplied by duration of smoking in years). Haber’s rule also is identical to time-weighted average (TWA), a frequently used measure of occupational exposure. The TWA is the air concentration of a substance, multiplied by its duration in air (Atherley, 1985). Fritz Haber established Haber’s rule in the process of making observations about chemical warfare agents (Witschi, 2000a). Some scientists believe that the rule best fits acute toxicity. For linear systems, no reason exists to suppose that Haber’s rule will not extend in time or expand according to a power law. Later experiments established these relationships for carcinogenic substances (Druckrey, 1967).

Haber’s rule commonly is understood to apply only to acute effects and only in its simplest form, \( C \times t = k \), where \( C \) is concentration, \( t \) is time, and \( k \) is a constant. In its expanded, power law version, the exponents applying to concentration and time can emphasize or diminish the importance given to exposure concentration and exposure duration for any substance in generating an observed toxic effect, \( C^a \times t^b = k \) (Institute of Medicine, 2000). If \( a > 1 \) and \( b > 1 \), applications of the unmodified form of Haber’s rule (\( C \times t = k \)) to high concentration, prolonged duration data yield upper bounds on concentration-duration relationships. For some substances the unmodified form may be inappropriate. Some toxicologists prefer using Haber’s rule and applying safety factors, when working with chronic data. Working with a pack-year relationship merits caution, because packs per day times years of smoking experience may not yield a valid estimate of exposure.

In this context, the number of cigarettes or packs of cigarettes smoked per unit of time, \( e.g. \), cigarettes per day or packs per year, is an exposure rate. An exposure rate equates to an air concentration during a period of time. The number of packs per day, multiplied by the number of years a smoker has smoked at that rate, is a measure of total exposure. For chronic exposure, the standard inhalation intake
rate has units of mg/kg/day. This rate is usually calculated, not measured (Byrd & Cothern, 2000). It is a product of concentration, inhalation rate, absorption factor, body weight, and an exposure factor, as follows;

\[
\text{air inhalation intake rate} = \frac{(C \times \text{IR} \times \text{AF} \times \text{EF})}{\text{BW}}
\]

where:
- \(C\) = contaminant concentration \((\text{mg/M}^3)\)
- \(\text{IR}\) = inhalation rate \((\text{M}^3/\text{day})\)
- \(\text{AF}\) = absorption factor (no units)
- \(\text{EF}\) = exposure factor (no units)
- \(\text{BW}\) = body weight \((\text{kg})\)

For cigarette smoking the absorption factor is known to be less than 1.0. (See subsection 4.3.2, below.) The exposure factor is a product of exposure frequency (events/year) and exposure duration (years/lifetime). Constant exposure over a lifetime generates an \(\text{EF} = 1\). For cigarette smoking, exposure frequency is equivalent to packs smoked per day, and exposure duration is the same as the number of years of smoking at that rate. So, introduction of pack-years into the standard inhalation intake rate calculation changes the units and the physical assumptions that go into the calculation.

Standard estimation of inhalation exposure assumes that concentrations in the external air will equilibrate with internal concentrations in the pulmonary air space. For cigarette smoking, this assumption cannot hold. Either for the gas or particulate phases, nonequilibrium circumstances prevail. Instead, the smoker rapidly inhales a series of puffs with little volume of air between the end of the cigarette and the surface of the lung.

Rozman’s theory of toxicity supports the use of the power law version of Haber’s rule \((C^a \times t^b = k)\) (Rozman, 2000). It suggests that mortality and morbidity from cigarette smoking, like other chronic health effects, should relate to both exposure rate and duration of exposure (Rozman, 1998). However, either exposure rate or duration of exposure may contribute more to a toxic effect. Even so, for cigarette smoking, an estimate of exposure rate should normalize chronic human exposure-response relationship data.

The potential inaccuracy of using pack-years is obvious (Kuper et al., 2002). Epidemiologists usually obtain exposure information about smoking from questionnaires or interviews. Retrospective, self-reported data will inaccurately characterize the cigarette exposure of an individual smoker. Measures of cigarette consumption cannot easily account for variable smoking, for example, in bars or at parties. However, replacing pack-years as an estimate of exposure rate in epidemiological surveys will not be easy. Cigarette smoking does not provide a
constant exposure but if fluctuations occur on a time scale very much shorter than the evolution of disease, such fluctuations may be unimportant. The chief difficulty with pack-years is estimating it.

Recently, the application of Haber’s rule to cigarette smoking has undergone scrutiny. Leffondré and coworkers (2002) used data from a case control study and Cox’s model to study smoking status, intensity, duration, cigarette-years, age at initiation, and time since cessation, using time-dependent variables and goodness of fit as their primary criteria. They found (a) that using cigarettes per day and duration separately improved fits with their model, compared to Haber’s rule and (b) that colinear effects declined, when they estimated the effects of time since cessation or age at initiation, if they used Haber’s rule.

### 4.2.3 Human Smoking Behavior

Human smoking behavior influences puff volume, puff duration, peak puff flow velocity (the maximum rate of mainstream smoke flow from cigarette to smoker), interval between puffs, number of puffs per cigarette, and residual butt length left at completion of smoking the cigarette. The puff profile graphically represents the rate of flow of mainstream smoke during a puff. Puff profiles can be used to calculate puff volume (International Organization for Standardization, 2000). Figure 4.1 shows the puff profiles of three subjects and a puff profile generated by standard smoking machine settings.

*The Health Consequences of Smoking. Nicotine Addiction* (1988) summarized data about puffing behavior, as a common measure of smoke exposure. The summary cited 29 separate studies of 8 to 517 subjects carried out between 1978 and 1986. The means (ranges) of the indices were:

- Puffs per cigarette: 11 (8-16; n=27 studies),
- Puff duration: 1.8 seconds (1.0-2.4 seconds; n=27 studies),
- Puff volume: 43 mL (21-66 mL; n=18 studies),
- Peak puff flow: 36 mL per second (28-48 mL per second; n=6 studies),
- Inter-puff interval: 34 seconds (18-64 seconds; n=21 studies),
- Total smoking time: 346 seconds per cigarette (232-414 seconds; n=13 studies),
- Total smoke inhalation volume per cigarette: 591 mL (413-918 mL; n= 5 studies).

Bentrovato and coworkers (1995) reported that puff volumes declined progressively with a lower average rate of mainstream smoke flow from the cigarette to the smoker (peak puff flow) than the Surgeon General’s report (1988). (See Figure 4.2) Bentrovato and coworkers found 18 mg of tar per cigarette delivered to the mouths of adult smokers.
Figure 4.1 Kinetics of human puff flows for three smokers. Modified from Bentrovato, et al., (1995).

Figure 4.2 Average puff volumes as a function of sequential puff number. Error bars represent one standard deviation. Modified from Bentrovato, et al., (1995).
The taste of cigarette smoke influences smoking (Huber et al., 1990; Nil & Battig, 1989). Ingredients added to cigarettes can affect taste. Extensive government reports discuss the factors contributing to initiation and maintenance of smoking, and they cite a few studies about the sensory experience of smoking, including human perceptual parameters, such as flavor and aroma, which added ingredients might alter (U.S. Department of Health and Human Services, 2001b) (U.S. Department of Health and Human Services, 1994) (U.S. Department of Health and Human Services, 2001a). Sensory cues, such as cigarette flavor and aroma, also might support the maintenance of smoking, as cues, through learned behavior. Smoking pairs a distinctive aroma with a pharmacological effect (Rose et al., 1993; Westman et al., 1996).

No individual smoker exhibits a constant pattern of cigarette smoking. An individual’s behavior changes, even during the smoking of a single cigarette (Bentrovato et al., 1995; Creighton & Lewis, 1978; Pillsbury, 1996). Smokers tend to draw larger puff volumes initially and taper down by the last puff (Adams, 1976; Reeves & Dixon, 1995). The decreases in puff volume correlate with decreases in puff duration, while smoking a single cigarette (Reeves & Dixon, 1995). As illustrated in Figure 4.2, average puff volume in one study decreased from approximately 60 mL for the first puff to less than 20 mL for the eighteenth puff (Bentrovato et al., 1995; Polydorova, 1961). Inhalation also contributes to variation in cigarette smoking. Depth of inhalation has been estimated by various methods: self-report, chest wall expansion, impedance magnetometers, and inductance plethysmography (US Consumer Product Safety Commission, 1993).

Variations in puff profiles lead to the notion that no two persons smoke the same brand of cigarette in an identical manner (Bentrovato et al., 1995; Creighton & Lewis, 1978; Nil & Battig, 1989). Among smokers of filtered cigarettes in France, men took fewer puffs per cigarette than women, but men had greater durations and volumes of puffs (Hee et al., 1995). Similar gender-specific findings were reported for smokers in the U.S., but the total volume of cigarette smoke inhaled per day did not differ significantly by gender (Djordjevic et al., 1998).

Nicotine binds to receptors on dopamine-releasing cells in the ventral tegmental area of the brain’s mesolimbic system, creating a reinforcing sense of satisfaction (Clarke, 1990; Corrigall et al., 1994) (Figure 4.3). Whether smokers consume cigarettes in amounts and ways, such that they obtain a constant level of satisfaction, is not clear. Individual smoking behavior, which varies, does determine the delivery of cigarette smoke and nicotine to the respiratory tract (Gori & Lynch, 1985). Benowitz and Jacob (1994) measured nicotine uptake with deuterium labeled nicotine and found an average uptake of 2.3 mg per smoker. However, these investigators decided that a prestudy period of abstinence influenced self-administration. They recommended the continued use of a standard 1 mg per
cigarette for nicotine delivery. Similarly, CNS levels of nicotine will vary among individuals in response to the same delivery, and satisfaction will vary among individuals with the same extent of receptor binding.

The delivered dose of nicotine from smoke affects smoking behavior. Benowitz and coworkers (1983) studied 272 subjects who smoked different brands of cigarettes. Smokers of low yield cigarettes achieved concentrations of blood cotinine similar to smokers of high yield cigarettes. Cotinine is a biomarker of nicotine exposure. The data are consistent with a biological feedback mechanism involving nicotine. The data also demonstrate compensation for lower nicotine yields. As nicotine yields declined, smokers inhaled more smoke in poorly understood ways. Investigators have documented that at least partial compensation occurs (Lee, 2001).

4.2.4 Smoking Compensation

Benowitz (2001) and Scherer (1999) used mathematical equations to define the extent of compensation based on proportional changes in biomarker levels and machine-determined yields of a constituent, before and after a change in brand of cigarette. One possible explanation of compensation is that an increase in the number of cigarettes consumed will compensate for the reduced amount of nicotine in the cigarette (Russell et al., 1980). Another option is a change in smoking behavior, so that the smoker obtains more nicotine per cigarette (Lee, 2001). Still another is the smoker blocking holes in cigarette papers, to reduce dilution with air. In addition, smokers could engage in all of these behaviors (increase cigarette consumption and smoke to obtain more nicotine per cigarette by inhaling deeper and blocking air holes).
Compensation need not be absolute. Burns and coworkers (2001) used data from an American Cancer Society cohort of 174,997 U.S. male smokers, which included brand and number of cigarettes smoked over a 12-year period from 1960 to 1972, and found a relationship between nicotine content and cigarette consumption. For each milligram decline in machine-measured nicotine yield per cigarette, the number of cigarettes smoked increased by 0.85 per day. Among 169,610 subjects, who switched brands of cigarettes in follow-up surveys, the number of cigarettes smoked per day increased by 2.3 cigarettes per day for each mg decline in nicotine yield (Burns et al., 2001).

Stepney (1980) analyzed the results of fifteen studies about switching from brands with medium nicotine deliveries (machine deliveries of 1-2 mg) to brands with decreased nicotine delivery. In twelve of the fifteen studies, the number of low tar and nicotine cigarettes smoked increased, compared with the number of medium tar and nicotine cigarettes smoked. These data produced a correlation of R = 0.59 between the percent reduction in nicotine delivery and percent increase in the number of cigarettes smoked per day. Regression analysis predicted that a reduction of 50% in nicotine delivery would result in a 9% increase in consumption. From a reanalysis of these results, Lee (2001) also concluded that brand switching showed only modest increases in number of cigarettes smoked per day relative to major decreases in delivery of tar and nicotine.

Garfinkel and coworkers (1979) reported data from a large prospective American Cancer Society study of 28,561 U.S. male smokers, 60% of whom smoked a lower yield brand of cigarette in 1972 than in 1959. Persons who increased consumption did so by ten cigarettes per day. Garfinkel and coworkers (1979) concluded that this increase did not relate to the yield of tar and nicotine. Of those who smoked less than one pack per day in 1959, 55% increased the number of cigarettes smoked per day in 1972 after changing to cigarettes with decreased tar and nicotine. However, the majority of other smokers consuming less than one pack per day in 1959, who selected cigarettes of the same or increased content of tar and nicotine in 1972, also increased the number of cigarettes they smoked daily by 1972. Lee (2001) cited these data to show that reduced tar and nicotine did not change the number of cigarettes smoked per day.

Smokers could compensate for reduced tar and nicotine by puffing more frequently, by inhaling deeper, and/or by blocking ventilation holes, impeding the dilution of smoke in the tobacco rod (Kozlowski et al., 2001b; Sutton et al., 1978; Sutton et al., 1982). All three behaviors increase smoke yields (National Institutes of Health, 2001). Changes in puff volume might explain why a 40% reduction in cigarette tar, obtained from smoking machine data, would result in only a 15% reduction in smoker’s tar exposure (Lee, 2001).
According to Scherer (1999), changing puff volume is most probably the dominant, if not only mechanism of compensation. Scherer (1999) reviewed sixty studies involving a total of more than 2000 smokers, to see whether low-yield cigarettes are smoked more intensely than high-yield cigarettes. He summarized the studies as compensation for tar (four studies), taste (six), irritation (one), draw resistance (five), nicotine content (29), and no compensation (eight). Puustinen and coworkers (1987) reported the eight volunteers who smoked cigarettes yielding 4-5 mg tar and 0.32 mg nicotine had greater puff duration (2.2±0.2 sec), greater puff volume (36±4 mL) and took more puffs per cigarette (18.4±1.4). The smokers inhaled a greater volume of smoke per cigarette (649±69 mL) compared to their earlier brand of cigarette, containing 14-15 mg tar and 0.97 mg nicotine (1.8±0.2 mL, 26±4 mL, 13.6±1.0 mL, and 345±46 mL, respectively).

Lynch and Benowitz (1987) obtained plasma cotinine measures from 197 smokers at two time points, six years apart. For those who switched to lower yield cigarettes, the number of cigarettes smoked decreased by 6.6 cigarettes (20%), from an average of 32.9 to 26.3 per day. Plasma cotinine concentration increased by ~5% for those who smoked cigarettes with similar yields (n=104), decreased by ~20% for those who smoked cigarettes with lower yields (n= 62), and increased by ~23% for those who switched to higher yield cigarettes (n= 31). For those who switched to lower yield cigarettes, total daily nicotine yields (nicotine yield per cigarette multiplied by the total number of cigarettes smoked per day) declined by ~50%. Because Lynch and Benowitz (1987) did not measure puff profiles, Benowitz (2001) estimated the intensity of smoking of study subjects by dividing the plasma cotinine concentration by the number of cigarettes smoked per day. Smokers who switched to lower yield cigarettes compensated by smoking more intensely, because their plasma concentrations of cotinine, relative to the number of cigarettes smoked, closely resembled levels obtained when the higher yield cigarettes were smoked previously.

Peach and coworkers (1986) measured urinary nicotine metabolites of 599 male smokers in a multi-site, randomized heart disease prevention project. The number of cigarettes smoked per day declined during the study for all subjects. Smokers excreted similar amounts of urinary nicotine metabolites, regardless of tar yield, consistent with compensation.

Benowitz (2001) summarized five studies of up to ten day durations and five longer term studies of five weeks to nine months durations, in which smokers were assigned a test cigarette and group mean compensation ranged from 20 to 100%, as defined by biomarkers (blood concentrations of cotinine or carboxyhemoglobin, expired carbon monoxide, or urinary nicotine metabolites). Guyatt and coworkers (1989), Robinson and coworkers (1983) and Zacny and Stitzer (1988) showed that some smokers increased the number of cigarettes smoked per day but that the primary means of compensation was accomplished by increased intensity of smoking. The
intensity of smoking could be increased by increasing puff volume, increasing peak flow, decreasing the inter-puff interval, and/or increasing the number of puffs per cigarette (Guyatt et al., 1989; Zacny & Stitzer, 1988). Some subjects blocked ventilation holes (Guyatt et al., 1989).

Thus, two reviewers, Lee (2001) and Benowitz (2001) generally agree that some compensation occurs but that a switch to a lower yield cigarette does not necessarily lead to an increase in the number of cigarettes smoked per day. Benowitz (2001) further concludes that such a switch probably will alter smoking behavior in the direction of extracting more nicotine from the cigarette, compensating to some degree. Burns reached a similar conclusion in a report of the U.S. Consumer Product Safety Commission (1993), suggesting that changes in design (draw resistance and filter perforation) could alter cigarette smoking.

Mechanisms of compensation for decreased cigarette yield, not involving consumption of more cigarettes, have not been fully characterized. Smokers can achieve higher nicotine yields through poorly understood smoking behaviors. Pritchard and Black (1984) reported a decrease in the total body and thoracic deposition of tar in 23 healthy male smokers who switched from a high tar (16-17 mg), to a low tar (8-9 mg) cigarette for six months, using cigarettes labeled with radioactive $^{123}$I-iodohexadecane, which has a half-life of thirteen hours and undergoes little pyrolysis during cigarette combustion. At baseline, and at one, three and six months following the switch to the lower tar cigarette, subjects smoked the labeled cigarette in their usual manner. Changing to the lower tar cigarette significantly reduced total body deposition of labeled particles by 25% at one month and more than 30% by six months (Table 4-1). However, deposition in the head and abdomen remained the same. Pritchard and Black (1984) suggested that a large part of this deposition would be in the mouth and throat, perhaps reflecting modifications in the smoking pattern to compensate for differences experienced smoking the lower tar cigarette.

<table>
<thead>
<tr>
<th>Region</th>
<th>Deposition (%)</th>
<th>Higher-tar (16-17mg)</th>
<th>Lower-tar (8-9mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Body</td>
<td></td>
<td>100.0 ± 6.8</td>
<td>67.1 ± 5.1</td>
</tr>
<tr>
<td>Extra Thoracic (head, abdomen)</td>
<td>13.9 ± 3.4</td>
<td>15.6 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>Thoracic</td>
<td>86.1 ± 6.8</td>
<td>51.5 ± 4.2</td>
<td></td>
</tr>
<tr>
<td>Tracheobronchial airway</td>
<td>51.1 ± 3.4</td>
<td>33.8 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Vascularized pulmonary tissue</td>
<td>35.0 ± 4.2</td>
<td>18.1 ± 2.1</td>
<td></td>
</tr>
</tbody>
</table>

Table 4-1. Regional deposition of tar 24 weeks after switching to a lower tar cigarette (mean ± SE).²

² Modified from Pritchard and Black (1984).

² Expressed as a percentage of the total deposited from the higher tar cigarette.
4.3 DOSIMETRY OF CIGARETTE SMOKE

When people inhale cigarette smoke, they expose their respiratory tracts to both the gas and particulate phases of the smoke. In addition to absorption of smoke constituents on the surface of the respiratory tract, many chemical substances circulate, further exposing internal tissues and organs. The scientific community has made progress in identifying the chemical substances in cigarette smoke. (See Chapter 3 of this report.) Quantitative assessment of human exposure is limited.

4.3.1 Structure of the Human Respiratory Tract

Some background information will aid the discussion of absorption and deposition of smoke constituents. Hollander and Stober (1986) based their descriptions on a task force report (Bates et al., 1966). A naso-pharyngeal (extra-thoracic) compartment begins at the anterior nares and extends beyond the anterior and posterior pharynx to the epiglottis. The respiratory tract divides into two regions: the tracheobronchial tree (the upper airway) and the pulmonary parenchyma (the vascularized tissue where gas exchange occurs). The tracheobronchial compartment consists of the trachea and the bronchi. These airways branch and subdivide over and over, approximately 25 times in the human, to reach fine divisions in the terminal bronchioles.

Ciliated epithelia characterize the tracheobronchial compartment, greatly increasing the surface area. Both glandular and nonglandular secretions bathe the lining of the airway (Bates et al., 1966; Hollander & Stober, 1986). The cilia move and transport secreted mucus and deposited particles up the tracheobronchial tree and out of the lungs. The pulmonary compartment consists of the nonciliated respiratory bronchioles, which extend from the terminal bronchioles, and their supporting vascularized tissue. The peripheral pulmonary compartment includes alveolar ducts, atria, alveoli attached to the respiratory bronchioles, and alveolar sacs. The vascularized air sacs of the alveoli serve as the interface where gases exchange between the airway and the perfusing pulmonary capillary compartment (the parenchymal tissue).

Leading from the trachea, the structure of the tracheobronchial tree is asymmetric (Yeh et al., 1976). A parental airway tube divides into two, uneven daughter branches, a major and a minor branch, distinguished by their differences in diameter. The airways in the human lung are more divided by branching, than the airways of dogs, rats and hamsters. In humans and dogs, angles of the major and minor branches increase progressively down the tracheobronchial tree. Data for variation in human tracheobronchial tree branch angles, daughter to parent tube diameter ratios, and daughter tube length to diameter ratios are available, as are data for some laboratory mammals. Yeh and coworkers (1976) suggest that species
differences in the structures of pulmonary lobes may explain the findings of uneven deposition of particles in respiratory tracts of different animals.

4.3.2 Absorption of Smoke

Absorption refers to the transport of a chemical or substance from the site of administration into the systemic circulation. In the case of inhaled cigarette smoke, constituents will transfer from the pulmonary air space of the respiratory tract onto its surface. Potentially, components of the smoke transfer further into the circulation through this highly vascularized tissue. Both the gas and particulate phases of smoke reach, and react with, the surface of the lung. Localized surface reactions lead to some of the biological effects of smoke. In addition to coating of lung surfaces, smoke includes unreacted chemical fragments created by pyrolytic and pyrosynthetic reactions associated with cigarette combustion. These fragments can react covalently with cellular elements of the lung, instead of each other.

Smoking studies sometimes use an alternative term for absorption, deposition. In deposition a fraction of inspired smoke is not expired (Brain & Valberg, 1979). By definition, the processes of absorption and deposition overlap. For cigarette smoking, the particulate fraction of captured smoke, deposited on a filter, can be weighed before and after inhalation, leading to an experimental measure of deposition. The mass entering and exiting an airway also can be calculated from aerosol concentrations and flow rates (Subramaniam et al., 2003). Several processes lead to deposition in the respiratory tract, including impaction, sedimentation and diffusion (Martonen, 1992; Yeh et al., 1976). Changes in breathing patterns alter deposition. An increased velocity of inhaled particulates increases deposition by impaction (Yeh et al., 1976).

Cigarette smoke is a significant environmental source of radioactive polonium-210 (210Po) (Little et al., 1965). Polonium-210 is a naturally occurring, alpha particle emitting element, with a half-life of 138 days, generated from decay of its parent lead-210 with a half-life of twenty years (Little & McGandy, 1968). In addition to postmortem examination of lung tissues, early studies of 210Po provided evidence that components in cigarette smoke were absorbed into lung tissues and into the systemic circulation. Little and coworkers (1965) found that smokers had 210Po in their lung tissue equivalent to the amount naturally occurring in seven packages of cigarettes. They considered this deposition low, because their smoking subjects consumed at least one-half pack of cigarettes per day. Because they found only small amounts of 210Po in lung parenchyma, they suggested that the lung cleared 210Po, which absorbed into the circulation rapidly (Little & Radford 1967). Smokers had blood concentrations of polonium-210 2.5 times higher than nonsmokers. Consistent with clearance from the lung into the circulation, blood concentrations

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of $^{210}$Po declined an average of 14% three-to-four days after smoking cessation and by 20.5% at 11 to 14 days after cessation (Little & Radford, Jr., 1967).

Minty and coworkers (1984) investigated whether cigarette smoking would increase the permeability of the pulmonary epithelial tissue. Five male nonsmokers breathed an aerosol containing $[^{99m}\text{Tc}]$diethylenetriamine pentacetic acid ($[^{99m}\text{Tc}]$DTPA) for two to three minutes, twice at baseline and on days one and three during the experimental period when they smoked ten cigarettes per day for three days. A probe scintillation detector was placed over the right upper zone of the chest to detect $[^{99m}\text{Tc}]$DTPA. Values were corrected for increasing radioactivity in blood and non-lung tissue. The half-time elimination of $[^{99m}\text{Tc}]$DTPA decreased by 26% to 53% for subjects by the third day of smoking. Smoking increased the permeability of pulmonary epithelial tissue, which permitted greater absorption of the aerosol from lung into blood (Minty et al., 1984).

No precise estimate of particulate deposition from inhaled cigarette smoke exists. Many factors could affect the deposition of smoke particulate, including smoking behavior, smoking experience, brand of cigarette, humidity, body size, age, gender, disease status, and previous injury, depth of inhalation, residence time in the lung, exercise, and life style. Table 4-2 (World Health Organization, 1986) shows estimates of the fraction of inhaled tar deposited in the respiratory tract from seven studies of exhaled particles conducted between 1923 and 1984. The method for measuring particle capture varied among these early studies. Subjects retained most of the inhaled tar (74% to 97%). However, one early study found 47% tar retention by fluorophotometric measures of 11 subjects (Hinds et al., 1983). All 13 measures of deposition by Hinds and coworkers (1983) in five female smokers were less than 50%, and their average of 40% was significantly less (p< 0.01) than the 57% average deposition for six male subjects in the study.

**Table 4-2. Some estimates of inhaled tar deposition using different exhaled particle collection methods.**

<table>
<thead>
<tr>
<th>RANGE</th>
<th>RETAINED (%)</th>
<th>CAPTURE TECHNIQUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>73-98</td>
<td>88</td>
<td>Electrostatic precipitation</td>
</tr>
<tr>
<td>70-90 (5-sec inhalation)</td>
<td>82</td>
<td>Balloon</td>
</tr>
<tr>
<td>94-99 (30-sec inhalation)</td>
<td>97</td>
<td>Balloon</td>
</tr>
<tr>
<td>79-97</td>
<td>87</td>
<td>Filter</td>
</tr>
<tr>
<td>22-89</td>
<td>74</td>
<td>Vacuum-assisted filter$^a$</td>
</tr>
<tr>
<td>22-75</td>
<td>47</td>
<td>Vacuum-assisted filter$^d$</td>
</tr>
</tbody>
</table>

$^a$ Modified from World Health Organization (1986).
$^b$ Not reported.
$^c$ Data from Polydorova (1961)
$^d$ Data from Hinds et al. (1983)
Particulate deposition may be important. Based on an assumption of 50% deposition, Gurney (1998) calculated that over a 40-year period, more than 6 kg of particulate matter would be deposited in the lung of a one-pack per day smoker, who smoked cigarettes containing 20 mg of tar. The potential tumorigenic activity of particulate deposition has been studied using particulate matter painted onto skin surfaces of animal models (Coggins et al., 1982; Dontenwill et al., 1972; Dontenwill et al., 1976). This is thought to create an analogy to the deposition of particles on the surface of the lung. Both lung and skin are epithelial surfaces.

Later studies suggest less deposition of cigarette smoke, approximately 15 to 50% of the inhaled particulate. Hollander and Stober (1986) suggested that up to 6% of smoke constituents might deposit in the tracheo-bronchial compartment and between 22% and 32% in the pulmonary region. Theoretically, only 15% to 30% of mainstream smoke particles of 0.2 \( \mu \)m average diameter would deposit in the human respiratory tract, based on the deposition of similar sized nontobacco particles inhaled by mouth or nose during typical low-activity conditions of 750 cm\(^3\) tidal volume and 15 breathes per minute (Hinds et al., 1983; National Council on Radiation Protection and Measurements, 1997). (See Figure 4.4.)

![Figure 4.4 Relative deposition of particles in a lung model](image_url)

Figure 4.4 Percent deposition of particles as a function of particle size under conditions of 770 cm\(^3\) tidal volume, 13 breaths per minute and functional residual capacity of 0.4 total lung capacity. Data from National Council on Radiation Protection and Measurements (1997).
Using a Weibel symmetric lung model under conditions similar to that of a smoker (750 mL tidal volume, 5-second inhalation, 3-second pause, and 2-second expiration), and assuming that the growth of saturated saline droplets represented the hygroscopic characteristics of cigarette smoke particles, Muller and coworkers (1990) calculated 31% to 53% deposition for mainstream particles and 6% to 28% deposition for sidestream particles.

Schultz and coworkers (2000) reviewed the physical mechanisms of particle deposition in the respiratory tract. Determinants of inhaled particle deposition in the respiratory system include particle size, shape, density, charge and hygroscopicity. These factors affected growth of particles (agglomeration) in the humidified respiratory tract (Kaufman et al., 1996; National Council on Radiation Protection and Measurements, 1997; Sethi & Rochester, 2000). Martonen (1992) and others (Chen & Yeh, 1990) thought the behavior of mainstream cigarette smoke in the lung related to particle cloud motion, an effect produced by high particle number (~ $3 \times 10^9$ per cm$^3$) and mass (~0.0001 g per cm$^3$) densities of the smoke. Particle cloud motion could explain why mainstream cigarette smoke would deposit at sites that differ from the sites of deposition expected for a dilute aerosol of similar sized particles (Martonen, 1992; Phalen et al., 1994).

The change in the size of smoke particles as they age may influence the amount deposited. The respiratory tract has high humidity, estimated at 99.5 per cent (Ferron, 1977). Subsequent coagulation of mainstream smoke caused by high humidity in the lungs might increase particle diameters, acquiring the physical characteristics of a smoke cloud with high concentrations of particles, exhibiting larger diameters, perhaps up to six or seven $\mu$m (Keith, 1982). In theory, a greater portion of larger particles would deposit than particles of 0.2 $\mu$m diameter, the initial average diameter of mainstream cigarette smoke particles (Figure 4.4). Disagreements exist about the maximum growth of smoke particles in the humid conditions of the respiratory tract and the effect of particle growth on deposition (McRae, 1990).

Ventilation parameters, such as tidal volume, rate of cigarette smoke inhalation, dilution with inspired air, and timing of inhalation, can influence deposition. Whether the breathing pattern is controlled or spontaneous may be an important consideration for comparative studies (Martonen et al., 2000). Lung anatomy and the size of study subjects, including morphological differences in the shape and size of the larynx and tracheobronchial airways, will alter cigarette smoke particle deposition in the respiratory tract (Yeh & Schum, 1980). Geometric parameters, such as airway segment diameters, lengths, branching angles and angles of inclination to gravity, vary between individuals and contribute to the complexity of lung anatomy.
The vapor pressure and solubility of chemical substances in cigarette smoke determine their partitioning between the vapor and particulate phases in smoke (Pankow, 2001). Figure 4.5 illustrates direct deposition of a substance from the vapor phase of a smoke aerosol onto a tissue and evaporation of the substance from the particulate phase into the vapor phase with subsequent deposition onto the tissue. Unlike ingestion, substances absorbed from the respiratory tract do not undergo first pass metabolism in the liver.

Dalhamn and coworkers (1968a; 1968b) published data on the disappearance of carbon monoxide, acetaldehyde, isoprene, acetone, acetonitrile, toluene, and total particulate matter from cigarette smoke by mouth-only and inhalation exposures (Table 4-3). They exposed volunteers to smoke from unfiltered cigarettes yielding about 30 mg of tar each. Smoke from a two-second, 35 mL puff was delivered into the mouth of the subject and either blown out by the subject without inhaling, or inhaled and then blown out. Inhalation of smoke resulted in disappearance of more than 85% of all volatile compounds and for 54% of the carbon monoxide, compared with only 3% disappearance of carbon monoxide by the mouth-only exposure. The authors stressed the importance of using exposure methods that approximate actual smoking conditions (Dalhamn et al., 1968a). For example, exclusive mouth breathing inhalation narrows the theoretical range of deposition of smoke particles to approximately 15% to 20%, compared to up to 30%, based on methods that include nasal inhalation (National Council on Radiation Protection and Measurements, 1997).
Limited data indicate that approximately half of inhaled cigarette smoke deposits in the middle zone of the lung, 25% in apical regions and a little more than 25% in base regions. The base regions receive the major portion of the inhaled breath. Martonen (1992) reported that localization of smoke particle deposits in airway bifurcations in ex vivo human cast models, specifically the carinal ridges, resembles the location of lung carcinomas in the upper tracheobronchial tree. Smoke particles do not show a uniform dispersion along tubular airways in human cast models, instead the particles concentrate in the posterior segments (Martonen et al., 1987; Martonen, 1992). (See Figure 4.6)
Compared with resting ventilation, Pearson and coworkers (1985) detected more deposition of cigarette smoke particles in the apical and central regions of the lung during smoking for eleven habitual smokers. For total deposition, the mid-to-lower zone of the lungs collected most of the technetium$^{99m}$ sulfur colloid particles in the experimental cigarette smoke (apex: 25%; mid zone: 47%; base: 27-28%), similar to the overall pattern for nonsmoking breaths (Pearson et al., 1985). Longer inhalation and breath holding might contribute to deposition of mainstream smoke in the lower airways (Muller et al., 1990).

During elective surgery or autopsy, Little and coworkers (1965) obtained fresh bronchial and parenchymal tissue (whole lung or single lobes) from thirty-six patients, including twenty-five who had smoked for 20-60 years and measured $^{210}$Po in selected sections of tissue. Centrally located parenchymal tissue was available for twelve cigarette smokers. The concentration of $^{210}$Po in central specimens was approximately twice that of the peripheral samples and generally two orders of magnitude greater in the bronchial epithelium than in parenchyma or lymph nodes. The average concentration of $^{210}$Po was highest at segmental bronchial bifurcations. No $^{210}$Po was detected in segmental bronchi or upper-lobe bifurcations of nonsmokers.

Churg and Stevens (1992) inspected lung tissue samples from autopsies of five smokers (30-90 pack years) and five age and gender matched nonsmokers, using a scanning microscope equipped with a wavelength dispersion x-ray spectrometer. They detected irregularly shaped particles (0.5 to 0.8 µm) containing calcium, carbon and oxygen in lung segments of smokers (23±10% of all particles) but rarely in nonsmokers (0.1±0.2%). It is not known whether these particles were deposited intact from smoke (e.g., calcium carbonate) or formed from smoke and tissue interactions (e.g., calcium oxalate). A portion of the particle burden in smoke may translocate to the lung interstitium and remain indefinitely.

Boykin and coworkers (1993) labeled filtered research cigarettes by injecting 1-2 µCi of $[^{14}C]$dotriacontane along the tobacco rod. They administered smoke from these cigarettes, using FTC protocol through tracheostomies to mechanically ventilated dogs. After exposure to two radiolabeled cigarettes, they sacrificed the dogs and systematically analyzed tissues for the isotope. The tracer deposited in regions of the lung in direct relation to tissue mass, the time smoke was delivered relative to inspiration, and animal posture. When they delivered a bolus of smoke at the start of an inhalation, peripheral deposition of $^{14}$C averaged twice the amount of central deposition. The distribution of $^{14}$C was more uniform when the bolus of smoke was delivered at mid-inhalation. A supine posture significantly increased deposition in the upper lobes compared to an upright position (Boykin et al., 1993). In contrast, Brain and Valberg (1979) suggested that for small mammals, deposition of aerosols was generally greater in the apex than at the base of the lung, regardless of whether the animal was standing, prone or inverted.
The tar and nicotine content of smoke may not affect the location of deposition. Phelps et al. (1984) exposed female rats to \[^{14}\text{C}]\text{dotriacontane}\) labeled smoke from three types of cigarettes that varied in tar and nicotine yields, as measured by the FTC smoking machine protocol. Although the composition of the smoke varied, patterns of deposition were similar for all three groups. The major portion of the inhaled smoke penetrated the upper airways and deposited deep in the lung (Phelps et al., 1984). The cigarettes yielding high tar and low nicotine generated significantly greater external deposition on the rat body than the other cigarettes that yielded low tar and high nicotine or high tar and high nicotine, suggesting that animals did not maintain nose-seal throughout the exposure and the results may have been confounded.

Yeh and Schum (1980) noted that for a given inspiratory flow rate, deposition in the region of the mouth increased as the particle size increased. Figure 4.4 illustrates the effect of particle size on regional deposition (National Council on Radiation Protection and Measurements, 1997).

Compensation may alter smoke distribution. Pritchard and Black (1984) reported a decrease in the total body and thoracic deposition of tar in 23 healthy male smokers who switched from a high tar (16-17 mg), to a low tar (8-9 mg) cigarette for six months, using cigarettes labeled with radioactive \[^{123}\text{I}]-\text{iodohexadecane}\) which has a half-life of thirteen hours and undergoes little pyrolysis during cigarette combustion. At a baseline, and at one, three and six months following the switch to the lower tar cigarette, subjects smoked the labeled cigarette in their usual manner. Changing to the lower tar cigarette significantly reduced total body deposition of labeled particles by 25% at one month and more than 30% by six months (Table 4-1). However, deposition in the head and abdomen remained the same. Pritchard and Black (1984) suggested that a large part of this deposition would occur in the mouth and throat, perhaps reflecting modifications in the smoking pattern to compensate for differences experienced in smoking the lower tar cigarette.

### 4.3.4 Smoke Elimination From the Lung

Smokers eliminate inhaled cigarette smoke particles in their lungs by exhaling or coughing, by swallowing particles cleared by the mucocilliary elevator in their lung, or by absorbing particles into their systemic circulation. For the most part, cigarette smoke particles have a nonaqueous, liquid composition, so systemic absorption should play a major role. Initially, when particle and vapor phase components come into physical contact with the epithelial surface of the lung, they encounter the respiratory tract lining fluids. The mucus fluid can entrap particles, which mucocilliary movement or phagocytosis then eliminates. The epithelial fluid lining the lung is mobile. Materials deposited in one area of the lung can transport to other areas. The fluid undergoes active secretion and resorption. Components deposited in the epithelial fluid may concentrate or dilute.
Albert and coworkers (1971) described a typical pattern of elimination of radioactive $^{198}$Au particles (1.5-4.1 µm) and $^{99m}$Tc particles (2.3-7.9 µm) in aerosol for human smokers and nonsmokers, detected by serial tracheal and chest measurements. Elimination consisted of two distinct phases. A period of rapid elimination was complete within a few hours, and a second slower phase took 3 to 24 hours. Considerable variability in elimination rates existed among human subjects, regardless of smoking habit. However, the size of the test particles apparently varied between subjects. Some smokers cleared particles rapidly compared to nonsmokers. Albert and coworkers (1971) suggested that inter-subject variability relates to the rate of the second phase mucociliary elimination of smaller-sized test particles (1.6 µm and 1.9 µm) from the lower portion of the bronchial tree. The test particles were markedly larger than cigarette smoke particles, which ranged from 0.18 µm to 0.7 µm diameter. Pritchard and Black (1984) used $^{[123I]}$-iodohexadecane as a tracer in a similar study of smokers and also characterized lung elimination as biphasic. Duration of phases was similar for twenty-three smokers of high tar (16-17 mg) cigarettes and twenty-seven smokers of lower tar (8-9 mg) cigarettes. The first phase had a half-life of approximately two hours, and the second phase had a half-life of 18 hours.

Smoking status influences the rate of smoke particle elimination from the lungs. Albert and coworkers (1975) studied the short-term effects of cigarette smoking on the elimination of radioactive test aerosols containing particles ranging from 2.7 to 4.7 µm in size. Six smokers and nine nonsmokers inhaled radioactive ($^{198}$Au or $^{99m}$Tc) aerosol for one minute at a rate of fourteen breaths per minute and a volume of approximately one liter per breath. After an interval of several hours, the subjects repeated this process with a different radioactive ($^{198}$Au or $^{99m}$Tc) aerosol. Then, all subjects smoked from two to five cigarettes, inhaling once every thirty seconds, with approximately a ten-minute interval between cigarettes. Cigarette smoking decreased the time required for 90% bronchial elimination by approximately 30% (an accelerated rate of elimination from 292 to 201 minutes for nonsmokers and from 257 to 173 minutes for smokers). Albert and coworkers (1975) speculated that an increased rate of mucous elimination from distal ciliated airways during the test smoking session may have accelerated the rate of bronchial elimination, possibly related to the effect of cigarette smoke on mucous production and ciliary elimination.

The location of deposition in the respiratory tract might contribute to elimination rate. Ilowite and coworkers (1989) pooled data from earlier studies of mucociliary elimination using $[^{99m}$TC$]$sebacate, $[^{99m}$TC$]$iron oxide and $[^{99m}$TC$]$albumin to determine inter-subject variation from activity time plots and the ratio of central-to-peripheral airway counts. The elimination of deposited aerosol was faster, the more central the location of the deposit (larger ratio). The coefficients of variation for particle retention at 120 minutes were 31%, 30% and 18% for sebacate, iron oxide and albumin, respectively.
Disease status influences the elimination of particles. Moller and coworkers (2001) measured the elimination of ferromagnetic tracer particles (1.35 µm diameter) by magnetopneumography for 300 days after controlled voluntary inhalation in 39 healthy adults and 42 patients. They described three distinct, sequential phases of elimination. In phase one, patients with chronic obstructive bronchitis rapidly cleared bronchial areas within 48 hours. This phase did not necessarily occur in healthy young nonsmokers. In phase two, particles redistributed but did not move from the lung during a translocation period lasting up to six weeks. In phase three, the remaining particles slowly cleared from the alveolar space. Nonsmokers with sarcoidosis (n=10) or idiopathic pulmonary fibrosis (n=5) had significantly longer alveolar elimination times than nonsmokers who were healthy (Moller et al., 2001). The half-life of alveolar elimination for healthy nonsmokers was 124±66 days. Alveolar elimination increased by 5.7±1.3 days per pack-year (p<0.01) in cigarette smokers. In addition to smoking status and disease, age influenced particle elimination. Alveolar elimination was faster for smokers younger than 40 years of age (n=13) 220±74 days and slower for smokers who were older 459±334 days (n=9).

Albert and coworkers (1971) also studied whether smoking and disease might contribute to variation in the elimination of radioactive aerosols from the lungs. They calculated the weighted average elimination, representative of the average residence time for particles deposited in, and cleared from, the bronchial tree, by dividing the area under the elimination curve, minus the residual activity remaining in the lung, by the total amount of bronchial elimination (Albert et al., 1971). Total elimination times averaged 126±14 minutes for the healthy nonsmokers (n=18), 170±18 minutes for the healthy smokers (n=19) and 238±33 minutes for patients with bronchitis (n=6), significantly longer than for nonsmokers. Several smokers with bronchial deposition levels of 90 to 100% had pronounced delays in the initiation of elimination. Patients with bronchitis had delays in elimination of up to five hours.

Coggins and coworkers (1980) showed that rats exposed for twelve weeks to whole cigarette smoke had marked loss of cilia at tracheal bifurcations compared to control rats (Figure 4.7a & Figure 4.7b). Based mainly on these data, Martonen (1992) speculated that dedicated bifurcation zones could slow mucociliary transport in response to cigarette smoke particles.
Figure 4.7a Ciliated epithelium at a tracheal bifurcation in an unexposed rat. SEM x 1044. Reprinted with permission from (Coggins et al., 1980).

Figure 4.7b Deciliation at a tracheal bifurcation in a rat exposed to cigarette smoke. SEM x 1044. Reprinted with permission from (Coggins et al., 1980).
4.3.5 Models of Smoke Dosimetry

In addition to the biological model of the rat, used by Coggins and coworkers, and others, investigators have employed isolated organs, physical models, and mathematical models of the human lung to understand the dosimetry of cigarette smoke. For example, Mitchell (1975) simulated normal breathing patterns in an intact excised human lung at room temperature within eight hours of death. By counting particles of diluted cigarette smoke inhaled and exhaled by the lung, he determined that deposition of particles increased as residence time of particles in the lung increased. Morawska and coworkers (1998) calculated that particle residence time in the human lung could vary from four seconds (one breath in and out) to approximately 24 seconds, assuming 3 L of alveolar gas exchanged at a rate of 7.5 L/min⁻¹. Phalen and coworkers (1994) reported no difference in deposition efficiency of sidestream cigarette smoke between different sized silicone models representing the tracheobronchial tree of young adults and children of four and seven years of age. They calculated deposition efficiency as the deposition in the cast model divided by the total amount measured in the cast, airways, and filters through which the smoke exited the model.

Aerosol deposition models first were developed to aid in establishing exposure limits for radioactive aerosols (Phalen & Oldham, 2001). To characterize the deposition of cigarette smoke following inhalation, investigators must define attributes of the aerosol, the morphology of the respiratory system, and ventilatory conditions (Martonen et al., 2000). Martonen (2001) advised that models of particle deposition include the naso-oral cavity for four reasons: (1) Humans are naso-oral breathers (unlike laboratory rodents). (2) The naso-oro-pharynx compartment filters inspired air. (3) The ratio of nasal to oral airflow may affect the aerosol mass delivered to deeper airways. (4) The ability to heat and humidify inspired air varies between the nose and mouth.

Yeh and Schum (1980) developed a model of human tracheobronchial airways based on detailed morphometric measurements. A silicone rubber replica cast was made by injecting liquid rubber into the lung of a deceased man who died of a myocardial infarction and whose lungs showed no abnormalities. After the silicone had cured in situ, the lung tissue was removed. Detailed measurements were obtained of the airway diameters, lengths, angles of branches and inclination to gravity. Using a similar approach to the Findeisen-Landahl computational method, their lung model predicted the deposition of airborne particles in different regions of the lung during different breathing patterns. They also wrote a computer program using geometrical data from the cast and estimated inhaled particle deposition for portions of the lung (i.e., lobe, or whole lung).

The National Council on Radiation Protection and Measurements (NCRP) developed a model in 1997 to predict dosimetry of particles inhaled by radiation workers and
the general public, based on the model of Yeh and Schum (1980), but modified to include more recent data (Yeh et al., 1996). Deposition predictions agreed reasonably well with data from nose breathing and mouth breathing (1997). Yu and Diu (1982) compared the Yeh and Schum model (1980) with models developed by Weibel (1963), Olson and coworkers (1970), and Hansen and Ampaya (1975). They determined that total deposition curves for nose breathing and mouth breathing did not differ substantially among these models and also compared reasonably well with other experimental data. For particles of 0.2 µm in diameter, they obtained agreement among models for regional deposition fractions for both nose breathing and mouth breathing.

The NCRP suggested that predictions from the Yeh and Schum model resembled predictions of the 1971 model developed by Horsfield and coworkers (1971). The NCRP model (Yeh et al., 1996) predicts slightly lower nasopharyngeal deposition for particles with diameters < 1-2 µm than a 1994 model by the International Commission of Radiological Protection (ICRP), which was based on a model by Egan and coworkers (National Council on Radiation Protection and Measurements, 1997). Table 4-4 compares the 1997 NCRP model to earlier models. Additional background information is available about methods to assess particle deposition in the respiratory tract (Schultz et al., 2000).

Table 4-4. Percentage of inhaled particles (diameter 0.2 µm) predicted to deposit in human lung regions by three models.

<table>
<thead>
<tr>
<th>DEPOSITION (%)</th>
<th>ICRP&lt;sup&gt;a&lt;/sup&gt; 1966</th>
<th>Yeh and Schum&lt;sup&gt;b&lt;/sup&gt; 1980</th>
<th>NCRP&lt;sup&gt;c&lt;/sup&gt; 1997</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naso-pharyngeal</td>
<td>0.0</td>
<td>0.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Tracheobronchial</td>
<td>2.7</td>
<td>3.3</td>
<td>4.9</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>28.1</td>
<td>14.8</td>
<td>16.2</td>
</tr>
<tr>
<td>Total</td>
<td>30.8</td>
<td>18.1</td>
<td>22.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> ICRP: International Commission on Radiological Protection (1966) using 750 cm³ tidal volume and 15 breaths per minute.

<sup>b</sup> Yeh and Schum (1980) using 750 cm³ tidal volume, 15 breaths per minute, and a functional residual capacity of 40% total lung capacity.

<sup>c</sup> NCRP: National Council on Radiation Protection and Measurements (1997) using 770 cm³ tidal volume, 13 breaths per minute, and a functional residual capacity of 40% total lung capacity.

Biomarkers of chemical substances in cigarette smoke intrinsically measure neither exposure nor dose. However, measures of biomarkers easily convert into exposure estimates, through calculating the exposures that should have led to those biomarker levels (Scott et al., 2001). Most studies of constituents of cigarette smoke in smokers measure substances in blood, urine, or expired air. Some typical substances

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measured include thiocyanate, nicotine, cotinine, carbon monoxide and benzene, or metabolites of nicotine, carbon monoxide and benzene, in blood or urine. Smokers have higher internal concentrations of these substances than nonsmokers (Table 4-5) (Jarvis et al., 1984).

### Table 4-5. Comparison of exposure biomarkers in nonsmokers and active smokers.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>NONSMOKERS (n=100)</th>
<th>SMOKERS (n=94)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon Monoxide</td>
<td>5.6</td>
<td>21</td>
</tr>
<tr>
<td>(ppm in expired air)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carboxyhemoglobin</td>
<td>0.9</td>
<td>4</td>
</tr>
<tr>
<td>(% in whole blood)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicotine (mg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>0.9</td>
<td>15</td>
</tr>
<tr>
<td>Saliva</td>
<td>4.8</td>
<td>673</td>
</tr>
<tr>
<td>Urine</td>
<td>8.3</td>
<td>1750</td>
</tr>
<tr>
<td>Cotinine (ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>1.5</td>
<td>275</td>
</tr>
<tr>
<td>Saliva</td>
<td>1.7</td>
<td>310</td>
</tr>
<tr>
<td>Urine</td>
<td>5</td>
<td>1391</td>
</tr>
<tr>
<td>Thiocyanate (µmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>50.8</td>
<td>123</td>
</tr>
<tr>
<td>Saliva</td>
<td>1300</td>
<td>2450</td>
</tr>
<tr>
<td>Urine</td>
<td>74.8</td>
<td>155</td>
</tr>
</tbody>
</table>

\(^a\) Modified from Jarvis et al. (1984).

### 4.4 BIOLOGICAL MONITORING OF SMOKING EXPOSURE

#### 4.4.1 Nicotine

Nicotine in cigarette tobacco transfers intact during smoking (Schmeltz et al., 1979). The boiling point for nicotine is 247°C. Thus, nicotine distills into smoke in advance of the burning cone, before it can undergo pyrolysis (Darby et al., 1984). The particulate phase of smoke contains most of the nicotine in smoke (Houseman, 1973; Jenkins, Jr. et al., 1971; Jenkins, Jr., 1990). Nicotine is a useful model for substances that transfer in smoke. Nicotine also is a tracer for the particulate fraction of cigarette smoke.

Tobacco contains approximately 1.5% nicotine by weight and a cigarette contains approximately 0.8 to 1.0 g of tobacco. So, cigarettes have approximately 12-15 mg of nicotine each. Tobacco contains several alkaloids, including nicotine, nornicotine, anabasine, myosmene, nicotyrine and anatabine. The alkaloids other than nicotine usually constitute approximately 10% of the alkaloid content (Piade
& Hoffmann, 1980). However, some varieties of tobacco have more nornicotine than nicotine (Schmeltz & Hoffmann, 1977).

The pKₐ of nicotine is 8.0. At the pH of blood (approximately 7.4), nicotine is 31% unionized. Smoke from flue-cured tobaccos is acidic, so little nicotine absorbs in the mouth (Gori et al., 1986). However, most of the nicotine in smoke absorbs rapidly in the human lung. Skin also absorbs nicotine base well. Nicotine absorbs poorly in the stomach but well from the small intestine. Blood concentrations peak approximately 1 hour after the oral administration of nicotine in capsules (Jenner et al., 1973).

The pharmacokinetics of nicotine absorbed from cigarette smoke has been reviewed (Benowitz et al., 1990). Peak blood concentrations of nicotine range between 10 and 50 ng/ml. Benowitz and Jacob (1984) found an approximately 40 mg daily intake of nicotine per smoker, or 2 mg per cigarette for a one pack a day smoker, approximately one-half of the value obtained by Feyeraband (1985). In a study with deuterium labeled nicotine, Benowitz, et al. (1991) found that smokers inhaled a dose of 2.3 mg of nicotine. However, these investigators suggested that a prestudy period of abstinence increased subjects’ inhalation and that a lower dose would be more appropriate.

Blood or urine nicotine levels are poor indicators of nicotine absorption (Benowitz et al., 1982). Little nicotine binds to plasma proteins. The initial half-life of elimination is approximately 2 hours and the volume of distribution is approximately 180 liters (Benowitz & Jacob, III, 1994). However, nicotine elimination is complex, and it changes with blood flow and smoking (Benowitz et al., 1990). Total clearance was approximately 1300 ml/min. Kidney, liver, heart, and brain had the highest concentrations of nicotine in rabbits and adipose tissue the lowest, after 24-hour intravenous infusions. In rats, kidney, liver, and brain had the highest concentrations of nicotine and adipose tissue the lowest (Benowitz et al., 1990). The pharmacokinetics of nicotine after smoking resembles the pharmacokinetics after rapid intravenous administration. At normal urinary pH, little nicotine reabsorbs (Borzelleca & Lowenthal, 1967).

In the dog, the liver metabolizes most absorbed nicotine, and most nicotine undergoes metabolism (Turner et al., 1975). Human renal elimination of unmetabolized nicotine accounts for only 5-10% of total absorbed dose. Urinary nicotine concentrations correlate poorly with nicotine intake. Human liver extracts most nicotine and metabolizes it to cotinine and nicotine-N-oxide in a ratio of the two metabolites of approximately 17:1. (Mice have a ratio of approximately 1:1, and rats approximately 1:2, which may make these common laboratory animals poor models for some purposes.)
Armitage and coworkers (1974; 1975) used cigarettes labeled with \([^{14}C]\) nicotine to show that smokers retain as little as 29% or as much as 92% of the nicotine in mainstream smoke, perhaps depending on how deeply they inhaled.

By subtracting the nicotine in exhaled air from the amount in mainstream smoke, obtained by duplicating the subjects’ puffing dynamics with a smoking machine, Frost and coworkers (1998) calculated 35% and 40% deposition of nicotine in the mouth after zero and ten seconds, respectively. Deposition of nicotine increased to more than 80% of smoke nicotine after a shallow inhalation of 70 mL and to 90% and 100% after typical inhalation volumes of 500 and 700 mL.

Nicotine concentrations in body fluids and tissues are relatively specific for exposure to tobacco. Some foods, such as tomato, cauliflower and tea contain nicotine. Of these, the highest concentrations of nicotine were measured in instant tea (up to 285 ng/g wet weight) (Davis et al., 1991). However, the influences of diet on biological measures of nicotine metabolites are negligible (Davis et al., 1991).

Because smoking involves multiple exposures to nicotine, nicotine accumulates in the body leading to persistent levels of blood nicotine for 24 hours each day. Measures of peak arterial nicotine concentration ranged from 8 to 93 ng/mL in smokers (Armitage et al., 1975; Gourlay & Benowitz, 1997; Henningfield et al., 1993). Blood concentrations of nicotine related to the volume of the puffs inhaled. In a review of the literature, Schneider and coworkers (2001) reported a low value for plasma concentrations of nicotine in cigarette smokers of 10-37 ng/L and a peak value of 19-50 ng/mL, indicating wide variation. Varying cigarette smoke puff volume from 15 to 60 mL influenced the concentration of plasma nicotine. Nicotine concentration increased as puff volume increased (Zacny et al., 1987). In contrast, plasma nicotine was not influenced by varying inhalation volume from 20% to 60% of vital capacity or by holding the breath from 5 to 21 seconds, suggesting that the inhaled cigarette smoke puff volume is the most important contributor to plasma nicotine concentrations.

The two major metabolites of nicotine are nicotine-N-oxide and cotinine. (For cotinine, see section 4.4.2 below). Both metabolites undergo additional metabolism. Unmetabolized, renal elimination of nicotine accounts for approximately 9% of an absorbed human dose, the nicotine-N-oxide metabolite accounts for approximately 4%, and 17% of absorbed nicotine has an unknown elimination pathway (Benowitz et al., 1990).
4.4.2 Cotinine

Human liver converts approximately 70% of nicotine to cotinine, and cotinine undergoes extensive metabolism. Little cotinine binds to plasma proteins. Despite extensive tubular reabsorption, renal elimination of unmetabolized cotinine accounts for approximately 17% of the total produced. The elimination half-life of cotinine is approximately eighteen hours and the volume of distribution is approximately ninety liters. Urinary flow and pH have little influence on urinary cotinine elimination, and urinary cotinine concentrations correlate well with cotinine production. These characteristics make cotinine a good biomarker of nicotine exposure over intermediate, and day-long periods of exposure (Galeazzi et al., 1985).

Cotinine concentrations in saliva and plasma correlate with cigarette smoke uptake in both active and passive smokers (Jarvis et al., 1984). Curvall and coworkers (1990) confirmed these correlations in two experiments that monitored the increase in cotinine in plasma, saliva and urine as indices of nicotine exposure. The plasma and salivary concentrations of cotinine demonstrated a positive linear association with the quantity of nicotine infused, $r \geq 0.97$. Individual urinary cotinine excretions, which did not reach a plateau level within five hours of a one-hour infusion, did not correlate well with nicotine dose. However, Andersson and coworkers (1997) found significant differences ($p < 0.01$) between salivary cotinine concentrations and cigarette consumption by Swedish smokers. For smokers consuming 11(±2), 18(±2) and 28(±6) cigarettes per day and receiving an estimated 11(±3), 19(±3) and 30(±4) mg nicotine per day, salivary cotinine concentrations were 174(±86), 238(±99) and 330(±105) ng/mL, respectively.

The National Health and Nutrition Examination Survey (NHANES) measured the levels of cotinine in the urines of nonsmokers representative of the U.S. population. Between 1988 and 1991, the median level was 0.20 ng/mL (Pirkle et al., 1996). By 1999-2000 the level decreased to 0.059 ng/mL. Smokers defined within the study as current smokers of greater than five cigarettes per day have urine cotinine levels of at least 10 ng/mL.

4.4.3 Carbon Monoxide


Because of carbon monoxide’s relatively short half-life, Benowitz (2001) suggested that measurements of expired carbon monoxide reflected only recent exposure (several hours). Physical activity affects the rate of carbon monoxide elimination.
Wald & Howard (1975) calculated, based on the mathematical modeling of data gathered by Coburn and coworkers (1965), that the half-life of carbon monoxide varies from four hours during sedentary activity to approximately one hour during vigorous physical activity. Carbon monoxide is not specific to cigarette smoke. Other sources could influence exposure, including air pollution. Humans produce small amounts of carbon monoxide endogenously, accounting for carbon monoxide normally found in exhaled air and blood. An advantage of using expired carbon monoxide as a biomarker of cigarette exposure is that the method is automated. Results are immediately available (Secker-Walker et al., 1997).

Bentrovato and coworkers (1995) measured the expired air of thirteen adult smokers, immediately prior to their 5th through 9th cigarettes of the day, for four consecutive days. The carbon monoxide concentrations of their expired air varied from 6 ppm to 44 ppm (mean 25 ppm) and correlated poorly ($R^2 = 0.44$) with the yields of carbon monoxide per cigarette as measured by a smoking machine programmed to duplicate the human smoking profiles (Bentrovato et al., 1995). Thus, oral delivery of carbon monoxide does not predict uptake from smoke. Secker-Walker et al. (1997) examined the association between self-reported cigarette consumption and expired carbon monoxide in pregnant women. Data were collected from 521 women at their first visit to a prenatal clinic for underserved women. For the second visit at 36 weeks, 365 of the women returned. The correlations between cigarette consumption (number of cigarettes per day) and expired carbon monoxide are 0.65 for the first visit and 0.70 for the second ($p < 0.001$ for both). Self-reports suggested that the participants provided crude estimates of cigarette consumption. Varying puff volume from 15 to 60 mL influenced the concentration of carbon monoxide in expired air, which increased with increasing puff volume (Zacny et al., 1987).

The carbon monoxide concentration of exhaled air is closely related to the carbon monoxide saturation of the hemoglobin. Carbon monoxide binds reversibly to hemoglobin to form carboxyhemoglobin in a tighter complex than the oxygen-hemoglobin complex. Therefore, carboxyhemoglobin compromises oxygen transport by hemoglobin.

Smokers have higher concentrations of carboxyhemoglobin than nonsmokers (Behera et al., 1991). A diurnal rhythm is evident in smokers, with higher concentrations during the day (Wald & Howard, 1975). The concentration of carboxyhemoglobin ranges from 3% in nonsmokers to 17% or higher in smokers (Behera et al., 1991). Under experimental conditions, inhalation of 7,000 to 24,000 ppm carbon monoxide by 18 healthy men for three to five minutes followed by exposure to 232 ppm carbon monoxide for up to 130 minutes, achieved carboxyhemoglobin levels of 16% to 23% with no significant oxygen deprivation symptoms (Benignus et al., 1987). In contrast, accidental exposure to approximately
500 ppm of carbon monoxide for 150 minutes or less resulted in complaints of illness from 160 of 184 exposed persons, whose carboxyhemoglobin levels were 30% or less (Burney et al., 1982). Increasing exposures result in symptoms of headache, dizziness, muscle weakness, visual problems, nausea, cardiovascular dysfunction, CNS derangement, coma and death (Burney et al., 1982; Crocker & Walker, 1985). For children less than 15 years of age, carboxyhemoglobin levels of 24% or higher are associated with syncope (Crocker & Walker, 1985). Although patients with serious carbon monoxide poisoning have carboxyhemoglobin values exceeding 25% (Sloan et al., 1989), carboxyhemoglobin concentration is not necessarily indicative of a prognosis (Burney et al., 1982; Raub et al., 2000).

Behera and coworkers (1991) measured blood carboxyhemoglobin in 58 healthy nonsmoking men, 25 asymptomatic male cigarette smokers and 20 symptomatic male smokers having persistent episodes of coughing for more than one month. Blood samples were drawn from smokers exactly 10 minutes after the subject finished smoking his own cigarette in his usual manner. Carboxyhemoglobin levels were 3.72 ± 1.15% for nonsmokers, 15.33 ± 1.98% for smokers, and 17.24 ± 3.41% for symptomatic smokers. For smokers, the self-assessed depth of inhalation was directly proportional to the blood carboxyhemoglobin level (p < 0.05). Because of its short half-life, measures of carboxy-hemoglobin reflected recent exposure, similar to measures of carbon monoxide in expired breath.

4.4.4 Other Substances

Cyanide occurs in the vapor phase of cigarette smoke, as a combustion product of tobacco, humans metabolize it primarily to thiocyanate. Thus, blood thiocyanate is a biomarker of cigarette smoke exposure. Blood thiocyanate concentrations positively correlated with the number of cigarettes smoked per day when expressed relative to ideal body weight (Gardner et al., 1984). Longitudinal studies tracking changes in repeated measures of blood thiocyanate for one individual are supportive of periods of smoking cessation and/or resumption of smoking (Pettigrew & Fell, 1972).

Blood thiocyanate concentration may not be useful for verifying whether a person is a smoker or nonsmoker, because of a considerable overlap between the groups. Cyanides are also found in common foods, such as leafy vegetables and nuts, which contribute to the high variability of serum thiocyanate concentrations among individuals (Borgers & Junge, 1979; Pettigrew & Fell, 1972; World Health Organization, 1986).

Concentrations of benzene in the homes of smokers exceed benzene concentrations in the homes of nonsmokers. The average overnight air concentration of benzene in homes with one or more smokers increased two-fold or more (Wallace et al., 1987). Higher indoor benzene concentrations in homes with smokers are consistent
with the hypothesis that cigarette smoke represents a source (Wallace et al., 1987). Jo and Pack (2000) recently used breath analysis to estimate exposure to benzene associated with active smoking involving five different brands of cigarettes, two each from Korea and the U.S. and one from Japan (Table 4-6). The five subjects ranged from 22 to 24 years old. The average breath concentration of benzene before smoking was 17.6 µg/m³ (range 15.9 to 19.2 µg/m³) and one minute after smoking was 69.9 µg/m³ (range 58.1 to 81.30 µg/m³). Thus, breath after smoking was three to five times higher in benzene, depending on the brand, than before smoking.

**Table 4-6. Breath concentrations (µg/m³) of selected smoke constituents by smokers (S) and nonsmokers (NS) [Data given as unweighted geometric averages]∗**

<table>
<thead>
<tr>
<th>Location</th>
<th>Season</th>
<th>n</th>
<th>Benzene</th>
<th>Ethyl-benzene</th>
<th>Styrene</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Jersey</td>
<td>Fall</td>
<td>150</td>
<td>188</td>
<td>21</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>66</td>
<td>76</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>26</td>
<td>23</td>
<td>-</td>
<td>2.3</td>
</tr>
<tr>
<td>California, Los Angeles</td>
<td>Winter</td>
<td>29</td>
<td>85</td>
<td>15</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>11</td>
<td>40</td>
<td>14</td>
<td>1.6</td>
</tr>
<tr>
<td>California, Antioch/Pittsburg</td>
<td>Spring</td>
<td>19</td>
<td>49</td>
<td>14</td>
<td>0.8</td>
</tr>
</tbody>
</table>

a Modified from Wallace et al. (1987). All comparisons of concentrations between smokers’ and nonsmokers’ geometric means differed significantly (P < 0.001). Reprinted with permission of Heldref Publications.
b Measurements for benzene may have been elevated due to permeation of exhaust fumes into van-mounted spirometer.
c S = smokers; NS = nonsmokers.
d Measurements during this visit may have been elevated due to permeation of exhaust fumes into van-mounted spirometer.
e Not reported.

Estimates suggest that the most important source of benzene exposure for smokers is their cigarette consumption (1989). Benzene concentrations in the exhaled breath of smokers were six- to seven-fold higher than for nonsmokers (Wallace et al., 1987). Smokers’ breath styrene concentrations were four-fold higher, and ethylbenzene concentrations were three-fold higher, compared with nonsmokers (Table 4-6). Breath concentrations of benzene correlated with the number of cigarettes smoked. As shown in Figure 4.8, the benzene concentrations varied from 9.4 ug/m³ for nonsmokers to 30 µg/m³ for smokers of 40 cigarettes on the day of measurement. For heavy smokers (>50 cigarettes per day) breath benzene was 47 µg/m³. Breath analysis of benzene also generated estimates of absorption efficiency, biological half-life, and exposure to passive smoke. Individuals smoking 33 cigarettes per day inhale approximately 2.0 mg per day of benzene compared to a nonsmoker, who inhales approximately 0.2 mg per day.
4.5 EXPERIMENTAL ANIMAL EXPOSURE TO CIGARETTE SMOKE

4.5.1 Methods for Experimental Animal Exposure to Cigarette Smoke

Thomas and Vigerstad (1989) cite many early reports of exposing animals to whole cigarette smoke, in contrast to fractions of the smoke. Historically, investigators preferred rodents, including rats, mice, hamsters and guinea pigs (Coggins, 1998; Witschi et al., 2000) (Wright & Churg, 2002a; Yamato et al., 1996). Under similar experimental exposure conditions, different animal species responded differently to cigarette smoke (Fishbein et al., 2000). Studies of exposure of animals to cigarette smoke have not replicated the diseases observed in human smokers in epidemiological surveys (Coggins, 2001).
Phalen and coworkers (1984) reviewed four methods of inhalation exposure for animal experiments, whole body, head only, nose or mouth only, and lung by intubation or tracheostomy. A common problem with voluntary breathing methods is that animals prefer to breathe air and reject cigarette smoke. They hold their breath and/or cough, when exposed to smoke (Battista et al., 1973). Whole body exposure contaminates the animals’ skin, fur and eyes with smoke (Kuller et al., 1989). The animals ingest these contaminants during grooming, making the exposure pathway different and dosimetry difficult.

Whole body cigarette smoke exposure systems modified from those developed by Escolar and coworkers (Escolar et al., 1995) (Figure 4.9) were used in murine models of acute pulmonary oxidative stress and chronic emphysema (Cavarra et al., 2001b; Cavarra et al., 2001a). Whole body cigarette smoke exposure systems have also been developed for use in murine models of smoke-induced lung tumors from machine-generated sidestream smoke (D’Agostini et al., 2001; Teague et al., 1994; Witschi, 2000b). In whole body studies, urinary cotinine may not be a meaningful indicator of smoke exposure, because oral ingestion during grooming contributes to nicotine exposure and metabolite level (Vanscheeuwijk et al., 2002).
The head only method of exposure has the advantages of minimal skin contamination, uniform individual exposure, conservation of smoke, and difficulty in avoiding inhalation. However, restraint adds stress to the design. The most popular method for delivering cigarette smoke to experimental rodents is by nose only exposure. Rodents are obligate nose breathers. Major advantages are the reduction of skin contamination (compared to whole body exposure) and the ability to pulse smoke exposure at rates similar to human puff patterns. The smoke is also easier to contain and less smoke is used than the whole body or head only methods (Battista et al., 1973; Phalen et al., 1984). Disadvantages include technical difficulties in sealing the face/nose, and labor intensiveness (Phalen et al., 1984).

Matulionis (1984) tested four early models of nose only exposure systems designed for use with rats, mice or hamsters. For the same species, responses varied between the different exposure systems. Nose only systems lend themselves to measuring smoke concentrations and processing smoke before exposure, including cooling and dilution of smoke with air (Baumgartner & Coggins, 1980).

Animal models present an opportunity to control experimental exposure variables, such as smoke concentration and duration. However, to date no animal model has replicated the major features of human smoking-related diseases. In addition, exposure of animals to whole cigarette smoke has a major, perhaps insurmountable problem, carbon monoxide poisoning.

4.5.2 Carbon Monoxide Pharmacokinetics in Animal Species

Like canaries in coal mines, small mammals like mice and rats have higher metabolic rates in relation to their body size than humans. Studies exposing rats to diluted cigarette smoke for 2 hours a day over 2 weeks reported COHb levels reaching 20 to 40% placing considerable stress on the rat (Carmines et al., 2003). They also reported the need to interrupt the 2 hour exposure with a 30 minute smoke free period to allow COHb dissociation due to concerns of potential CO related mortality in the rodent. Smaller animal species, such as rodents are considerably more sensitive to CO levels than humans. For human sensitivity to carbon monoxide, see the preceding Section 4.4.3. No lasting and important functional effects occur at exposures that generate carboxyhemoglobin up to 17%. Exposure-response relationships for carbon monoxide and various biological effects have been reviewed for other species (Benignus et al., 1990).

Efforts to understand the toxicology, physiology, and pathology of acute carbon monoxide poisoning have proceeded for more than a century (Penney, 1990). Even so, whether the effects of carbon monoxide result from hypoxia secondary to carboxyhemoglobinemia or direct entry into critical organs and cells, where it binds key components, remains unknown (Winneke, 1992). Further, the overall literature about the exposure-response relationship between carboxyhemoglobin and carbon...
monoxide effects, including human behaviors, is inconsistent (Benignus et al., 1990). Across many species, the exposure-response relationships are nonlinear. Besides mortality when carboxyhemoglobin levels exceed 20%, effects on the cardiopulmonary system, metabolism, and the central nervous system are most prominent. Short of mortality, the most exposure sensitive morphological indicator is damage to the white matter in the central nervous system. Carbon monoxide induces acidosis and a prominent hypotension, which compromises blood flow and worsens the acidosis.

4.5.3 Absence of Animal Models of Human Health Effects

The notion of biological continuity between species forms the basis of any effort to obtain an animal model or to extrapolate results between species (Winneke, 1992). However, the idea that animal models, particularly chronic bioassays in rats and mice, predict the human health effects from smoking represents confusion between regulatory policy needs and scientific needs for animal models. Regulatory policy is based on the notion that carcinogenic results in animal models can be directly applied to humans. This policy forms an inadequate basis for scientific research, which requires a replication of the modeled phenomenon in all essential aspects, to achieve predictivity. Otherwise, research findings in an animal model cannot provide useful scientific information (Bukowski et al., 2001).

Some studies have implicated isolated substances in lung cancer induction based on their presence in cigarette smoke and their pulmonary carcinogenicity in lab animals (Hecht, 1999). It is implied that studies of animal exposures to whole or fractionated cigarette smoke are predictive of the disease observed in human smokers. A statement that a particular substance, typically a polycyclic aromatic hydrocarbon or tobacco-specific nitrosamine, causes lung cancer in laboratory animals provides important information when put in an appropriate context. The quantitative presence of the substance in cigarette smoke should be related to the carcinogenic potency of whole cigarette smoke, or, should qualitatively explain disease properties. In reality, potencies of the identified carcinogens in cigarette smoke sum to a small portion of the quantitative potency of smoking (Fowles et al., 2000; Menzie Cura & Associates, 1999). No single or group of chemicals in smoke has been identified which can explain the full range of human health effects observed in epidemiological studies of cigarette smokers.

Studies of exposure of animals to cigarette smoke have not replicated the range of diseases observed in epidemiological surveys of human smokers (Coggins, 2001). Most studies of the effects of cigarette smoke in animal models have concentrated on carcinogenic effects. One example is the Witschi model developed to study the induction of pulmonary adenomas by cigarette smoke (Witschi et al., 1997b; Witschi, 1998). Witschi et al. (1997b; 1999; 2000; 2002a) developed a smoke exposure procedure using the A/J strain mouse model to screen potential chemopreventive
agents. The authors subjected strain A/J mice for five months through whole-
body exposure to a mixture of mainstream and side stream smokes, better replicating
human smoking conditions (Witschi et al., 1997b). After a recovery period of
exposure to room air for four months he found excess pulmonary adenomas. Later,
Witschi exposed female A/J mice to unfiltered or HEPA-filtered smoke and found
that the volatile or gas phase of smoke caused as many tumors as full smoke
(Witschi et al., 1997a; Witschi, 1998).

An increase in adenomas in mice is not a compelling finding. Cigarette smoke
apparently accelerates the appearance of a mouse tumor that usually occurs at a
high incidence in strain A/J. However, pulmonary adenomas have acquired new
prominence in studies of the epidemiology of cigarette smoking. The proportion of
lung adenocarcinomas to squamous cell carcinomas of the lung has recently
increased among cigarette smokers. Some investigators link this trend to an
increased use of cigarettes with filters (Hoffmann et al., 1996). The argument is
two-fold: (1) Lower nicotine yields increase smoking intensity, leading to more
smoke deep in the peripheral lung, a site more prone to develop adenocarcinomas.
(2) Smoke from filtered cigarettes produces more of a lung-specific carcinogen, 4-
(N’-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone) (NNK).

4.6 SUMMARY

This chapter outlines the exposure, and some of the dosimetry, of human smoking.
The previous chapter covered the properties of cigarette smoke, and the following
chapter explains some of the adverse human health effects observed in
epidemiological studies. Thus, the three chapters form a logical progression. Other
than this linkage, the chapter should discourage sophisticated exposure or dose
measurements as a way of characterizing the relative risk of a cigarette with an
ingredient compared to an identical cigarette without the same ingredient. However,
simple measurements, such as smoking rates (the number of cigarettes smoked
per unit of time) or biomarkers of smoking exposure, given some smoking rate,
hold promise.

Dose and duration of exposure are the primary determinants of the mortality and
morbidity associated with cigarette smoking. The relationship between inhalation
exposure and dose is scientifically challenging. Exposure can either be calculated
or measured. Dose often is inaccessible, and direct measurement is not possible.
For a mixture with thousands of chemical components, the analysis of dose of
cigarette smoke is particularly daunting. However, biomarkers of exposure often
come closer to dose estimates. In addition, some information is available about the
human absorption, distribution, and elimination of whole smoke or the particulate
fraction of cigarette smoke. Smoking affects the condition of the body. Therefore,
exposure changes over time.
No two individuals smoke cigarettes in the same way. A smoker’s pattern of smoking differs while smoking the same cigarette. Smoking behavior is affected by yield of nicotine, tar, taste, irritation, and draw resistance. Smoking behavior influences smoke substance delivery to the respiratory tract. When cigarettes are altered through engineering design, smokers may compensate to obtain the same nicotine yield. Compensation appears to involve small increases in the number of cigarettes consumed and large changes in inhalation.

Monitoring of chemical substances to which an individual is exposed provides some information and may be achieved with biomarkers of smoke exposure, such as cotinine or carboxyhemoglobin. Experimental animal exposure has several disadvantages: (1) the animal models do not replicate the diseases that smokers have, (2) the animals die of carbon monoxide poisoning, when exposed to whole smoke in ways that mimic human exposure and (3) the animal’s life span limits the long term latency period necessary for production of some of the observed human health effects.
ADVERSE HUMAN HEALTH EFFECTS

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5.4 SUMMARY
ADVERSE HUMAN HEALTH EFFECTS

5.1 INTENT & PURPOSE

The feasibility of testing an ingredient added to cigarettes relates to the potential health effects of the ingredient, which could arise from any of three processes: (1) inhalation of the ingredient or its pyrolysis products, (2) changes the ingredient induces in the chemical and physical structure of the surrounding matrix of cigarette smoke, and (3) changes the ingredient induces in smoking behavior. Changes in smoking behavior could affect smokers’ health indirectly through, for example, the consumption of more cigarettes or deeper inhalation.

To exert direct health effects after inhalation, an ingredient would have to survive pyrolysis and either transfer intact in cigarette smoke or have a pyrolysis product that would transfer into smokers’ lungs. The presence of an ingredient could change the adverse outcomes of cigarette smoking, either by creating a new outcome, directly related to the presence of the ingredient or its pyrolysis products in the smoke, or by changing the known outcomes of smoking. This chapter summarizes the adverse human health effects of cigarette smoking.

For LSRO, one question remains preeminent. Will ingredients added to cigarettes in some way change the human health risks already associated with cigarette smoking? If so, how will testing allow detection of (and reduction in) the amounts of those ingredients? Many studies document the adverse health effects of cigarette smoking. Detailed descriptions and analyses of these studies fall beyond the scope of this report. Instead, this chapter refers readers elsewhere for more detailed information (National Institutes of Health, 1997; Wilson & Crouch, 2001).

LSRO finds that epidemiological information has provided almost all of the available information about the human health effects of cigarette smoking. In LSRO’s view, tests that predict the results of epidemiological studies of human cigarette smoking are unavailable. Animal models of human health effects are also unavailable. Even so, epidemiological studies also have limitations.

Epidemiology is a crude instrument to detect differences, like the differences expected between a population smoking cigarettes without an ingredient and a similar population smoking nearly identical cigarettes with the ingredient. Because the primary approach, comparison of populations depends on stochastic measures, statistical considerations limit the kinds of possible comparisons. Small differences
lie beyond practical means; they require unmanageably large populations. Studies that seek to measure outcomes that require long durations of observation also appear impractical; members of the study populations would migrate and/or change their smoking habits. Epidemiological data seldom, if ever, directly inform mechanism or causation, although they can disprove hypotheses. Instead, scientists require a surrounding context of information from different sources. Epidemiological studies generate associations.

Other reasons exist to conduct more kinds of studies than human epidemiological studies. For example, many screening toxicology studies are established parts of regulatory practice, essentially a form of conventional wisdom. Despite their lack of predictivity for human health effects of cigarette smoking, these tests are thought predictive of general health effects. If an ingredient added to a cigarette transfers into human lungs in smoke, as some will, screening to detect novel effects will come into play, using the standard methods of inhalation toxicology. Decisions to employ these methods will be matters of scientific judgment. LSRO can devise no science-based rationale to apply these methods in uniform ways to all ingredients added to cigarettes, nor should they be uniformly excluded.

### 5.2 Premature Mortality from Cigarette Smoking

Overall, chronic cigarette smoking causes premature mortality. Premature mortality means that, on average, a group of persons with a defined characteristic has a different, shorter average life span than persons without this characteristic (Cohen, 1991). For example, a population of smokers lives for fewer years than an otherwise similar population of nonsmokers. Data from many epidemiological studies supports the idea that chronic cigarette smokers have shorter life spans than nonsmokers (Doll et al., 1994; Doll & Peto, 1976; National Institutes of Health, 1997). No credible epidemiological studies contradict this conclusion. Premature mortality is most easily determined by taking otherwise similar persons (with similar incomes, body weights, etc.) of approximately the same age and dividing the death rate of the smokers by the death rate of nonsmokers. If this ratio exceeds 1.0 (100%), then subject to statistical qualifications, the smokers experienced premature mortality.

The best way to avoid the adverse health effects of cigarette smoking is never to begin smoking. However, once smokers begin, the second best health protective approach is to cease smoking as soon as possible. After smokers cease smoking, all-cause premature mortality begins to decline (Kuller et al., 1991). The rate and extent of this decline is influenced by a number of factors including the number of cigarettes smoked and the duration of smoking. Whether this decline depends on the age of cessation is unknown. Thus, smoking cessation remedies some, but not all, of the increased incidence of premature mortality. Chronic obstructive pulmonary

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disease (COPD) outcomes decline little, if at all, after smoking cessation (Davis & Novotny, 1989).

5.2.1 Communication of Premature Mortality

To understand the effects of an ingredient, LSRO relies most on a perspective of premature mortality. The most important objective is that the ingredient should not contribute to, or increase premature mortality experienced relative to smoking the same cigarette without the ingredient. The ingredient also should not contribute to, or increase, any of the diseases or causes of death associated with premature mortality from cigarette smoking, including cardiovascular diseases, COPD, and cancer. Evidence that the ingredient does not contribute to, or increase, one of these diseases is not sufficient. An ingredient might be shown hypothetically, to decrease carcinogenesis, but might increase one of the other diseases associated with premature mortality from cigarette smoking. Thus, a meaningful “no detectable change” in premature mortality has appeal in the context of ingredients added to cigarettes.

Risk assessors argue about the best way to communicate premature mortality. Expressions vary, particularly depending on the population and time of application. In this chapter, LSRO refers to the current U.S. population. Cigarette smoking would cause little premature mortality in a human population with a short life expectancy. As life expectancy of the U.S. population increased over the past century, medical authorities have eliminated some previously notable causes of death, mostly infectious diseases (Amler & Eddins, 1987). The list of most prominent preventable sources has changed.

Each year the Centers for Disease Control and Prevention (CDC) present a tabulation of nationwide death certificate reports (Hoyert et al., 2001). The National Center for Vital Statistics processes this information and reports total mortality in the United States, age-adjusted death rates, as well as enumerating life expectancy at birth and leading causes of death (Hoyert et al., 2001). This information provides the backbone for other calculations that rank the importance of all the risk factors for diseases.

The CDC follows guidelines set by the World Health Organization (WHO) that require member organizations to classify and code deaths using the International Statistical Classification of Diseases and Related Health Problems (ICD) (World Health Organization, 1992). WHO guidelines allow mortality statistics from all participating countries to be compared, by installing a uniform format for the reporting of cause of death on a death certificate in the form of an ICD code. The WHO continually revises the ICD guidance. Revisions can lead to some discrepancies in the data from year to year, because the definition of a disease may change or because the code for a disease may change. Thus, part of the CDC’s annual vital statistics report must address the similarity between mortality statistics from year to year, due to changes in the definitions of diseases.
The basic way to capture statistics about the cause of death is through death certificates (Hoyert et al., 2001). The issuer of a death certificate determines the disease or injury that lead to death, or the circumstances of the accident or violence which produced the fatal injury (National Center for Health Statistics, 1991). The CDC tabulates data from all death certificates and creates a rank-ordered list of causes of deaths (Hoyert et al., 2001). Age-adjusted death rates are also critical to the analysis of the data presented in the cause of death tables, because those rates correct for the effects of age. The age-adjusted data represent a more accurate way to indicate relative incidences of diseases and examine changes in incidence of death from specific diseases over time.

Mathematical formulae are applied to the vital statistics data to attribute mortality to risk factors (Centers for Disease Control and Prevention, 1993). This information is important, because the causes of all diseases are multifactorial. For example, to determine what number of heart disease deaths to attribute to smoking, the CDC uses a formula based on smoking prevalence and relative risks for heart disease that compares current and former smokers with never-smokers. The result is known as the Smoking-Attributable Mortality (SAM) (Centers for Disease Control and Prevention, 2002b). SAM is made up of three types of deaths: death from smoking-related diseases for adults aged 35 years or older, deaths from smoking-related diseases for infants and deaths from cigarette-related fires (Centers for Disease Control and Prevention, 1999).

The CDC uses data on the prevalence of current and former smokers (Centers for Disease Control and Prevention, 1997). By obtaining data from the Behavioral Risk Factor Surveillance System, combined with relative risk estimates from the American Cancer Societies Cancer Prevention Study II (1982-86), the number of deaths from smoking-related diseases from Vital Statistics Data, the number of deaths associated with cigarettes from the National Fire Protection Association and population estimates from the U.S. Census, the CDC can prepare estimates of the number of deaths caused by smoking for each state. This information is part of a national database called the Smoking-Attributable Mortality, Morbidity and Economic Costs database (SAMMEC) (Centers for Disease Control and Prevention, 1999).

Cohen (1991) suggested that the traditional measure of risk assessors, events per million persons (or events per million persons per year) may generally distort comparisons between risks. He developed loss of life expectancy (LLE) as an alternative expression instead. For cigarette smoking, he estimated the risk of smoking one to two packs of cigarettes per day over a lifetime for men at approximately -6.8 years lost life expectancy per smoker (Cohen & Lee, 1979). A more recent calculation independently supported this estimate: approximately -6.6 years LLE for one or more packs per day per lifetime male smoker (Lew & Garfinkel, 1987). A similar government-approved statistic is person years of life
lost (YPLL). Calculations of YPLL show how many productive life years, premature mortality takes. The CDC estimates YPLL due to smoking, by multiplying age- and sex-specific smoking attributable mortality (SAM) by remaining life expectancy. YPLL figures for the 1995-1999 period indicate that SAM translated into a loss of 3,332,272 YPLL for men and 2,284,113 YPLL for women (Centers for Disease Control and Prevention, 2002a). These figures include smoking-attributable fire deaths but exclude any deaths due to second-hand smoke.

The precision or usefulness of the LLE, or YPLL, may be debated. The method used by Federal agencies to calculate premature mortality, inevitably generates some double counting. Smoking related premature mortality is currently set in excess of 400,000 deaths per year in the U.S., or an annual rate of approximately 1.4 X 10^-3 (Centers for Disease Control and Prevention, 2002a), others disagree and set the death toll lower at approximately 250,000 deaths per year, for an annual rate of approximately 9 X 10^-4 (Choi & Pak, 1994; Siegel et al., 1994; Sterling et al., 1993). Whatever the most precise estimate, the seeming avoidable and self-inflicted nature of these deaths and the associated morbidity makes them nearly intolerable to the public health community.

5.2.2 Changes in Cigarette Related Mortality

Two large-scale U.S. prospective studies, Cancer Prevention Study-I (CPS-I) and Cancer Prevention Study-II (CPS-II), conducted under the auspices of the American Cancer Society (ACS), examined death certificates of white middle-class smokers and nonsmokers (Thun et al., 1995; Thun et al., 1997). Between the 1960s to the 1980s, when ACS conducted the two studies, overall premature mortality doubled among women and continued approximately the same among men. However, the diseases contributing to mortality changed. Lung cancer surpassed coronary heart disease as the major cause of death in the second study (Thun et al., 1997). Thus, the exact magnitude of premature mortality, and the precise magnitude of the major contributions to it, varied from study to study, even when the studies were conducted under analogous study conditions. However, the conclusion that cigarette smoking causes premature death is consistent over all epidemiological studies.

In addition, causes of death can change from study to study. For example, from CPS-I to CPS-II, an increase in smoking-related deaths from cancers and COPD more than offset a decrease in deaths from vascular diseases in male smokers of all ages (Thun et al., 1995). Premature mortality of male smokers increased from 1.7 to 2.3 between CPS-I and CPS-II (Thun et al., 1995). Table 5-1 gives a summary of smoking related mortality taken from Smoking and Tobacco Control Monograph No. 8 (National Institutes of Health, 1997) which includes the CPS I and II studies as well as the Kaiser Permanente study (Friedman & Tekawa, 1997). Numbers of deaths for the four major smoking-related diseases represent approximately 50% of the total smoking related mortality in all three of these studies.
Table 5-1 Summary of Smoking-Related Deaths

<table>
<thead>
<tr>
<th>STUDY</th>
<th>Total Mortality</th>
<th>Lung Cancer</th>
<th>COPD</th>
<th>CHD</th>
<th>Stroke</th>
<th>% death from 4 major SRDs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Woman</td>
<td>Total</td>
<td>Men</td>
<td>Woman</td>
<td>Total</td>
</tr>
<tr>
<td><strong>CPS I</strong></td>
<td>13551</td>
<td>4921</td>
<td>18472</td>
<td>1035</td>
<td>157</td>
<td>1192</td>
</tr>
<tr>
<td><strong>CPS II</strong></td>
<td>9899</td>
<td>6232</td>
<td>16131</td>
<td>1781</td>
<td>1014</td>
<td>2795</td>
</tr>
<tr>
<td><strong>Kaiser</strong></td>
<td>405</td>
<td>208</td>
<td>613</td>
<td>53</td>
<td>54</td>
<td>107</td>
</tr>
</tbody>
</table>

Summarizes smoking related deaths as reported in Smoking and Tobacco Control Monograph No. 8 (National Institutes of Health, 1997) which includes data from three epidemiological studies. Values are given for total smoking-related mortality as well as figures for the four major smoking-related diseases (SRDs); lung cancer, chronic obstructive pulmonary disease (COPD), coronary heart disease (CHD) and stroke.

Cancer Prevention Study (CPS-I and CPS-II) (Thun et al., 1997) data represent the age adjusted death rates from all cause mortality as well as the data for the individual four diseases. The figure for total mortality (all cause mortality) consists of all men and women listed as current smokers that died of a smoking-related disease.

Kaiser Permanente (Friedman & Tekawa, 1997) data are age specific (35 and older) smoking-related deaths due to all smoking-related causes including the four major SRD’s. Data is based the number of deaths from male and female current smokers.
Studies in other countries have confirmed these broad conclusions and show that the effects of cigarette smoking on premature mortality do not vary greatly with culture (Forey et al., 2002). For example, Yuan and coworkers (Yuan et al., 1996) showed that middle-aged male smokers of 20 or more cigarettes per day in Shanghai, China, had a 60% greater risk of death relative to lifelong nonsmokers. More generally, the seven countries study, specifically examined cross-cultural factors in the mortality effects of cigarette smoking by looking at the pooled mortality experience of men who smoked ten or more cigarettes per day followed for twenty-five years. They found that the association between all cause mortality (1.8; 95% confidence interval, 1.7-1.9) did not vary across the studied countries (Jacobs, Jr. et al., 1999). These investigators attributed a few instances of reversal of the association of the four causes of premature mortality within one of the studied countries, to the statistical effects of random variation associated with inevitably smaller numbers of subjects within one country, occasionally low prevalence of disease among never smokers, and multiple comparisons.

Because cigarette smoking amplifies the background incidence of at least three of the diseases that contribute to premature mortality, hypothetically cigarette smoking might accelerate aging, prematurely harvesting deaths. Two approaches show that this hypothesis is not true. Cigarette smoking exerts an overall adverse effect and does not merely accelerate normal mortality experience. First, the overall mortality experience of smokers changes relative to that of nonsmokers, as a cohort ages and becomes deceased. The smokers die earlier. The diseases associated with cigarette smoking have greater prominence among smokers within a cohort that has aged sufficiently that almost all of its members have died. Second, epidemiologists have studied the effects of smoking cigarettes among the elderly. A small study of individuals age 65 or older in communities of three U.S. states (Massachusetts, Iowa and Connecticut) with significant prevalence of smoking showed that the premature mortality effects of smoking extend to older ages (LaCroix et al., 1991). A recent study in France confirmed these findings. Deceased elderly persons smoked more cigarettes than those still living during an eight-year follow-up, and smoking was associated with mortality among current smokers (Tessier et al., 2000).

Gender-specific effects of cigarette smoking remain uncertain. Cultural factors clearly account for differences in the prevalence of smoking rates between men and women in different countries and in cohorts born at different times. So, men and women experience different adverse health effects of cigarette smoking. Some investigators have looked for gender-specific target organ effects. A ten year follow-up of 117,557 U.S. women who initially described their smoking habits revealed no association between smoking of 25 or more cigarettes per day and breast cancer (London et al., 1989). Such studies help to define the specificity of the adverse human health effects of cigarette smoking.
When investigators controlled for age, duration of smoking, and smoking rates between men and women, gender-specific sensitivity appeared elusive. Prescott and coworkers (1998) examined a Danish cohort and found both total and cause-specific mortality similar in male and female smokers relative to nonsmokers. After excluding non-inhalers, female smokers had a slightly higher relative incidence of respiratory and vascular diseases than male smokers, but no gender differences were found in the relative incidence of cancers. However, nonsmoking Danish women had lower mortality rates of respiratory and vascular diseases, so the investigators noted that their results should be interpreted with caution (Prescott et al., 1998). Further, these investigators did not adjust for gender specific differences in body size.

Most investigators agree that smoking cessation produces desirable health effects. Yet, the effects of smoking cessation remain poorly understood, and different studies produce conflicting results. In the Multiple Risk Factor Intervention Trial, cigarette smoking associated weakly with approximately one-half of all deaths, and smoking cessation rapidly eliminated deaths from all causes associated with cigarette smoking, compared to continued smoking (Kuller et al., 1991). However, smoking cessation did not yield diminished lung cancer incidence in this cohort during the ten-year follow-up period (Kuller et al., 1991). Further, Enstrom and Heath (1999) found the effects of smoking cessation in CPS-I to have less of an effect than commonly believed. Of 51,343 male Californians enrolled in CPS-I in 1959 and followed for 38 years, who had largely ceased smoking, the all cause death rate dropped from 20.67 to 18.68 for smokers and from 10.51 to 9.46 for never smokers. Of 66,751 women the all cause death rate increased from 9.54 to 10.14 for smokers and decreased from 6.95 to 6.44 for never smokers.

### 5.2.3 Detection Bias

Little ambiguity usually exists about whether a person died or continued to live, or the date their death occurred. Thus, in principle, data about premature mortality is associated with less uncertainty than data about cause of death, which requires some determination, usually taken from a death certificate. The uncertainty arising from the number of deaths is associated with the counting of objects. Inconsistencies in diagnosing specific disease and assigning cause of death may result in detection bias, which is the preferential detection of disease in individuals within a specific group (Feinstein & Wells, 1974). Mortality risk assessments are often based on diagnoses. Hence, ultimately detection bias may compromise the validity of risk assessments calculated from death certificate based data.

Upon death of an individual, the immediate cause of death (the final disease injury or complication directly causing death), the underlying cause of death (the malady or injury which began the events that led to the immediate cause of death) (National Center for Health Statistics, 1991), and other significant conditions contributing to death but not resulting in the death of an individual (U.S. Department of Health and
Adverse Human Health Effects

Human Services, 1981) are noted. Data from Grulich et al. (1995) suggest that death certification and coding errors resulted in an approximately 46% increase in reported prostate cancer mortality in England and Wales between 1970 and 1990. In cases where individuals have multiple diseases (Hoel et al., 1993), the validity of death certificate data may be further compromised (Smith Sehdev & Hutchins, 2001).

Calculations of mortality risk from disease on exposure are often derived almost entirely from information about the underlying cause of a disease. The contribution of smoking to lung cancer is one such calculation (Sterling et al., 1992). Determinations of risk based almost solely on information about underlying cause of disease presuppose that when lung cancer is recorded as the underlying cause of death, it is duly noted for all groups of individuals, regardless of varied smoking habit. If the presupposition is not upheld, a “cause of death attribution bias” is said to have occurred (Sterling et al., 1992). A cause of death attribution bias could result from assumptions made by a physician, the lack of further investigation by a physician if a decedent is known to have been a smoker or performance of a more intense search for other possible causes of death when a non-smoker is diagnosed with lung cancer. Detection bias of this type could result in an overestimation of the risk of death from smoking (Sterling et al., 1992).

Sterling et al. (1992) analyzed data from the 1986 National Mortality Followback study and the 1954-1962 Dorn study and determined that the relative frequency of recording lung cancer as an underlying cause of death was lower for known never smokers and higher for known smokers. No cause of death attribution bias could be attributed to smoking for other diseases associated with cigarette smoking, such as cardiovascular and cerebrovascular disease. However, a sex specific bias has been reported for the diagnosis of COPD, with men more likely to be provisionally diagnosed as COPD than women, whose symptoms are more likely to be attributed to asthma (Chapman et al., 2001). For never smokers, other cancers were more often listed as underlying cause of death and lung cancer as contributory, whereas the reverse was true for current and former smokers (Sterling et al., 1992). Since knowledge of smoking history may affect a physician’s diagnosis, epidemiological data may, as well, be affected by physician’s diagnostic bias (Feinstein & Wells, 1974). This diagnostic bias would result in excess attribution of mortality risk of lung cancer for smokers. Sterling et al. (1992) proposed that reasonable estimates of the effect of this bias on the relative risk is to, in using this data, treat each individual for whom there is mention of lung cancer as having lung cancer as an underlying cause of death.

Detection biases may also occur in live subjects. McFarlane et al. (1986) showed decreasing gradients of non-detection of lung cancer according to amount of cigarettes smoked by the patient. Lung lesions detectable by radiology were more likely to signal lung cancer in smokers than in non-smokers (McFarlane et al., 1986). Feinstein and Wells (1974) investigated whether knowledge of the smoking habits of a patient influenced diagnosis of lung cancer during life. To determine
the relationship between extent of use of clinical diagnostic test and the detection gradient, the search rate for a pap smear of lung sputum (the number of patients for whom a pap smear test had been ordered divided by the number of patients in that category) was determined. The data showed a significant increase in search rate as the amount of cigarette smoking increased. Whether a cough was absent or present, the search rate increased with amount of smoking. In this study, pap smears were more likely to be ordered for men than women. Their data suggest that the stronger the smoking habit of an individual, the greater the probability of lung cancer diagnosis within a lifetime (Feinstein & Wells, 1974).

5.2.4 MATHEMATICAL CANCER MODELS

Premature death associated with cigarette smoking does not result from a generalized increase in all causes experienced in the U.S. population. Instead, three principal causes of death account for most smoking-related premature mortality among smokers: cardiovascular diseases, cancer, and COPD. All of these diseases occur in nonsmokers. COPD comes closest to being a disease characteristic of smoking (U.S. Department of Health and Human Services, 1984). Moreover, cigarette smoking essentially increases the incidence of diseases that would otherwise occur at lower incidence.

Susceptibility to the diseases attributed to cigarette smoke varies with age, even in nonsmokers (Thun et al., 1997). However, little information exists about the effects of age of initiation of smoking, other than younger age of initiation often results in increased duration of smoking. In addition, the expression of different smoking-related diseases occurs at different ages in any cohort with similar smoking experience. For example, the incidence of coronary heart disease increases first among smokers, later they experience a rise in lung cancer incidence, and still later, the incidence of COPD increases (Thun et al., 1997). Since cohorts living at the same time experience similar historical events, the incidence of smoking also varies with the chronological year of observation.

Besides duration of smoking, adverse health effects also increase with exposure rate. The more cigarettes consumed per unit of time, the greater the resulting premature mortality. Thus, the incidence of premature mortality reflects trends in two factors, duration and smoking rate. Multiplying average daily cigarette consumption by duration of smoking generates an estimate, which epidemiologists sometimes define as cumulative exposure. Toxicologists refer to the same measure of duration of exposure, times intensity of exposure, as Haber’s rule (Rozman & Doull, 2001). However, definitions of cumulative exposure and Haber’s rule are equivalent, and both make several unwarranted assumptions (Wilson & Crouch, 2001). Right now, separate analyses of smoking rate and smoking duration may lead to a better understanding of adverse health effects than the assumption of cumulative exposure (Leffondre et al., 2002).
Human mortality results from exposure to multiple chemical substances in cigarette smoke and results in multiple causes-of-death (Fuchs et al., 1996). However, the scientific community has produced little information about mathematical models that describe overall cigarette-associated mortality. Instead of models of overall cigarette-associated mortality, research has focused on models of cigarette-associated lung cancer (Doll, 1971; Doll & Peto, 1978; Greenfield et al., 1984; Moolgavkar & Knudson, Jr., 1981; Wilson & Crouch, 2001). The disease characteristics of lung cancer aid the development of quantitative models. Subjects with the disease die rapidly and are seldom misdiagnosed (Feinstein, 1967). Prevalence is usually well documented (Thun et al., 1997). The medical community seldom fails to record a case, in part because of the efforts of tumor registries. Even so, lung cancer is a complex of several diseases. Changes in adenocarcinoma incidence may differ from changes in bronchiolar carcinoma incidence.

Many carcinogenesis models suppose that each human being begins life with a certain capacity for damage. Damaging events, invisible “hits” or “bets,” arrive at some rate. These models are based on the ‘Gamblers Ruin’ stochastic process that supposes that a person starts with some capital and places bets at a steady rate, which are won or lost, until bankruptcy occurs (ruin) or a certain amount of money is won. Mathematicians have thoroughly worked out many variations of the model (Herrero-Jimenez et al., 1998). Gambler’s Ruin is also intuitive with many practical applications. In the case of cancer modeling, the bets only have a losing payoff with smoking causing an increase in the rate of betting.

In their classic studies, Sir Richard Doll and his colleagues gathered data about lung cancer among British physicians who smoked (Doll & Peto, 1976). In general, lung cancer prevalence increased as the exposure increased. Smoking more cigarettes within the same time period or smoking for a longer time increased the odds ratio for lung cancer. Doll (1971) developed the multistage model to explore the general effects of age on carcinogenesis. Doll and Peto (1978) used this model to describe overall lung cancer incidences by age in the British physicians cohort. Lung cancer incidences increased in proportion to the square of dose rates (number of cigarettes per day). This attempt to fit a mathematical model to the lung cancer data from human epidemiological studies of cigarette smokers did not comply with Haber’s rule. However, Doll and Peto excluded the highest dose rate group (40 cigarettes or more per day), which showed a lower increase in lung cancer incidences than expected from this relationship. Doll and Peto (1978) pointed out some serious systematic biases in the relationship.

Lung cancer incidences in the cohort increased as fourth to fifth power of age, even among smokers. This result is consistent with four to five stages in the progression of events to identifiable lung cancer. Smoking appeared to amplify the background (spontaneous) incidence of lung cancer. Other investigators have attempted to specify which stages smoking alters, including Brown and Chu (1987).
who believed that smoking primarily affects the first and last stages. Freedman and Navidi (1990) criticized the multistage model, based on data about lung cancer among cigarette smokers. For data from three studies they showed that lung cancer incidence declined after smoking cessation, a result at odds with predictions of the multistage model.

Other groups applied alternative mathematical models to the same data from the British physicians study. Moolgavkar and his colleagues developed a different conceptual model, sometimes called the MVK model, which has two mutational steps with an intermediate proliferation of mutated cells, as modified by the first mutation (Moolgavkar & Knudson, Jr., 1981). Analysts have extensively explored the MVK model and the concepts that it incorporates, such as effects of carcinogens on cell proliferation (Greenfield et al., 1984). In the basic MVK model, the population of intermediate cells can expand or contract and these intermediate cells are the targets for the second mutational step. While this model limits the number of stages to two, it resembles a multistage model in some ways. In particular, the overall process still is a Markov chain, in which the first step is obligatory for the second. Mathematically, the MVK model is more flexible than the multistage model, making it possible to fit incidence data, for example, from pediatric cancers. The MVK model also makes the consideration of temporal patterns of exposure (age and duration) much easier.

Overall, a two mutation model with a cell proliferation compartment fit the data from the British physicians study well (Moolgavkar et al., 1989; Moolgavkar et al., 1993). Moolgavkar and coworkers assumed a quadratic model of dose rate, taken from Doll and Peto (1978). Thus, their model does not comment on the dose rate-response relationship. However, their work reemphasizes the important impact of the daily dose of smoke. If the model is an accurate portrayal of biological events in the cohort, age has an independent and prominent effect on lung cancer incidence, independently from the effect of duration of smoking. In addition, their model showed prominent effects of cigarette smoke on the first mutation rate and the proliferation of intermediate stage cells, but not on the second mutation rate. This result is consistent with many possibilities, such as a loss of the ability of intermediate stage cells to respond to potential mutagens in cigarette smoke.

Wilson and Crouch (2001) utilized a multistage model approach, much like that of Doll and Peto (1978) and applied it to a different source of data about smoking outcomes, the Cancer Prevention Study-I (CPS-I) and Cancer Prevention Study-II (CPS-II) (Thun et al., 1995; Thun et al., 1997). CPS-I took place between 1959 and 1965 and included more than one million U.S. residents and eleven million person-years of observation. CPS-II took place between 1982 and 1986 but has not received reporting as thorough as CPS-I. In part, Wilson and Crouch had to infer data for CPS-II from follow-up reports to CPS-II which extend the observation period to 1988 (Thun et al., 1997) and to comparisons of CPS-I to CPS-II.
Like Doll and Peto, Wilson and Crouch (2001) found that lung cancer incidence followed approximately the same power law of age dependence in men and women up to an age of 80, but Wilson and Crouch found differences in several parameters, including cigarette potency, between men and women (Wilson & Crouch, 2001). They found a different shape for the dose rate-response relationship: a linear function of cigarettes per day at low doses, and a square root of cigarettes per day at higher doses. Thus, the shape Wilson and Crouch found differs from the shape found by Doll and Peto (1978). It agrees with Haber’s rule at dose rates below 20 cigarettes per day, and it fits higher dose rates in CPS-I and CPS-II well. Wilson and Crouch provide a detailed explanation about the limitations on the low dose shape they found. Because the data they modeled collapsed smokers of 1 to 9 cigarettes per day into one group, it is not possible to dissect the low dose response in any greater detail. In essence, Wilson and Crouch imposed a linear dose rate-response relationship on the data. Further, Wilson and Crouch (2001) remain circumspect about the shape of the dose rate-response relationship for more than 40 cigarettes per day, based on the same rationale.

5.2.5 Interactions with Other Carcinogens

Several efforts to quantitatively model human lung cancer prevalence developed during the processes of studying other substances, which interact with cigarette smoking, including asbestos, radon and beverage alcohol consumption. For example, lung cancer from exposure to asbestos is extremely rare in the absence of cigarette smoking (Selikoff & Hammond, 1979). Chase and coworkers (1985) remodeled the same dose rate data that Doll and Peto summarized and applied it to data about cigarette smokers exposed to asbestos. They found that asbestos modified the effects of cigarette smoking on lung cancer incidence.

Unlike asbestos, radon is a carcinogen for the human lung. Moolgavkar and coworkers (1993) applied a more exact version of the MVK model to the British physicians study. They applied this model, essentially as a modifier, to data from a study of Colorado uranium miners to explore the interaction between radon and cigarette smoke. Surprisingly, both radon and cigarette smoke increased the first mutation rate and the intermediate cell division rate, but neither changed the second mutation rate. Joint exposure to radon and cigarette smoke resulted in a more than additive but less than multiplicative increase in lung cancer incidence. The initial age of exposure significantly increased radon and/or smoke induced lung cancer incidence in this model. This work confirms other observations that fractionation of radon dose increases lung cancer incidence, suggesting a nonlinear dose-response relationship. In the MVK model, duration of exposure is more important than exposure rate in increasing cancer incidence through effects on the proliferation of the intermediate cell compartment. This effect also would apply to cigarette smoke.

Smoking and beverage alcohol also interact, but the effects of each substance on consumption behaviors, exposures, and tissue sensitivities of the other substance
have proven difficult to dissect (Bien & Burge, 1990). In addition, some of the interactions apparently involve socioeconomic variables, such as socio-economic class (Cummins et al., 1981; Williams & Horm, 1977). Extensive covariance of consumption occurs; heavy drinkers tend to be heavy smokers. Epidemiologists have made some efforts to differentiate the effects of beverage alcohol consumption in the absence of smoking (Longnecker, 1995). These efforts depend on correction for the effects of cigarette smoking. However, if the relationship between dose rate and response is not linear, most of these efforts will have failed. In a nested case-control study, Murata and coworkers found an effect of beverage alcohol consumption on lung cancer independent of cigarette smoking but a “synergistic” effect of the two substances on cancers of the upper aerodigestive tract and bladder (Murata et al., 1996).

5.3 CIGARETTE-RELATED CAUSES OF PREMATURE MORTALITY

5.3.1 Cardiovascular Diseases

Some medical authorities cite heart attack, stroke and diabetes as the major smoking-related cardiovascular diseases (Amler & Eddins, 1987). Others cite stroke and cardiomyopathy as major subdivisions of smoking-related cardiovascular diseases (National Institutes of Health, 1997). Currently, scientific experts disavow any such summarization, as these subdivisions imply unproven disease mechanisms. Regardless of the division, cardiovascular diseases are one of the major causes of premature deaths among the smoking population.

Thirty percent of all deaths for Americans are attributed to coronary heart disease (CHD) (Hoyert et al., 2001). Smoking is associated with both a two- to four-fold increase in the risk of CHD and an elevated risk of sudden cardiac death (Lakier, 1992). CHD related changes induced by smoking include alteration of serum lipids, changes in platelet aggregation, and damage to the vascular wall (Krupski, 1991). In addition, smoking increases the risk of coronary artery vasospasm (Sugiishi & Takatsu, 1993). Patients with existing CHD who are smokers have more severe disease than nonsmokers, as demonstrated by more frequent and longer episodes of myocardial ischemia (Barry et al., 1989). Women who smoke have a higher risk of CHD, myocardial infarction, and angina pectoris (Willett et al., 1987). Smoking also relates to the incidence of atherosclerosis, transient ischemic attacks, intermittent claudication and aortic aneurysm (Freund et al., 1993).

CHD and type 2 diabetes have many common causal factors (Jarrett & Shipley, 1988; Perry et al., 1995), however there is some debate over the association, if any, between diabetes and smoking. Recently conflicting reports citing smoking as an independent and modifiable risk factor for diabetes have been published (Rimm et al., 1995; Wannamethee et al., 2001; Wilson et al., 1986). Further epidemiological evidence is required before a causal link between smoking and diabetes can be established.
In a meta-analysis of 32 studies, Shinton and Beevers (1989) showed that cigarette smoking status increased the overall incidence of stroke by fifty percent. Because stroke is a major contributor to overall mortality in the U.S., even small increases in ischemic disease significantly increase excess mortality. Thus, cigarette smoking contributes to overall U.S. mortality by increasing stroke, as documented in a major longitudinal U.S. health study, the Framingham Study, in which smoking two packs or more per day independently increased the incidence of stroke two-fold (Wolf et al., 1988). In the meta-analysis, cigarette-related mortality varied by infarction subtype. The relative incidence of subarachnoid hemorrhage was 2.9 (Shinton & Beevers, 1989). Younger smokers exhibited greater effects of cigarette-related mortality, showing a nonlinear exposure-response relationship with higher prevalence at higher pack-years (Shinton & Beevers, 1989). The risk of ischemic stroke declines rapidly within two to four years after smoking cessation (Wolf et al., 1988).

5.3.2 COPD

Smoking is a significant cause of pulmonary disease and death in the U.S. Studies have shown increased mortality among smokers from pneumonia, influenza, asthma, and emphysema. Smoking is the single most important risk factor for chronic obstructive pulmonary disease (COPD) (Sherman, 1992) and is the primary determinant of COPD-associated mortality (Kuller et al., 1989). Although the prevalence of smoking has declined among American adults, a concomitant decrease in mortality from COPD has not occurred (Davis & Novotny, 1989). The residual risk of COPD mortality from smoking persists after cessation.

Cigarette smoking contributes to pulmonary disorders such as chronic bronchitis and emphysema. It also relates to other forms of interstitial lung disease, such as desquamative interstitial pneumonia, respiratory bronchiolitis-associated interstitial lung disease, and pulmonary Langerhans’ cell histiocytosis (Hartman et al., 1997; Ryu et al., 2001). Smoking leads to more severe asthma outcomes (Ulrik & Lange, 2001). Smoking exacerbates influenza infections and other infectious pulmonary diseases (Finklea et al., 1969; Petitti & Friedman, 1985).

5.3.3 Cancer

Smoking contributes to the risk of many cancers. Organs in direct contact with smoke are at greatest risk. A 26-year follow-up health study of almost a quarter of a million U.S. veterans revealed that more than 50% of cancer deaths among smokers and 23% of cancer deaths among former smokers were attributable to cigarette smoking (McLaughlin et al., 1995). Mortality rates from these cancers have increased for smokers, and the risk of lung cancer has increased more rapidly for women than for men. Lung cancer may be the greatest cause of excess mortality among male and female smokers (Shopland et al., 1991). Lung cancer death rates among smokers increased from 26 to 155 per 100,000 among women.
and from 187 to 341 per 100,000 among men (Thun et al., 1995). Cigarette smoking is the predominant risk factor for lung cancer, and shows a dose-response relationship with all major lung cancer cell types (Kabat, 1996).

In addition to its position as a risk factor for lung cancer, smoking is also associated with cancers of the pharynx, esophagus, stomach, pancreas, kidney, urethra, and bladder, with the strongest risk associated to cancers of the mouth and larynx (Kuller et al., 1991). Smoking was associated with more than half of the deaths in women from cancers of the oral cavity, esophagus, pancreas, larynx, lung, and bladder (Stellman & Garfinkel, 1989). Some forms of adult leukemia also associate with smoking (Brownson et al., 1993). There is also a reported increased risk of cervical cancer associated with smoking (Slattery et al., 1989).

5.3.4 Mortality From Other Diseases

The 1989 Surgeons General report showed that out of an estimated 390,000 smoking related deaths, 52,900 were due to smoking related conditions other than cardiovascular diseases, COPD, or cancer (U.S. Department of Health and Human Services, 1989). In a cross-sectional analysis of premature death, Amler and Eddins (1987) stated among the 338,022 tobacco related deaths in 1980, an estimated 1,897 deaths were due to peptic ulcer disease. The ratio of mortality rates for peptic ulcer disease ranges from 3.0–4.6 in smokers when compared to the non-smoking population (Doll et al., 1994; Korman et al., 1983) and a greater relapse rate of ulcers of the stomach and duodenum (Lane & Lee, 1988; Sontag et al., 1984). A 26 year follow-up of the US veterans study (1954-1980) (Hrubec & McLaughlin, 1997) listed 50 cause of death groups. Diseases that show excess risks associated with cigarette smoking are asthma (2.3), tuberculosis (1.6), cirrhosis (1.5) and chronic nephritis (1.3). The figures in parentheses show the relative risks based on smokers compared to never smokers for the entire follow-up period (1954-1980). These conditions are often grouped together in studies of tobacco related mortality as the numbers of deaths involved are relatively low when compared to the three smoking related conditions; cancer, COPD and cardiovascular disease.

5.3.5 Infant Death

An indirect effect associated with smoking is the negative effect of maternal smoke on perinatal and postnatal mortality. A 1980 report by the Surgeon General (1980) lists many adverse outcomes of pregnancy associated with maternal smoking, including fetal growth restriction, increased spontaneous abortion, greater incidence of bleeding during pregnancy, premature and prolonged rupture of amniotic membranes, abruptio placentae and placenta previa. Smoking related increases in childhood behavioral and psychiatric illness (Brook et al., 2000), blood pressure (Blake et al., 2000) and respiratory disorders (Gilliland et al., 2001) have been reported. Children from mothers who smoked during the pregnancy have more hospital admissions, compared to non-smoking mothers (Harlap & Davies, 1974).
Based on combined data from 2000-2001, 19.8% of women were reported to have smoked during pregnancy (National Institute on Drug Abuse, 2001). Among these mothers, the perinatal mortality (PNM) rate exceeds that of non-smokers. Kleinman (1988) assessed Missouri vital statistics records, which included data from 360,000 live births, 2500 fetal deaths and 3800 infant deaths. Among women in their first pregnancy, the relative risk of fetal mortality associated with maternal smoking was 1.36 (95th CI, 1.16-1.59) for less than 1 pack per day smokers and 1.62 (95th CI 1.34-1.97) for those smoking more than 1 pack per day. A similar dose-related increase was observed for neonatal mortality. The Ontario Perinatal Mortality study included data from 51,490 births during 1960-1961 (Meyer & Tonascia, 1977). It showed that PNM increased significantly (p<0.00001) as maternal smoking increased. Meyer (1977) showed that most smoking associated PNM related to anoxia or unknown causes (fetal deaths) or premature deliveries (neonatal deaths). These deaths are strongly associated with a smoking related increase in the incidence of bleeding during pregnancy, abruptio placentae, placenta previa or premature and prolonged rupture of the membrane.

Maternal smoking and sudden infant death syndrome (SIDS) interrelate. The Ontario Perinatal Mortality study showed that sudden infant deaths were strongly associated with the frequency and level of maternal smoking during pregnancy (p<0.001). In a prospective study of 24,986 births, the odds ratio for SIDS in children from smokers was 3.5 (95th CI, 1.4-8.7), in comparison to children of non-smokers (Wisborg et al., 2000). The incidence of SIDS was dose related (p<0.05). Reports link SIDS to smoking related changes in respiration and cardiovascular control (Browne et al., 2000; Milerad & Sundell, 1993).

5.3.6 Morbidity and Other Health Effects

Using estimates from 1980 of approximately 350,000 attributable deaths per year and 1,500,000 years of life span lost before age 65 as indicators, parallel rough estimates are that, as a population, smokers experienced approximately ten days of hospitalization per year of life span lost or fifty days of hospitalization per death (Amler & Eddins, 1987). Days of hospitalization do not function well as an indicator of health status. Efforts, primarily by economists, to produce weighting factors for various indicators that yield more satisfactory expressions are controversial. Quality adjusted life years (QALYs) weight the number of years of future life span a person will spend in each state of health, with perfect health as one and death as zero. Leaving aside the problems of polling people about their preferences for various states of health and assigning numerical weights to them, economists use an entirely different approach, willingness-to-pay (Hammit, 2002).

When discussing the adverse human health effects of cigarette smoking, the concept of morbidity is important. Besides increasing premature deaths, cigarette smoking
contributes to nonfatal morbidity. Thus, morbidity could have effects on the quality of life experienced by smokers, independently from premature mortality effects. For example, tobacco influences periodontal health and disease. Smokers experience more periodontal disease, including greater bone- and attachment-loss, increased pocket formation and calculus formation. Depending on the criterion, smokers are 2.6-6.0 times more likely to exhibit periodontal destruction than nonsmokers (Bergstrom & Preber, 1994; Grossi et al., 1994; Haber et al., 1993; Page & Beck, 1997). The mechanisms involved in smoking associated periodontal disease are not known. Smoking may affect the periodontal vasculature, inflammation, or immunity, including humoral immunity (Christen, 1992; Haber, 1994; Palmer et al., 1999). However, periodontal disease, even severe periodontal disease, does not necessarily lead to premature death.

Whether any morbidity, not leading to premature mortality, might occur at lower exposures remains uncertain and speculative. In the sense that LSRO knows of no predictive tests for such non-lethal morbidities, such testing is not feasible. Only if a smoking-related morbidity occurs out of relation to (independently from) smoking-related mortality, and if an ingredient exerts an effect on this morbidity through one of the three pathways summarized in section 5.1, could a smoking-related morbidity become a health concern associated with the presence of an additive.

5.3.7 Diseases and Exposures not Covered in This Report

LSRO has not attempted to cover all health effects of cigarette smoking in a comprehensive way. For example, scientists have observed some beneficial effects, such as improvements in mortality from Alzheimer’s disease (Lee, 1994). In addition, the relative prevalence (or relative incidence) of some effects or some exposure pathways is so low that even the most comprehensive and extensive of studies could not detect the changes an ingredient might cause. For example, environmental tobacco smoke (ETS) remains a controversial subject, but the maximum relative prevalence of mortality of diseases noted in only a few studies is near 1.3 (Tweedie & Mengersen, 1992; Vutuc, 1984).

5.3.8 Relationship of Risk Analysis to Safety Assessment

The process that LSRO initiates with this report is based on the available evidence and involves identifying levels of ingredients added to cigarettes such that expert scientists do not expect unfavorable incremental health effects. This process has parallels with the safety assessment process used by the U.S. Food and Drug Administration (FDA) to set levels for additives and pesticides in the food supply (Raiten, 1999). While based on scientific principles, FDA’s safety assessment process is administrative and regulatory in nature. Therefore, this report describes how the processes differ.
The U.S. Environmental Protection Agency (EPA) also has a new policy, one modified from FDA’s policy presented as a process of science-based, regulatory risk estimation. Mechanically, operationally, and scientifically, the policies of these two agencies differ. For example, under regulatory risk assessment, EPA generally assembles data from available sources to estimate potencies and exposures for all environmental carcinogenic substances, including agricultural pesticides, which enter the U.S. food supply. However, FDA essentially excludes carcinogenic substances from consideration as additives to the U.S. food supply.

For non-carcinogenic substances, both agencies seek a no-observed-adverse-effect-level (NOAEL) based on experimental animal data, essentially a regulatory determination of dose, or of experimental exposure, which the agency can later convert into some estimate of human exposure. Both agencies divide these NOAELs by additional factors, called safety factors at FDA, or uncertainty factors at EPA, to decrease human exposure still more.

Under a regulatory risk assessment (Byrd & Cothern, 2000):

1. some person or organization, usually a governmental agency, assesses the risks of exposures of a substance, process or use of a product, which should be specified as the probabilities of future losses under specified conditions,

2. the agency usually publishes the assessment for public comment, often through a process that mimics informal rulemaking, takes comments on the assessment, and issues a final assessment, and

3. the agency usually will engage in some form of communications program about the agency’s assessment and the agency’s perceptions of the risk.

Note that for cigarette smoking, the primary loss would be lives or life span under specified conditions. EPA tries to keep risk management, the process of regulating the sources of risk specified in the assessment, well separated from the process of assessing the risk. While the implications of the risk assessment for a pesticide tolerance, whether from a carcinogenic potency or from a maximum allowable amount of a non-carcinogen, may seem obvious to all, issuing and enforcing a regulation will come later. However, under a safety assessment (Byrd & Cothern, 2000):

1. a manufacturer submits required data about a candidate, non-carcinogenic substance under specified conditions in a petition to a governmental agency, either to FDA as a proposed additive to the food supply or to EPA as a proposed agricultural pesticide,

2. the agency reviews the data and sets a tolerance, as subject to public comment, to allow or not to allow the substance in the food supply under certain conditions, and
(3) the agency publishes its decision in the Federal Register and the Code of Federal Regulations.

Both EPA’s and FDA’s paradigms have undergone analysis, and differences have been noted (Carrington & Bolger, 2000). Thus, the essential difference between safety assessment and risk assessment is that the functional objective of a safety assessment is an administrative action, whereas the functional objective of a risk assessment should be a statement of a population risk.

EPA has confused the separation of these two processes by redefining a safety assessment-like process, as applied to non-carcinogenic substances in the environment, as a risk assessment. However, EPA does not generate a probability statement as part of its non-cancer assessment procedure. Instead, EPA demarcates a point of exposure, which after division by an uncertainty factor, the agency asserts will have no risk whatsoever at lower exposures.

Often, the same individual at FDA carries out both steps 1 and 2 of a safety assessment. No division of risk assessment and risk management exists. EPA strives to separate risk assessment and risk management. Both agencies rely heavily on animal test data in making decisions about tolerances for non-carcinogenic substances allowed in the food supply. For non-pesticides, EPA often limits itself to data available in the scientific literature. Often, the safety or uncertainty factors are described as two independent tenfold factors, one for interspecies variation and the other for intraspecies variation. However, the tenfold safety factors of safety assessment cannot function independently of each other. FDA really has a hundredfold safety factor for everything – species differences, human variation, “uncertainty,” and everything else.

Regardless of the reasoning used by FDA’s regulators to achieve the first use of the safety assessment process, on balance, the policy has worked for food additives. The U.S. and other Western nations now have nearly fifty years of experience with the process. Wilson (1998) has made an accounting of its performance. One significant failure occurred. When Canadian regulators allowed the use of cobalt salts in beer, they miscalculated the prodigious amounts of beer that brewery workers consumed, and several workers died of heart failure presumably due to cobalt toxicity. Otherwise, the track record is clear over perhaps 6,000 substances allowed as food additives worldwide. So, the failure rate of additives submitted to the worldwide safety assessment process is perhaps $2 \times 10^{-4}$. This rate is per chemical substance or a risk of administrative failure. It is not a risk in the sense of loss of human lives or reduction of human life span under specified conditions.

FDA may, on average, over-regulate food additives. Scientists have no way to tell. The failure estimate cited above has a large associated uncertainty, one difficult
to estimate. The number of regulated substances does not change the estimate or its uncertainty by much. For example, if only 3,000 substances have undergone evaluation as food additives, the failure rate changes to $1 \times 10^{-4}$. Instead, the uncertainty resides in a lack of knowledge about the numerator of the fraction. Further, fifty years of regulatory experience provide data about a changing process, in the sense that FDA keeps making improvements and expanding the data manufacturers have to submit. The net effect has been lowering of NOAEL exposures and decreases in the uncertainties of these NOAELs.

The analytical procedures that LSRO contemplates for ingredients added to cigarettes, and the tests to support these procedures, are not analogous to either safety assessments or regulatory risk assessments. Instead, the scientific basis for the feasibility of testing resides in three recommendations.

1. Scientists can evaluate data from tests that compare highly similar, virtually identical test cigarettes, which differ only in the presence of specific amounts of an ingredient or ingredients.

2. LSRO will not confine itself to a review based primarily on animal toxicology data. Instead, LSRO will proceed with an evaluation of tests balanced among various approaches (physical, chemical, cell culture, animal model, clinical, and epidemiological), setting priorities and using cost of information approaches to adjust resources to the tests most likely to provide informative data. LSRO also will seek post-marketing surveillance information.

3. Some analogies exist between a NOAEL and a point of no detectable change in an exposure-response relationship. However, the two concepts differ. Although subject to some statistical clarification, LSRO essentially relied on the concept of no detectable change in deciding that testing ingredients added to cigarettes was feasible. Under specified experimental conditions and detection limits, scientists can define a point of no detectable change such that a difference cannot be detected between smoke from an experimental cigarette and smoke from a reference cigarette.

5.4 SUMMARY

Chapter 5 primarily summarizes the adverse health effects of cigarette smoking. If an ingredient changed the cigarette smoke matrix in such a way that it changed the known adverse health effects of cigarette smoking, as documented in epidemiological studies, LSRO would like a test program that at least signaled such a possibility for further investigation. In addition, a test program should signal an ingredient that changed human smoking behavior, such that known adverse health effects of cigarette smoking might change. In LSRO’s view, such a test program is feasible.
Adverse human health outcomes are biological measures of the cigarette smoke exposure. To this end, one purpose of this chapter was to establish an approximate point in the human exposure-response relationship in readily observable units. Most smokers consume approximately one pack of cigarettes per day, seldom more than two packs per day. Using this exposure rate from the date of initiation for a lifetime smoker and Cohen’s estimate, the typical male lifetime smoker loses a significant portion of his lifespan, approximately seven years.

The annualized chance of a lifetime cigarette smoker dying prematurely is approximately one-in-one-thousand, and the chance of that lifetime smoker dying from lung cancer is approximately 13% of the overall excess risk of death, or 0.13 x 10−4. Over the typical one pack a day smoker’s lifetime, the cumulative lifetime risk of death from cigarette smoking-related causes becomes approximately 7 x 10−2 (nearly one-in-ten), the cumulative chance of dying of lung cancer becomes 9 x 10−3. Pathologists observe few deaths from lung cancer among nonsmokers.

Rate-based estimates, like a probability of 10−3 of premature death for male smokers of more than one pack per day per year, dramatize the fates of deceased smokers but do not convey all of the risks of smoking. Overall, lifetime smoking degrades lung performance in all members of the smoking population, whether their death is premature or not (Davis & Novotny, 1989). Pathologists observe that the lungs of smokers are colored black with tar and lack many cilia, regardless of their causes of death (Auerbach et al., 1979). Further, the driving forces in human health responses to cigarette smoking are dose and time, not exposure rates or pack-years. Both dose and time are complex factors (Rozman & Doull, 2000).

Overall, the remarkable characteristics of cigarette-related mortality are (a) smoking does not generally increase all causes, or even most causes, of mortality seen in the nonsmoking population, and (b) smoking increases the incidence of mortality from diseases already seen in the nonsmoking population. Deaths associated with cigarette smoking are not associated with causes not seen in nonsmokers. Chapter 7 addresses the possibility that epidemiological studies might practically reveal useful information about the effects of ingredients on the human health effects of cigarettes. However, this chapter shows that the most important outcome to study for direct effects on smokers’ health is premature mortality, not the causes of premature mortality. For epidemiological studies, direct observation of premature mortality will produce the highest quality of data in the shortest period of time with the smallest populations under observation, compared to any associated causes of cigarette smoking-related mortality and morbidity.
6

TESTING: ISSUES AND METHODS

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6.5 SUMMARY
TESTING: ISSUES AND METHODS

6.1 INTENT & PURPOSE

Scientists can test an ingredient added to cigarettes in many ways relevant to establishing whether the substance might change the morbidity and premature mortality associated with smoking. Added ingredients could change the relative risks of cigarette smoking through different mechanisms. Added ingredients could change the exposure to other substances in cigarette smoke, intensify the biological activity of cigarette smoke, or create potentially new biological effects. Thus, scientists need to investigate the potential adverse health effects of added ingredients with a testing program that comprehensively explores many possible mechanisms.

Manufacturers currently have available the technology to prepare approximately identical test cigarettes for scientific study, only lacking one or more ingredient(s). However, such test cigarettes have been used in only a limited number of published experiments. The capacity to prepare paired test cigarettes is key to the experimental design and overall strategy adopted by LSRO. Such pairs exactly fit with a relative risk approach to added ingredients. By comparing data from a cigarette with an ingredient to data from a nearly identical cigarette without the ingredient, scientists can understand relative risks and can identify whether a detectable change has occurred, given that the test is appropriate and that scientific testing principles have been applied.

An aim of this Phase One report is to provide a basis, developed from a review of the literature, that a feasible process can evaluate whether, given certain conditions, ingredients added to cigarettes change the relative risks of smoking. Test data will support this process. In this regard, the rigor of the testing program is important. Therefore, the tests should be precise and accurate. Moreover, test performance should be standardized based on reference materials.

Section 6.2 describes conditions relevant to the use of test data in risk assessments and decisions based on those assessments, some of the issues that scientists generally encounter in determining the quality and accuracy of data. These issues include: establishing criteria for rendering a test (or a test result) scientifically valid, limitations of test methods, and suggestions for improving a testing process. In addition, a definition of scientific testing and a description of the parameters of
testing are necessary adjuncts to a discussion of the feasibility of testing. Section 6.2 also bridges to the previous chapter, which concerned direct observation of adverse human health effects of cigarette smoking, and to the subsequent chapter, where areas of research that might merit further testing are highlighted. Additional research needs, described in Chapter 7, clearly will be subject to the principles laid out in section 6.2.

Section 6.3 reviews and addresses the limitations of tests and testing programs traditionally used to determine the toxicities of specific substances, for example, as added to the food supply. Section 6.3 inquires whether LSRO can usefully adapt these programs to the review of ingredients added to cigarettes.

LSRO briefly examined several examples of added ingredients in preparing this report, deliberately choosing substances used in greater amounts, including sucrose and cocoa, to facilitate the discussion of feasibility. LSRO focused on menthol as an example of an added ingredient to explore the feasibility of testing for changes in smoke and biological activity relevant to the toxic effects posed by smoking cigarettes because menthol transfers with smoke and because some epidemiological data are available. Published studies compare smoke chemistry or potential health effects of menthol containing cigarettes to similar cigarettes without menthol. Section 6.4 summarizes some salient points of this exploration.

### 6.2 TESTING ISSUES

#### 6.2.1 Gold Standards

A “gold standard” is a test, or a material used in a test, that scientists consider reproducible, accurate, and reliable. Data from gold standard tests often form databases to evaluate other tests (Faraone & Tsuang, 1994). Gold standard data may be compared to other data about an added ingredient. Subsequently, new tests also undergo comparisons for their ability to replicate data obtained with the gold standard test or material.

In testing for adverse human health effects of smoking cigarettes (and in this report, for the adverse human health effects of ingredients added to cigarettes), the epidemiology of human health effects of smoking is the gold standard. The existing knowledge base about the adverse health effects of smoking comes almost entirely from human epidemiological studies. LSRO needs tests that predict human adverse health effects to insure that the test results will provide useful information about an added ingredient. To have usefulness, a test must predict an outcome of a human epidemiological study, or an outcome from the aggregated evidence of many epidemiological studies of the health effects of cigarette smoking.
Some tests are conducted in attempts to predict the presence of a disease (Kraemer, 1992). Investigators select two groups of subjects, some believed to possess the disease, some thought not to possess it. The validity of a test is defined by its relative ability to identify the patients with the disease and to avoid detecting the patients without the disease. No one pretends that the diagnosticians have infallible skills. However, test evaluation usually proceeds as if no uncertainty attaches to classification into the disease and control groups.

The application of a test and its evaluation in the above situation, involves defined reference populations. It should be distinguished from a situation frequently addressed in this report, attempting evaluation of a test without knowledge of any prior, defined reference populations. The distinction is crucial. In the first situation, the test predicts a known reference. In the second situation, the only characteristic defining the two groups is the result of the two tests, e.g., a ninety-day inhalation toxicity test in rats.

In many situations in this report, LSRO had to evaluate test data, or the potential performance of a test, in the absence of reference populations. For example, LSRO usually lacked information about the prevalence or incidence of diseases between populations that smoked cigarettes with added ingredients compared to similar populations that smoked cigarettes without the same ingredients. In this circumstance, test data often become self-referential.

6.2.2 Predictivity

Human studies directly generate human health data, as opposed to test methods involving animal models, which require interpretation when applied as indicators of potential human health effects and which do not necessarily yield definitive information about humans. One potential limitation of human studies, for example, clinical studies, is that the number of subjects may be small. Small numbers of subjects make the resulting data more dependent on chance (Bukowski et al., 2001). The number of subjects determines statistical precision and limits the ability to test hypotheses. Investigators can achieve greater sensitivity in each individual subject (See Appendix H). However, many epidemiological studies have many-fold more subjects than traditional animal bioassays.

Human epidemiological studies usually have lengthy durations - at least several years - because most human diseases of interest have long latencies. Diverse human populations, different living conditions and environments, and variable background rates of diseases, all limit quantitative epidemiological observations. Susceptibility factors, such as predisposing genetic traits, age, ethnicity, gender, or nutritional status, vary in human populations (Perera, 1997).
As an example of test predictivity consider the concordance of rodent cancer bioassays. For new substances, mixtures and processes, epidemiological data do not exist; therefore, studies that utilize model systems are used as substitutes. In the absence of human epidemiological studies of human carcinogenic effects of chemical substances, regulators have used lifetime animal bioassays to predict the carcinogenicity of chemical substances, particularly new substances and/or mixtures entering commerce. Generally, these studies employ two rodent species, rat and mouse. The status accorded to these tests suggests that the scientific community and society in general regards them as good quality tests. However, even the performance of good tests needs to be analyzed (Kraemer, 1992). Test evaluation includes specification of both internal and external validity. The two-year rodent carcinogenicity assay has received criticism for many reasons, including the high exposures involved in testing (McConnell, 1989). The test design explicitly substitutes high exposures to compensate for small numbers of subjects. However, this substitution often is not expressed when communicating the results of carcinogenicity bioassays, and whether the substitution is scientifically valid remains unclear.

Higher exposures do not necessarily reflect effects observed at lower exposures, yet, lower exposures typify human exposures. For example, at high exposures the normal metabolic pathway may saturate and an overflow pathway may generate a carcinogenic metabolite, one not seen at lower exposures. Other arguments favor testing at higher exposures, including use of the maximum tolerated dose (MTD), often defined as “the highest dose of the test agent during the chronic study that can be predicted not to alter the animals’ longevity from effects other than carcinogenicity” (Sontag et al., 1976). The MTD is used to increase the probability of a statistically significant number of animals with tumors in a small sample (Krewski et al., 1993). According to Omenn (2001), concerns about the use of the MTD include whether it is valid to extrapolate results down to the doses that are likely to be relevant. Additional criticism of lifetime cancer bioassays derives from their duration. Experiments that could estimate human carcinogenic risks within a shorter time might prove preferable. Even with lower predictivity, a tradeoff exists (Omenn & Lave, 1988).

Scientists sometimes regard a qualitative response of tumor induction in one species as raising a question about the test substance but insufficient evidence of carcinogenic activity to classify the substance for regulatory purposes. At present, carcinogenicity tests continue to use two rodent species, rats and mice. Depending on the regulatory scheme, chemicals that cause tumors in one or both rodent species are considered potential human carcinogens (Lave et al., 1988). In the U.S. National Toxicology Program experiments, however, results in rats do not predict results in mice very well, and vice versa (Lave et al., 1988). The rodent bioassay detects all known human carcinogens as rodent carcinogens (World Health Organization, 1987), thus it is a highly sensitive assay. However, the value of a test is measured by its specificity as well as its sensitivity (Coburn et al., 1965). Specificity is lacking,
since the concordance of data for rats and mice was 70% of chemicals (Zeiger, 1987).

The term, “concordance,” can have two different meanings. In toxicology, investigators often compare the responses of different species to the same chemical substance. When doing so, a quantitative concordance is a similarity between the exposures that produce any biological response in two species. Quantitative concordance differs from qualitative concordance, in which chemical substances produce the same biological responses in two different species. For qualitative concordance, the exposure inducing a response in one species predicts the exposure that induces a different response in a second species.

One way to improve on the gold standard approach to testing is to take multiple tests and evaluate the combined results of the tests (Kraemer, 1992). Although individual tests of carcinogenic effects may fallibly predict the risk of human diseases, if these tests provide information about different mechanisms of toxicity, analysts may combine test results and improve the quality of information (Rosenkranz et al., 1990). However, the inability to predict results of chronic bioassays of one rodent species, based on results in the other rodent species, when both bioassays have met high standards of internal validity, does not inspire confidence in predictions about human carcinogenic effects, using bioassay results with both rodent specie.

6.2.3 External Validity

In this report, LSRO’s goal in testing is to determine the relative health risks of ingredients added to cigarettes. Although tests on human subjects will yield the most scientifically valuable information, many considerations limit the allowable human tests. Despite close biological and genetic similarities, LSRO remains skeptical of animal to human extrapolation. The available animal models lack external validity for the adverse human health effects of cigarette smoking. Currently, no animal model predicts smoking related premature mortality or morbidity well enough that we could be secure in saying that an added ingredient would not increase human risk if it does not increase risk to the animal.

6.2.4 False Negative and False Positive Results

Minimally, tests of health effects should distinguish between individuals with and without a disease. An assessment of the validity of a test involves the determination of the relationship between the expected results — the prediction, and the measured results — the outcome. Test accuracy (or validity) and precision are indices of test performance. Precision is the extent to which multiple measurements under specified conditions agree (Dybkaer, 1995). Repeatability (intra-laboratory) and reproducibility (inter-laboratory) are two types of precision (Greiner & Gardner, 2000b).
Error may be defined as the difference between a value obtained and a true value of that which was measured (Dybkaer, 1995). Random error is the difference between the measured value and a large number of measurements of the same parameter that was measured (Dybkaer, 1995). Random error compromises precision (Jekel et al., 2001).

Accuracy defines the agreement between a measurement and its true value (Dybkaer, 1995). Thus, test results may be accurate and imprecise, or precise and inaccurate. For a disease, the accuracy of a test is described by its sensitivity, defined as the probability of a positive test outcome in the presence of the disorder, and its specificity, the probability of a negative test result from individuals that do not have the disorder (Kraemer, 1992). The true positive rate of a test is equal to its sensitivity, and the false positive rate of a test is equal to 1-sensitivity (Baker, 1995). If a test indicates, for example, presence of disease when disease is absent, this is referred to as a false positive result, type I error or alpha (α) error. If a test indicates disease absence when a diseased state is occurring, this is a false negative result, type II error, or beta (β) error (Jekel et al., 2001). A highly sensitive test is important for identifying ill individuals. A highly specific test is especially useful, when the cost of missing individuals without the disease is high (Faraone & Tsuang, 1994).

An ideal test will exhibit both high sensitivity and high specificity (Kraemer, 1992). However, a test that is only highly sensitive or highly specific is not necessarily a legitimate test. Such terms sometimes fail to show a frame of reference. The predictive value of a test shows the performance of the test (Smith & Slenning, 2000). The positive predictive value of a test describes the probability of that an individual with a positive test result will have a disorder. The negative predictive value of a test refers to the probability of not having a disorder, given a negative test result.

As an example of false negative and false positive test results, consider the use of the Ames test, a classic assay of genotoxicity of chemical substances, specifically, of bacterial mutagenicity to predict carcinogenic effects (Ames et al., 1973). Certain mutations in Salmonella typhimurium render the bacterium unable to synthesize the essential amino acid, histidine, and the mutated bacteria cannot grow in the absence of histidine. Some substances directly, or after metabolic activation by enzymes from mammalian liver homogenates, can additionally mutate the bacteria, which revert to histidine independence.

One theory of cancer is somatic mutation (See Chapter 3). If so, substances which cause mutations in bacteria and in mammals should prove positive in the Ames test and also carcinogenic in lifetime bioassays. Thus, any catalogue of substances tested for carcinogenic effects and substances tested for Ames positive
results should reveal a portion of the tested substances negative in the Ames test but positive in the lifetime rodent bioassay (false negative with respect to the Ames test) or positive in the Ames test, but negative in carcinogenicity tests (false positive with respect to the Ames test). Multiple factors could contribute to the lack of concordance between assays such as the shortcomings of each assay and the distinct criteria needed to give rise to the two measured endpoints (Elespuru, 1996). For example, a substance with activity in bacteria but not in mammals would classify as false positive in this example. For additional information, see results of Ashby and Tennant (1988). Chapter 3 describes tests that have been conducted on cigarette smoke and on the urines of smokers, using bacteria from the Ames test.

### 6.2.5 Combinations of Tests

If a group of tests is to outperform the individual tests, analysts must choose combinations of tests to outperform individual tests not simply based on the sensitivity and specificity, but on the qualities of these values. Combinations of tests will often yield less specific and less sensitive results than individual tests, because the results of different tests may disagree (Kraemer, 1992). Before assuming that a group of tests will perform better than an individual test, the investigator should have knowledge about test efficiency, and the extent to which tests agree. The information value of an individual test depends on the results obtained with other tests in a group. The new combined group result attempts to predict some gold standard, but its information value depends on many factors, including the true positive test rate, the false positive test rate, and the prevalence the gold standard (disease) in the population (U.S. Food and Drug Administration, 1994a).

Test results with Ames test strains, both using liver enzymes and not using liver enzymes, and using strains with sufficient loci representing sites like those involved in mutational processes which cause carcinogenic alterations, should detect almost all substances that cause cancer through somatic mutation. Thus, if all carcinogenic events are somatic mutations caused by exposure to chemical substances in the environment, scientists would expect the Ames test to detect all carcinogens also detected by the whole animal bioassay. However, the Ames test will not detect processes that derange the replication apparatus, such as substances that cause genomic instability or aneuploidy (Barrett & Shelby, 1986). In addition, not all carcinogenic events may involve somatic mutations, even somatic mutations through processes of DNA adduction (Ashby et al., 1989; Elcombe et al., 2002; Moolgavkar & Knudson, Jr., 1981).

Recently, the U.S. Food and Drug Administration (FDA) made progress in computationally predicting the results of Ames tests from structural characteristics of chemicals, by analyzing the results with various chemical structures and individual test strains, instead of using the standard Ames test result, achieved by aggregating the results of several different test strains (Matthews et al., 2000). FDA’s approach is to aggregate the Ames test results after predicting results for individual
Salmonella strains. Thus, the structure-activity relationship with one Salmonella strain is not like the structure-activity relationship of another strain.

Similarly, exposure to substances that selectively kill subpopulations of mammalian cells will increase cell turnover, as damaged organs replace lost cells. In this circumstance, cell replacement can fix normal background mutations, some of which give rise to carcinogenic alterations. Instead of direct mutation, as detected by the Ames test, cell killing could induce cigarette-smoke associated lung cancer (Moolgavkar et al., 1989; Moolgavkar et al., 1993). However, testing for both effects, direct mutation through adduct formation and cell killing, would detect both potential mechanisms of carcinogenicity.

6.2.6 Signal-to-noise

Important goals of any test are to detect, discriminate and describe a signal. The signal is a property that one intends to measure. Noise may be defined as fluctuations associated with the signal. Noise usually arises from multiple sources. In biological testing, it may arise from the sample itself, the equipment used to measure the signal, or factors in the external environment (Sherman-Gold, 1993). The signal-to-noise ratio of a test is, therefore, crucial to the quality of information of the test. This ratio delineates the limitations of the test and provides a framework for interpretation of results. The signal to noise ratio reflects the ability of an assay to detect a signal. A test with a high signal to noise ratio implies minimal distortion and an ability to detect small changes in the signal. Conversely, a low signal-to-noise ratio suggests a compromised ability to discern subtle changes in the signal from large effects of noise. Such measurements will be less reliable. An ideal test will have a high signal to noise ratio.

Given the omnipresence of noise in testing, no test achieves a perfect assessment of its signal. Each test needs to undergo an assessment of the reliability of its data. One approach to such an assessment is to consider the signal analogous to a mean, and the noise analogous to the standard deviation of the mean. Within this framework, the signal-to-noise ratio and the mean to standard deviation ratio become equivalent. This relationship may be expressed as:

\[
\frac{S}{N} = \frac{\bar{x}}{sd}
\]

where S is the signal, N is the noise, \(\bar{x}\) is the mean and sd is standard deviation of the mean. The assessor needs to compare data from the two groups and determine whether the groups differ in a statistically significant way. For example, does the concentration of a biomarker in individuals who smoke cigarettes with an added ingredient differ from the concentration of the same biomarker in individuals who smoke an otherwise identical cigarette without the ingredient? The student’s t-test allows the assessor to estimate whether the two groups of individuals differ significantly with respect to the biomarker.
The numerator of the equation reflects the change in the signal, that is, the difference between the average value of the signal obtained under standard or untreated conditions (e.g., cigarettes with an added ingredient) and the average value of the signal obtained upon deviation from this condition (e.g., cigarettes without the added ingredient). The denominator reflects the average fluctuation of the difference in the average value of the signal. This ratio may be compared to tabled values to determine if the observed difference is too large to be plausibly explained by chance fluctuations.

Experimental design is an important topic involving signal-to-noise consideration. In the presence of noise, assessors need to establish a standard for the level of noise that significantly alters the signal.

6.2.7 Internal Validity

Traditionally, when scientists discuss test validity, they mean internal validity, the ability of a test to predict itself. The ability to repeat tests independently, either in the same laboratory (the original laboratory) or in other laboratories (independent laboratories), and obtain the same results, is the essence of science. The characteristic of internal validity requires good test design as a first step. The scientific validity of tests within a program for testing ingredients added to cigarettes will contribute critically to its acceptance. For example, scientists should find the tests reproducible and accurate. Test performance also should undergo calibration.

Internal validity of tests is a necessary, but not final, step in development of valid tests. Tests may have internal validity, but lack external validity (Omenn & Lave, 1988). Gad and Rousseaux (Gad & Rousseaux, 2002) outlined the criteria for good test design. In this section, LSRO summarizes some commonly discussed elements of internal validity.

6.2.7.1 Experimental details and specifications

The first requirement for replication of a test is adequate description of test methods and materials, such that well-trained but independent scientists can conduct the test. For example, in animal toxicology experiments, the authors must adequately specify the test species and/or strain. Different animal species and strain have different metabolic pathways and rates, sensitivities to toxicants, differences in disposition, lifespan, and background prevalence of diseases (MacGregor et al., 1995). Strain is an important consideration in testing. For example, the A/J mouse strain possesses a genetically based susceptibility to lung cancer (Manenti et al., 1997), whereas the C57BL/6 mouse strain does not (Dontenwill et al., 1973). Knowledge of this strain characteristic helps in experiment design and interpretation of results.
In this report, LSRO has called for investigators to test reference cigarettes in parallel. When reviewing literature that use “cigarettes” as the substrate, it is not possible to discern whether unique characteristics detected are correctly attributed to, for example, the analytical method, or to the brand of cigarette analyzed. In principle, inclusion of reference cigarettes for calibration purposes will prove worthwhile, even when comparing a matched control and experimental cigarettes.

Similarly, LSRO has requested that experimenters use FTC smoking machine conditions as a standard reference method to generate cigarette smoke, when smoking itself is not the subject of investigation, as when mimicking human smoking patterns. The rationale for this request is that although no simple smoke generation conditions replicate human smoking, the FTC conditions are standardized and have been used for many years in many publications. FTC conditions generate mainstream and sidestream smokes for analysis. Thus, a large body of reference information exists about smoke chemistry and physics as generated under standardized conditions. If FTC conditions are included in a paper that uses a different smoke generation system, readers have a much better chance of understanding the experimental setup.

6.2.7.2 Sampling

In epidemiology, sampling refers to the selection of individuals from the population for study participation (Rothman, 1986). Approaches to sampling individuals or materials for studies include restriction, random sampling, and stratifying (Kleinbaum et al., 1982). Restriction refers to the decreasing the eligibility of individuals or materials for inclusion in the study. Random sampling refers to choosing individuals or test samples for a study so that the probability of their inclusion is known (Hansen et al., 1953). In stratified sampling, study participants are organized into subgroups that differ with respect to a covariate which is a source of variability (Chow & Liu, 1998). The goal of sampling techniques is to remove the effect of extraneous factors from affecting the data obtained (Kleinbaum et al., 1982). In probabilistic modeling, elaborate methods exist to attempt to sample data randomly, including Latin square methods. These approaches (and the terminology) also apply to chemical and physical analyses, and to the study materials of any test.

6.2.7.3 Sample size and power to discriminate

Two groups may be compared using the null hypothesis that the two groups are identical for the characteristic of interest. The power of a statistical test has been defined as the probability that a test will not accept a null hypothesis if a different hypothesis is correct (Campbell & Machin, 1993) (See Appendix H). The power of the test is equal to (1 - β), where β is the Type II error. The power of a test may be increased with a larger sample size when α (Type I error) is constant (Sokal &
Rohlf, 1987b). Statistical formulas for calculation of these values are available (Hall & Going, 1999; Jekel et al., 2001).

An important objective in experimental design is to minimize, to as great an extent as possible, Type I and Type II errors and to determine a balance between the two of them that will preclude committing the type of error that is considered to be the more serious.

**6.2.7.4 Adequate and appropriate controls**

Experiments should identify and include appropriate controls. Controls refer to individuals (or materials) who (or which) have similar, if not identical, qualities as the experimental group, differing only in the experimentally manipulated exposure (Friis, 1996). Proper controls reduce the effects of confounding factors. For example, human epidemiological studies should include studies of never smokers as a negative control for health effects observed in current and previous smokers. In determining the relative risk of ingredients added to cigarettes, the obvious control for a cigarette with an ingredient is the identical cigarette with a different amount of the same ingredient.

**6.2.7.5 Gradient of effect and kinetics**

Useful tests often allow investigators to measure a variable signal, such that the investigator can measure a change in signal over time. Because LSRO contemplates the use of “no detectable change” to set excursion limits on the amounts of ingredients in cigarettes, for tests which LSRO wants to investigate, inclusion of exposure-response relationships and pyrolysis products in the test data become important.

**6.2.7.6 Relevant experimental conditions**

Conditions under which experiments are conducted influence applicability of results. For example, to determine precursor-product relationships between cigarette ingredients and other ingredients, experiments should be conducted under conditions relevant to cigarette smoking.

**6.2.7.7 Measurable biological endpoints**

Histopathology is a traditional endpoint for toxicology studies (MacGregor et al., 1995). Approaches are being developed to measure biological endpoints other than histology. Such approaches include the study of damage-inducible (stress) genes, fluorescence in situ hybridization, transgenic animal models, single cell gel electrophoresis, and accelerator mass spectroscopy studies (MacGregor et al., 1995). Since such endpoints may be measurable in both humans and laboratory...
animals, these approaches could yield information about the applicability of animal toxicology data to human health effects.

### 6.2.7.8 Minimization of bias and differential error

Bias, the systematic interference that results in the misrepresentation of a measured effect, may compromise the internal validity of experiments (Greiner & Gardner, 2000a; Last, 1988; Nurminen, 1983). Biases may be divided into three subtypes: selection, information and confounding bias. Selection bias refers to the misrepresentation of the estimated influence of an exposure on a specific health effect (Last, 1988). The source of selection bias is the method employed in choosing individuals from the population to participate in a study (Choi & Noseworthy, 1992). Information or observer bias takes place during the collection of data and results in misassignment to categories or incorrect measurement (Feinleib, 1987). Confounding represents the confusion of the effects of two variables, such that the effect of the variable of interest is partly indistinguishable from the effects of the confounding variable (Rothman, 1975). A confounding variable in an epidemiological study is related to the exposure of interest, and is a disease risk factor. It is not a component of the process between exposure and disease development (Rothman, 1986). Confounding bias arises around data when investigators omit methods for control such as stratification (Choi & Noseworthy, 1992). Incorrect estimation of the effect of exposure occurs from mixing exposure and other factors independently associated with an outcome (Choi & Noseworthy, 1992). Good experimental design and interpretation require careful attention to, and minimization of potential sources of bias. [For a more detailed review of each type of bias, see Choi and Noseworthy (1992)].

### 6.2.7.9 Randomization and control

Randomization refers to a specified probability of individuals in a population to undergo inclusion in an experiment and assignment to a control or treatment group (Glantz, 1992). Random number tables or random number generators may achieve these assignments. Many investigators consider randomization an important aspect of study design. Some describe it as the sole assurance of unbiased selection and obtaining a representative sample of the population (Glantz, 1992). Many kinds of randomization exist. Simple random studies refer to those in which each member of a sampling unit has a similar probability of being included in a study (Forthofer & Lee, 1995). Simple random studies do not guarantee equal representation of members of different groups in the sample. A stratified random sample and/or random block experimental design attempts to correct for sampling that results in unequal representation of members of specific groups. After dividing members of a population into mutually exclusive groups, a within group random sampling is done (Elston & Johnson, 1987b). However, many Bayesian statisticians believe that control, not randomization, is the essential element of scientific experiment design (Howson & Urbach, 1993).
Unlike deliberate control (see above), randomization may control for variables that might influence experimental results but remain unknown to the investigator. Some authorities, however, discount randomization in favor of controlling factors known to influence test outcomes. Blinding is a method of controlling for effects of experimenter or study participant knowledge of treatment (or control) status. When both the experimenter and the participant are unaware of the treatment, the experiment is double-blinded (Elston & Johnson, 1987b).

6.2.7.10 Negative Results

A bias against the publication of scientific articles in which investigators report negative results or results that suggest that a particular exposure will yield minimal or no benefit has been noted (Gad & Rousseaux, 2002). This tendency could contribute to an inaccurate expectation of some process (Gad & Rousseaux, 2002). According to Omenn (2001), for results of epidemiological studies where there is no statistically significant difference between groups, it is important to note the difference between groups that would have been necessary in order to have a statistically significant difference (Omenn, 2001). This type of approach will help to provide a framework for understanding the results of the experiments, and underscores the need for good experiment design.

6.2.8 Interpretation of Data

Whatever the experimental model employed to test ingredients added to cigarettes, some common approaches are employed in the interpretation of results. The importance of data interpretation here relates to setting an excursion limit on the amount of an ingredient per cigarette, which in turn is crucial to providing assurances about the lack of change in risk from smoking a cigarette with the added ingredient.

6.2.8.1 Association, correlation, regression and concordance

Several statistical terms describe the relationships between parameters. When two variables are interdependent, they are associated. Tests of association also are known as tests of independence (Sokal & Rohlf, 1987b). Variables may have distinct types of relationships. One variable may lead to a change in another, changes in both variables may arise from another variable, or changes in the two variables may come from several other variables in common (Sokal & Rohlf, 1987a). Correlation is a measure of the extent to which two variables vary together. (See Appendix B). Variables correlate positively if they have a direct relationship (an increase in one variable relates an increase in the other) and correlate negatively, if they have an inverse relationship (an increase in one variable relates to a decrease in the other). Both association and correlation have formal statistical definitions. Regression refers to the determination of the relationship between variables by expressing one variable as functions of the other variables (Sokal & Rohlf, 1987b).
Concordance is a less formal measure of the agreement or similarity between two or more sets of data. (See Appendix B).

### 6.2.8.2 Classical (qualitative) criteria for causation

In scientific studies, one objective is to learn whether exposures “cause” the observed effects, meaning that elimination of the exposure will eliminate the observed effects (Byrd & Cothern, 2000). In epidemiology, confounding factors make discovery of causality particularly difficult (Elston & Johnson, 1987a). The 1964 Report of the Advisory Committee to the Surgeon General outlined the criteria necessary to show causality, including consistency, strength, specificity, temporal relationship and coherence of the association (U.S. Department of Health Education and Welfare, 1964). Hill later expanded these criteria to include demonstration of a biological gradient, biological plausibility, experimental control, and reasoning by analogy (Hill, 1971).

For example, a temporal relationship, having the exposure precede the effect, is the most important and most accepted component of these causality criteria (Kozlowski et al., 2001a). Consistency of association between event and outcome is another criterion for causality. Here, investigators would probe whether the ingredient produces an effect in only a few studies, in most studies, or in all studies and whether that effect is observed with multiple approaches. Specificity of association provides support for a relationship between an exposure to a specific substance and specific health effect, as opposed to linkage between many substances, especially without apparent chemical linkage, and panoply of effects, especially without some biological linkage. Coherence of explanation describes whether the biological explanation adduced to explain the association between the putative cause and effect is supported by other data and prevailing theories (Susser, 1991). As test data support more of these criteria, they lend more support to causality.

### 6.2.8.3 Bayesian criteria for causality

Many scientists and mathematicians find the above “criteria based” approach to causation unsatisfactory. Except for the temporality criterion, each of the other criteria has known exceptions. More comprehensive and systematic approaches are used to elicit causality from data. Cox (2002) outlined methods to address existing loopholes and proposed an alternative tripartite process to detect causality, which includes evidential reasoning, hypothesis-testing and law-based explanation. Evidential reasoning refers to a comparison of the consistency of the body of evidence in support of causation with the consistency of evidence of non-causation. Hypothesis-testing is the evaluation of whether data supports the larger theory behind the espoused causal theory. Law-based explanation uses known scientific mechanisms to explain the hypothesized causal relationship. Law-based explanation may suggest a causal relationship in isolation (Cox, Jr., 2002). However, statistics approaches also are useful for evidential reasoning and theory testing (Nurminen,
A gradient of evidence supports causation. As the evidence along the gradient increases, evidence for a causal relationship increases (Cox, Jr., 2002). Shared components of the Bayesian and traditional epidemiological approaches are refutation of potential bias and multiple levels of experimentation with the objective to reject alternate explanations of causality.

### 6.3 TESTING METHODS

#### 6.3.1 Programs of Toxicity Testing

The regulation of added ingredients to cigarettes varies. The United Kingdom program for regulation of new additives to cigarettes includes toxicity testing. The Government of British Columbia, requires disclosure of ingredients added to tobacco (Government of British Columbia, 1998). In addition, there are a number of toxicology testing programs in the United States that are overseen by the government and states. These testing and regulatory programs are described below.

The National Toxicology Program (NTP) is an arm of the U.S. Department of Health and Human Services that coordinates toxicology testing conducted by the National Institute of Environmental Health Sciences, the National Institute for Occupational Safety of the Centers for Disease Control and the National Center for Toxicological Research of the Food and Drug Administration. NTP has refined and standardized protocols and statistical analysis of lifetime bioassays. NTP protocols apply to inhalation studies (National Toxicology Program, 2002a).

The U.S. Food and Drug Administration (FDA) provides guidance for toxicological testing of food ingredients for ingestion (U.S. Food and Drug Administration et al., 2001). In addition to providing guidelines for specific types of testing, the FDA provides considerations for statistical and other common problems encountered in reviewing such data. FDA's approach relies extensively on animal models. Although methods for testing foods are not directly transferable to testing ingredients added to cigarettes, the two situations overlap sufficiently, when the test substance is shown to transfer in smoke and when the animal model is appropriate for inhalation. Guidelines for inhalation toxicity testing may also be applicable to studies of added ingredients in cigarettes. For example, De George and coworkers (1997) recommended general guidelines for toxicology studies for respiratory drug development, which provide scientific considerations relevant for the testing of added ingredients in cigarettes.

Testing of ingredients added to cigarettes also may be subject to state laws. Currently, State of Massachusetts (2001) regulations establish procedures for manufacturers to test cigarette smoke from their brands for specified constituents and report them. Massachusetts modified the FTC smoke generation protocol by increasing the required puff volume, puff frequency and number of blocked
ventilation holes. The State of Massachusetts (2001) also requires that manufacturers analyze urinary metabolites in samples from sixty smokers per brand tested. Subjects must have smoked for at least five years, currently smoke at least fifteen cigarettes per day, and smoke the brand tested or a brand with equivalent levels of tar and nicotine, for at least three months. Massachusetts specifies the analytic methods required for measuring human urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, its glucuronide, the urinary benzene metabolite, trans, trans-muconic acid, and additional relevant background information.

The United Kingdom Department of Health established guidelines for approval and permitted use of new additives in tobacco products in the UK (UK Department of Health, 1997; UK Department of Health, 1998; UK Department of Health, 2002). The approval process included a review of toxicological evidence of the ingredient, its pyrolysis products, and the condensate of a reference cigarette containing the new additive. The Department may require biological studies to identify the principal metabolites and to test for genotoxicity and changes in clinical, biochemical, anthropometric and histopathological parameters after inhalation exposure in an animal model. Required tests are determined on a case-by-case basis, depending on the composition, activity and quantity of the added ingredient.

The government of British Columbia has implemented the Tobacco Testing and Disclosure regulation which mandates that Canadian manufacturers of tobacco submit to the Minister of Health Planning a list of all ingredients (including added ingredients) of cigarettes of each brand of cigarette “sold, offered for sale, distributed, advertised or promoted for sale”. Manufacturers are also required to provide annual reports of the levels of specified smoke constituents in each brand of cigarette as well as test the efficiency of the filter and pH for cigarettes that have more than a 1.25% market share of cigarettes sold in British Columbia (Government of British Columbia, 2001).

### 6.3.2 Tests of Smoke Composition and Deposition

The addition of an ingredient to a cigarette might change the chemical and/or physical nature of its smoke. Thus, analyses of smoke chemistry and physics will be important. For example, the size distribution of particles affects the location and magnitude of smoke deposition in the respiratory tract (Baker, 1999). Changes in deposition might alter adverse health outcomes of cigarette smoking.

Burning an ingredient in isolation by heating it in the presence of oxygen and identifying the chemicals produced using sophisticated gas chromatographic mass spectrometry or related instrumentation can identify pyrolysis products. Cocoa powder, a more complex mixture, produced more than one hundred pyrolysis products (Chung & Aldridge, 1992). However, the identification of pyrolysis products after pyrolysis of a pure ingredient does not assure that the ingredient will produce
the substances, when incorporated into the more complex matrix of a cigarette prior to smoking.

The quantitative identification of added ingredients and their pyrolysis products in the cigarette smoke matrix is markedly more difficult than measuring the pyrolysis products of an isolated substance. For example, pyrolysis of an added ingredient containing organic compounds results in cleavage of the molecular compound into one, two, or three carbon units. Theoretically, these ingredients should yield carbon dioxide, formaldehyde, acetaldehyde, acrolein, 1,3-butadiene, and similar smoke components.

As indicated in Chapter 3, tobacco yields thousands of compounds when smoked, including carbon dioxide, formaldehyde, acetaldehyde, acrolein, and 1,3-butadiene. If an ingredient adds only a small increment of a substance to the smoke, the resulting low signal-to-noise ratio would be analytically difficult, if not impossible, to detect. Thus, specification of reasonable limits on detection limits, using modern isotopic labeling and sensitive instrumentation, will be important. Jenkins and coworkers (1977) provided recommendations for standard methods to produce $^{14}$C labeled cigarettes for test purposes. LSRO also will engage in some specification in the Phase Two report of this series.

Data about the pyrolytic fate of the added ingredient during cigarette smoking conditions will be uniformly desirable for all ingredients to understand whether the ingredient changes the amount or type of pyrolysis products in smoke. Strategies for testing added ingredients may differ depending on whether the ingredient is volatilized and transferred directly into smoke or undergoes complete combustion, resulting only in products of pyrolysis and pyrosynthesis.

The conditions for both isolated pyrolysis experiments and machine smoking of cigarettes can differ from those inside a burning cigarette, as smoked by humans. Analytical tests of cigarette smoke provide no definitive information about the biological relevancy of the chemical components of the smoke and only limited information about the potential toxicity of the smoke components. Additional data derived from alternative tests will contribute to an understanding the relative risk of the inhalation exposure of an ingredient and its pyrolysis products.

6.3.3 Tests of Biological Activity and Toxicity

Tests of biological activity have been conducted on cultured cells, tissue samples, isolated organs, model animals, and human subjects. Investigators have studied the acute effects of smoking on human biomarkers, such as altered expression of LFA-1 and HLA-DR II molecules by human alveolar macrophage and lymphocytes obtained from bronchoalveolar lavage fluids (Mancini et al., 1993). Whether these acute changes relate to long-term chronic disease associated with smoking remains
Figure 6.1 Biomarker categories along the continuum from exposure to disease. Reprinted with permission from (Rundle & Schwartz, 2003). Copyright 2003 American Association for Cancer Research.

unknown. At present, no test or group of tests can predict the human response to smoking.

Whole animal models can identify the absorption, distribution, metabolism and excretion of ingredients added to cigarettes within the smoke matrix (Farrell et al., 1980). When kinetic data also are available, physiological modeling of the data to the species of origin will permit meaningful extrapolation to humans, by substitution of human physiological and anatomical variables in the model (Andersen, 1995). Moreover, such models can elicit the effects of an ingredient, if any, on the dosimetry of nicotine or other smoke constituents.

For testing the toxicity of food ingredients, the U.S. FDA recommends a battery of short-term tests to evaluate induction of both gene mutations and chromosomal aberrations (U.S. Food and Drug Administration et al., 2001). The standard three-test battery used to test genotoxicity of a substance includes a test for gene mutation in bacteria, an in vitro cytogenetic test (chromosomal damage in mammalian cells or mouse lymphoma thymidine kinase gene mutation assay), and an in vivo test for chromosomal damage using hematopoietic rodent cells (MacGregor et al., 2000).

Investigators often use short-term genotoxicity tests to indicate whether a substance causes damage to DNA or reduces repair of DNA damage. Chromosomal damage has the potential to induce cancer or lead to an inheritable mutation. Point mutations involving substitution, addition, or deletion of one or a few DNA base pairs have been detected using bacterial tests (U.S. Food and Drug Administration et al., 2001). Many bacterial strains have been used to test for mutagenicity of substances (International Conference On Harmonisation Steering Committee, 1995). Selecting several strains and tests helps reduce the risk of false negative results for substances with genotoxic potential. However, a positive assay result in cell culture does not necessarily indicate that the substance poses a genotoxic/carcinogenic risk to humans.
For \textit{in vitro} evaluation of food ingredients to detect structural chromosomal aberrations, the FDA recommends a functional test, the mouse lymphoma thymidine kinase gene mutation assay, instead of a cytogenetic test of chromosomal damage to mammalian cells. The mouse lymphoma assay measures heritable genetic damage in living cells and can identify test substances that induce either gene mutations or heritable chromosomal events associated with carcinogenesis (U.S. Food and Drug Administration \textit{et al.}, 2001). Heritable chromosomal damage detected by this assay is not necessarily lethal. The International Conference On Harmonisation (1995; 1997a) has reviewed cytogenetic analysis of chromosomal aberrations of hematopoietic rodent cells in bone marrow and peripheral blood samples for the assessment of genotoxicity.

According to the Society of Toxicology (1999), in the absence of human data, research with experimental animals is the most reliable means of detecting important toxic properties of chemical substances and for estimating risks to human and environmental health. Animal models can be useful in evaluating differences in dosimetry, local toxicity (biochemical, functional or morphological changes), systemic toxicity (clinical observation, clinical pathology, necropsy, histopathology) and premature mortality from cigarettes with added ingredients. For the development of drugs intended for human inhalation, general guidelines for animal toxicology studies include consideration of the subject population and the route, level and duration of exposure (DeGeorge \textit{et al.}, 1997; U.S. Food and Drug Administration \textit{et al.}, 2002). The general principles outlined in these documents are relevant to testing the effects of inhaled smoke with animal models.

In testing the carcinogenicity of drugs, the FDA has chosen the experimental approach of coupling one primary long-term carcinogenicity rodent study with one or more other supplemental studies designed to provide additional relevant information that is not easily yielded by the long-term carcinogenicity rodent study. FDA bases inclusion of additional studies on their relevance to the potential human mechanism of carcinogenesis (Klaassen, 2001). Tissue assessment applies morphological, histological and functional criteria to determine whether the substance has caused changes at the cellular level (International Conference On Harmonisation Steering Committee, 1997b). The National Toxicology Program (2002a) described five categories of evidence for reporting results of carcinogenic activity: clear evidence, some evidence, equivocal evidence, no evidence and inadequate study. Overall evaluation of the carcinogenic potential of a substance would include consideration of the kinetics of the substance in the animal model, tumor incidence and latency, relative carcinogenic and toxic potencies, dose-response characteristics, and an evaluation of relevance to human carcinogenicity.

In a review, Coggins (2002) emphasized that long periods of exposure to high concentrations of cigarette smoke did not induce the cancers evident in epidemiological studies of human smokers during inhalation studies with animal models (rats,
mice, hamsters, dogs, or nonhuman primates). Differences in laboratory animal responses between cigarettes with added ingredients and similar cigarettes without added ingredients would interest LSRO. Since animal models can lack predictivity for the endpoints of interest, of cigarette-related human diseases, LSRO will restrict the interpretation of such results to the species tested.

The National Cancer Institute conducted three series of experiments to investigate differences in the tumorigenic activity of cigarette smoke condensate collected from machine smoked cigarettes (Gori, 1977). Reference cigarettes included the 1R1 University of Kentucky cigarette and standard experimental blends (33% flue-cured, 27% reconstituted sheet, 20% Burley, 11% Turkish, 1% Maryland tobaccos, 5% invert sugar, 3% glycerol) that resembled the 1970 market blend but varied by crop year of tobacco used. Either 12.5 mg or 25 mg of dry smoke condensate in 0.1 mL of solution was applied daily to mouse skin, i.e., skin painting bioassay, for 18 months or until death. In addition, some mice received only vehicle control (acetone), negative control (hair clipping) and positive control (benzo[a]pyrene in acetone) treatments. Tumorigenic activities of the condensates of the standard experimental blend (SEB III) of cigarette (1) with humectant, without sugar (2) with sugar, without humectant, (3) without sugar and humectant (4) with powdered cocoa and without sugar and humectant (5) with magnesium nitrate (6) with zinc oxide and (7) with magnesium nitrate and zinc oxide were determined. There was statistically significant less tumor growth when mouse skin was painted with 12.5 mg of condensate of SEB III cigarettes plus magnesium nitrate (at least a 5% confidence level) as compared to SEB III with humectant and without sugar; SEB III without sugar and humectant; SEB III with zinc oxide or SEB III with powdered cocoa, without sugar and humectant (12.5 mg and 25 mg condensate). When mouse skin was painted with 25 mg of condensate of SEB III with powdered cocoa, without sugar and without humectant, there were significant differences in number of tumors as compared to SEB III without sugar, without humectant and SEB III with magnesium nitrate and zinc oxide. Cigarette concentrate (25 mg in 0.10 ml) from smoke of cigarettes that contained powdered cocoa but were without sugar and humectant were significantly more tumorigenic than smoke from cigarettes that lacked both powdered cocoa and humectant or contained magnesium nitrate and zinc oxide (Gori, 1977). Statistically significant differences in tumorigenesis were found between groups of mice painted with condensate of SEB III without sugar, without humectant and SEB III with sugar and without humectant. In addition, there was a statistically significant difference in tumorigenesis between groups of mice painted with condensate of from cigarettes with zinc oxide and 1R1 cigarettes.

Roemer and Hackenberg (1990) also investigated whether addition of cocoa alters biological effects of cigarette smoke condensate. All experimental cigarettes were American blend filter cigarettes made with 36.5 % Flue-cure tobacco, 23.7% Burley tobacco, 12.4% Oriental tobacco and 27.4% reconstituted tobacco. Whereas the
reconstituted tobacco used in the NCI experiment (Gori, 1977) consisted of 43% flue-cured stems, 18% flue-cure scrap, 28% Burley stems, and 12% Burley scrap. The reconstituted tobacco in this experiment was composed of 26% flue-cured stems, 9% flue-cure scrap, 57% Burley stems, and 8% Burley scrap. To the tobacco, 0, 1, or 3% cocoa powder was added. Cigarettes were smoked using a smoking machine. Smoke condensate from each type of cigarette was collected separately and three concentrations of cigarette smoke condensate from each type of cigarette were prepared. Mice were randomly divided into treatment groups. After an 8 week dose adaptation period, each mouse received five applications per week of either acetone (control) or a total of 60, 90, or 125 mg dry cigarette smoke condensate prepared from one of the 3 types of cigarettes to an area of the back that was clipped twice weekly. Mice were monitored for general health status, behavior, tumor or other lesion development and mortality for 75 weeks.

No differences were noted in the general health and behavior of mice treated with acetone and condensates from cigarettes with varying amounts of cocoa nor between condensates from cigarettes with varying concentration of cocoa. Based on these results, Roemer and Hackenberg (1990), concluded that there is no significant difference in the biological activity of condensates from cigarettes with the concentration of cocoa tested in this study and condensate from cigarettes without cocoa.

Multigenerational rodent studies have been used to test the reproductive toxicity and teratogenicity of a substance by examining its effect on reproductive systems of males and females, postnatal maturation of offspring and the reproductive capacity of offspring (U.S. Food and Drug Administration et al., 2001). Multigeneration studies measure parameters of reproductive and developmental health, including estrous cycles, conception, parturition, neonatal growth and mortality. Study designs include direct exposure of the parents, in-utero exposure followed by direct exposure of offspring, in-utero only exposure of the pups born to those offspring, and concurrent controls. Treatment consists of exposing parental male and female rodents to the test substance during the period of mating (conception), gestation and through weaning of offspring. Randomly selected rodents from the first litters of offspring are continuously exposed to the test substance from weaning through their adult mating period. Of this group, randomly selected pregnant females continue to be exposed until the weaning of their pups. Some of the pregnant females is euthanized one day prior to the expected date of parturition and fetal pups are examined for fetotoxic effects of the test substance. Primary manifestations of developmental toxicity in the rodent fetus are: death during gestation, structural anomaly, altered growth and functional deficiency. The dose, timing of exposure and duration of exposure contribute to the developmental toxicity of a substance.
A preliminary study with pregnant animals may be required to determine whether the substance’s toxicity varies according to reproductive status and to select three levels of test doses. A sufficiently high dose is necessary to elicit reduced body weight or rate of weight gain in the parental rodents, but not cause more than 10% parental mortality. The lowest dose should not produce any observable adverse effects in parental rodents.

Cigarette smoke is associated with early menopause in women. Hoyer and coworkers (2001) speculate that this may be due to the destruction of oocytes by contaminants in smoke, which are known ovotoxicants. Once the oocyte is damaged, the result is ovarian failure because the oocyte cannot be regenerated. Hoyer and coworkers (2001) developed a generalized rodent model to study ovotoxicity, which may be of use in testing the reproductive effects of added ingredients to cigarettes.

Cigarette smoking increases the risk of delivering low birth-weight offspring. In a recent review, Carmines et al. (2003) summarized data on effects of exposure to cigarette smoke. Studies suggest that babies whose mothers smoked during pregnancy weigh less and are smaller for gestational age than babies whose mothers did not smoke during pregnancy (U.S. Department of Health Education and Welfare, 1964; Working Group Party of the Royal College of Physicians, 1992). While some studies report no association between maternal smoking habit and malformation of offspring (Malloy et al., 1989), other studies suggest an association between cigarette smoking and oral clefts (Beaty et al., 1997; Werler et al., 1990) and the possibility of a weak association between maternal smoking and congenital reduction limb defects in children (Aro, 1983; Czeizel et al., 1994; Kallen, 1997).

Numerous groups report that female rats or mice that are exposed to cigarette smoke give birth to pups of lower birthweight than those not exposed to cigarette smoke (Carmines et al., 2003; Essenberg et al., 1940; Reznik & Marquard, 1980; Seller & Bnait, 1995) while others report no effect of maternal smoking on birthweight of offspring (Bertolini et al., 1982; Peterson et al., 1981; Reckzeh et al., 1975; Wagner et al., 1972). One rabbit study revealed a decrease in birthweight due to exposure to cigarette smoke (Schoeneck, 1941). Rodent studies also report developmental delays (Seller & Bnait, 1995) and malformations, while other studies report no malformations (Bertolini et al., 1982; Peterson et al., 1981; Reckzeh et al., 1975). It is generally held that cigarette smoking does not lead to congenital malformations in offspring (Schardein, 1985). Further characterization of the relationship between cigarette smoking and birth outcomes are needed (Carmines et al., 2003).

Neurotoxins directly and indirectly elicit diverse biochemical, structural and functional abnormalities (U.S. Food and Drug Administration et al., 2001). For example, seven chronic smokers with toxic rhinitis had changes in their nasal nerve mucosa

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compared to control subjects without toxic rhinitis, who did not smoke tobacco products (Groneberg et al., 2003). Specifically, smokers with toxic rhinitis had increased expression scores for neuropeptide tyrosine and vasoactive intestinal peptide in mucosal nerve fibers. U.S. FDA suggests a tiered approach to testing substances for neurotoxicity, which screens substances for potential neurotoxic effects, followed by more in-depth testing of the screen positive substances to characterize effects and dose-response relationships (U.S. Food and Drug Administration et al., 2001). Screening tests should encompass a variety of pathological changes and functional disorders of the nervous system. Components of the neurotoxicological screen can be integrated into other toxicological testing.

Accumulating evidence implicates cigarette smoking in immunosupression, overactivation of the immune system and promotion of autoimmune reactions (Gardner, 2000). Sopori and Kozak (1998) concluded that chronic exposure to cigarette smoke was associated with T cell unresponsiveness in human smokers. A number of studies demonstrate enhanced leukocytosis (Corre et al., 1971; Silverman et al., 1975; Tollerud et al., 1989), increased percentages of total T lymphocytes (Ginns et al., 1982) and decreased natural killer cell activity (Ferson et al., 1979; Hersey et al., 1983; Hughes et al., 1985; Tollerud et al., 1989) in cigarette smokers. Data on effects of cigarette smoke on immunoglobulin levels show inconsistent results (Gardner, 2000). Decreased levels of IgA have been reported in rats after inhalation exposure to cigarette smoke (Miller et al., 1996) and in smokers (Barton et al., 1990; Sato et al., 1993; Sopori et al., 1989). Cigarette smoking could therefore lead to increased susceptibility to respiratory infections (Gardner, 2000).

There are conflicting data about mechanisms of immunotoxicity. Whether cigarette smoke inhibits or stimulates mucociliary clearance is not known (Gardner, 2000). Reviews of data suggest that the effects of factors such as age, sex, and smoking intensity and duration are not consistent (Johnson et al., 1990) and the severity and breadth of the effects of smoking cigarettes on the immune system have not been fully described (Johnson et al., 1990). Tiered approaches to screen chemicals for immunotoxic effects have been outlined (Karol, 1998; National Research Council, 1992). A major limitation to detecting changes in immune system function is that a healthy individual typically has a large functional reserve, therefore, only a major impairment may reveal an effect on immune function. Additional information about the effects of cigarette smoking on the immune system would be beneficial.

Only human studies can generate relevant data about variations in smoking behavior in response to added ingredients. Without considering other factors, such as acceptability, an ingredient added to a cigarette in an amount that changed the number of cigarettes smoked in a detectable way would fall within the scope effects considered undesirable in this report.
Short-term clinical studies with designs limited to the assessment of other biological effects may be useful on a case-by-case basis for specific ingredients, depending on the organ system affected. For example, a useful animal model of the human cardiovascular or respiratory system may not be available. The assumption underlying the use of short-term clinical studies is that the changes observed, if any, will relate to changes observed after chronic exposure to smoke from cigarettes with added ingredients.

Detection of biomarkers of effect could have great clinical significance, allowing for early detection of predisposing lesions, especially if measuring the biomarker were non-invasive (Srinivas et al., 2001). Once discovered in humans, biomarkers would open research agendas with animal models. Researchers often have suggested potential biomarkers, for example, lipid peroxidation or markers of platelet activation as early events in the pathways of cardiovascular and lung disease. Clearing the Smoke contains a list of potential biomarkers of harmful effects of cigarette smoking including several strengths and limitations of each (Institute of Medicine, 2001).

Lacking suitable animal models, only observational studies of chronic smoking can reveal human health effects associated with ingredients added to cigarettes. Epidemiologic studies of smokers identified the diseases associated with smoking and enabled quantification of the health risk of exposure to cigarette smoke (Thun et al., 2002). The benefits and limitations of retrospective and prospective epidemiological studies for assessing the risks and toxic effects of substances have been discussed from a regulatory perspective (Byrd & Cothern, 2000; U.S. Food and Drug Administration et al., 2001). General guidelines exist for the design and reporting of epidemiological studies, including selection of study populations, control subjects, assessment of exposure, control of confounding variables and statistical analyses (Verhalen et al., 1981). Some of the limitations of such approaches are discussed below.

Observational studies of chronic smoking must include adequate information about exposure. Leffondre and coworkers (2002) reviewed outcomes associated with smoking among ethnic and regional populations. Measures of smoking history varied markedly. Some investigators reported a simple categorization of smoking status (never, smoker, former smoker). Others provided detailed information about smoking intensity (i.e., cigarettes per day) and duration. Even studies that reported smoking history in similar units analyzed the data differently. They suggest that reported smoking intensity and duration be treated as separate variables. Simmons (2002) emphasized the importance of obtaining yields directly from smokers by collecting data about the length and amount of each cigarette typically smoked and by including an index of body mass index. Lee (2001) also reported considerations for improving the design and analysis of epidemiological studies of smokers.
Epidemiological studies are capable of providing sufficient information to evaluate the added risk of an ingredient added to a brand of cigarette. Such studies will have limitations, as even to detect large differences, they will impose immense logistical demands, require prolonged exposure periods, and need large study populations. Harris (1993) cautioned that human epidemiological studies are not practical for short-term assessment of small differences in the toxic effects of various cigarette prototypes.

Data from previously conducted studies, particularly multiple studies investigating a similar outcome can be reanalyzed in combination using meta-analysis. Meta-analysis is a method of analyzing results from different studies without the need to access raw data from experiments (Putzrath & Ginevan, 1991). Such analyses are conducted with the assumption that combining data from multiple experiments will provide more accurate information about exposure-response relationships than the analysis of individual experiments. Meta-analysis may improve statistical power, identify differences in effects of studies and research needs (Blair et al., 1995). The use of appropriate statistical procedures in meta-analysis protects against incorrect analysis of results (Putzrath & Ginevan, 1991). Among others, Thacker (1988), Sacks and coworkers (1987), and Blair and coworkers (1995) have published recommendations for conducting meta-analysis. In a regulatory context, meta-analysis can provide useful data integration (U.S. Food and Drug Administration et al., 2001). For example, Lee (2001) conducted a meta-analysis of 54 epidemiological studies, each reporting over 100 lung cancer cases with the objective of examining effects of type of cigarette smoked. Lung cancer frequency was lower in smokers of filtered than plain cigarettes. This review usefully illustrates the limitations on detecting differences in effects on smokers of different kinds of cigarettes.

6.3.4 Overall Programs and Methods for Testing

Except for direct observation (epidemiological studies), no tests correlate with human health effects of smoking cigarettes. Therefore, the contribution of any program of toxicity testing in cell culture or animal models of cigarettes with added ingredients would arise from the detection of potential novel effects, added to the existing adverse health effects associated with cigarette smoking. Limitations exist to the ability to observe the human health consequence of adding ingredients to cigarettes, which need to be considered when developing review criteria for testing the health effects of added ingredients to cigarettes. (See Appendix H). Cigarette smoke is a complex, highly variable, chemical mixture of gaseous and particulate agents formed by the combustion of cigarette ingredients. Therefore, detecting effects of exposure at low concentrations of mixtures, examining interactions among components in smoke, and identifying components of smoke that cause disease is challenging (Samet, 1995).
6.4 SOME TEST DATA: MENTHOL AS AN EXAMPLE

6.4.1 Background Information About Menthol

Eccles (1994; 2000) published authoritative reviews of menthol. The Joint FAO/WHO Expert Committee on Food Additives (1976) published a Toxicological Evaluation of Menthol and promulgated an acceptable daily intake for humans of 0 to 0.2 mg/kg body weight, obtained from a no-adverse-effect-level of 200 mg/kg body weight/day (derived from an unpublished 1961 German 5.5 week rat feeding study and a safety factor of 1000). The Cosmetic Ingredient Review Expert Panel reviewed the safety of peppermint for use in cosmetics and included a discussion of menthol, as a component (Nair, 2001). Other reviews of menthol toxicity have been published (TNO Bibra International Ltd., 1990).

The National Toxicology Program summarized lethal doses for different species and routes of exposure to dl-menthol, including rat oral LD$_{50}$ = 2,900 mg/kg and mouse LD$_{50}$ = 3,100 mg/kg (National Toxicology Program, 2002b). Bernson and Pettersson, (1983) studied the toxicity of menthol in several short-term assays and found 50% inhibitory concentrations for the cellular and subcellular systems ranged between 0.32 mM and 0.76 mM. Perfetti and coworkers (1993) summarized doses of menthol corresponding to irritation or lack of irritation of skin or eyes of rabbits and guinea pigs. Mentholated products, including cigarettes, are associated with sensitivity reactions in humans (Martindale, 1996; McGowan, 1966; TNO BIBRA International Ltd., 1990). Menthol at concentrations of 2%-5% can have an irritant or local anesthetic effect (Eccles, 1994; Lu et al., 1992). Among 877 patients with contact dermatitis or unclassified eczema in Poland, 1% tested positive at 48 h or 96 h after application of a skin patch containing 5% menthol (Rudzki & Kleniewska, 1970).

The biological action of menthol in producing a cooling sensation is mediated through members of the TRP family of receptors, specifically TRPM8 (Peier et al., 2002). Menthol and capsaicin interact with the same receptors, although capsaicin produces a sensation of heat. TRPM8 receptors occur prominently on nerve fibers of dorsal root ganglia in the spinal cord (Zuker, 2002).

Manufacturers add menthol (C$_{10}$H$_{20}$O, m.w. 156) to flavor cigarettes, to distinguish brands, to reduce throat irritation, and to impart aromas (Fisher, 1999). Both natural and synthetic menthol are used to flavor smoking tobacco (Gutcho, 1972). Menthol is widely distributed in herbs (fennel, coriander). It is obtained commercially from oils on the leaves of plants in the mint family, Mentha aversis or synthesized by hydrogenation of thymol (Budavari, 1989). Standard regulatory methods for colorimetric and gas chromatographic analyses of menthol in cigarette filler have been published (AOAC, 2000). Menthol is highly lipophilic (Wokes, 1932).
Menthol is metabolized in the mammalian liver by conjugation to a glucuronide (Eccles, 1994).

Menthol is a “Generally Recognized As Safe” (GRAS) flavoring additive for food. It is added to baked goods, frozen dairy products (e.g., ice cream), candies, puddings, alcoholic and non-alcoholic beverages, and chewing gum. The U.S. FDA permits the addition of menthol to over-the-counter, non-prescription drug products, however, due to inadequate data to establish safety and effectiveness, the FDA no longer permits manufacturers to add menthol as an ingredient in over-the-counter drugs used for smoking deterrence (U.S. Food and Drug Administration, 1994b).

Efforts to determine the amount of menthol that would evoke a 50% reduction in respiratory rate by Swiss-Webster mice differed by less than four-fold depending on the method used to estimate exposure (Alarie, 1986). The reduction in respiratory rate was calculated by subtracting the minimum respiratory rate during 30 minutes of exposure from the rate at baseline prior to exposure. In all cases, recovery to baseline respiratory rate was incomplete for 10 minutes following exposure. Mean percent reduction in respiratory rate for the seven levels (n = 4/group) ranged from 16% to 52%. However, only one group of mice reduced their respiratory rate by as much as 50%.

Menthol was first added to cigarettes in the 1920s (Covington & Burling, 1981; Gaworski et al., 1997). Substantial effort in the form of patented technologies did not occur until the 1960s (Perfetti et al., 1993). Presently, menthol is added to inner foil wrappers, to filters, or to tobacco during the latter stages of the manufacturing process. Because menthol is volatile, it does not remain where applied. For example, when 2.7 mg menthol was applied directly to tobacco, only 70% remained on the tobacco after four weeks of storage, and when the same amount was applied to the cellulose acetate filter tow of a cigarette, 40% migrated to the tobacco by four weeks of storage (Riehl et al., 1972).

For cigarettes containing between 2 and 3 mg of menthol, approximately 20%, 30% and 40% migrated from the tobacco rod to the cellulose acetate filter by the first, second and eighth month, respectively, of storage at room temperature with individual cigarette packs contained in cartons (Brozinski et al., 1972). Alternative menthol-like flavorants were developed and tested to reduce the amount of menthol lost during manufacturing and storage. Examples include 1-menthyl chlorocarbonate, menthyl acetoacetate, and benzaldehyde dimethyl acetal (Gutcho, 1972).
6.4.2 Effect of Smoking on Menthol

Several studies provide examples of heating menthol, in isolation and measuring the product yield. Van Duuren and coworkers (1968), reported results of thermal degradation experiments of approximately 1 g of dl-menthol heated in a tube for 5 minutes. Menthol did not pyrolyse at 200-500 °C, and more than 98% remained at 500-700 °C. The authors concluded that brief pyrolysis of menthol does not yield any appreciable amount of degradation products. Schmeltz and Schlotzhauer (1968) reported lower recoveries of unreacted menthol with higher temperatures of pyrolysis. They recovered nine times more unreacted menthol at 600°C than 860°C. Menthol produced six times more phenol at the higher temperature than the lower temperature. The predominant pyrolysis products at 860°C were benzene, toluene, styrene, naphthalene, and ethyl benzene.

With a boiling point of approximately 100 °C, menthol is highly volatile. Theoretically, added menthol would be swept up from tobacco downstream from the fire cone and transferred directly into smoke rather than being subjected to 600 to 950 °C temperatures in the combustion zone of a burning cigarette (Eble et al., 1987). Therefore, little menthol would be available for pyrolysis at the higher temperatures present in a burning cigarette. Eble and coworkers (1987) determined that the maximum transfer of menthol from unburned tobacco occurred 5 mm from the fire cone and that transfer declined linearly up to 30 mm from the fire cone. These data show that menthol transfers in cigarette smoke. The results of Van Duuren and coworkers (1968) also are consistent with the transfer of menthol in smoke. Using four commercial cigarette brands containing 1.6 to 2.2 mg menthol, Mitchell and coworkers (1963) reported that 19% to 26 % of menthol in each cigarette transferred into mainstream smoke, as measured in the total particulate matter fraction by gas-liquid chromatography.

Riehl and coworkers (1972) applied 2.7 mg menthol to cigarettes during manufacturing and stored them up to seven weeks. After 28 days, some of the cigarettes were smoked under FTC conditions and mainstream smoke total particulate matter was collected and analyzed for menthol. Approximately 0.43 mg menthol (16% of total application) from the smoked cigarette (FTC protocol) transferred to mainstream smoke particulate matter. However, the rate of transfer differed by location of initial application. For menthol applied to tobacco, transfer to mainstream smoke increased in a uniform manner from approximately 40 µg/puff for puffs 1-3 to approximately 58 µg/puff for puffs 7-8. For cigarettes in which the menthol was applied to the filter, the transfer of menthol into mainstream smoke was lower, approximately 35 µg/puff for puffs 1-3, but higher, approximately 90 µg/puff for puffs 7-8. Riehl and coworkers (1972) hypothesized that the temperature of the filter and the concentration and surface area of the aerosol, all factors that increase with increasing puff number, influence the transfer of menthol into mainstream smoke.
Jenkins and coworkers (1970) used 1.1 μCi of biosynthesized [14C]menthol, added to the tobacco of individual cigarettes, to measure the distribution of menthol in smoke and butt. They added additional, nonradioactive menthol to adjust to a constant 3 mg per cigarette. After a four-week equilibration period, machines smoked the cigarettes. For [14C]menthol added to cigarettes, 44% was detected in sidestream smoke, 29% in mainstream smoke and 27% remained in the butt. They found most (96.5%) of the menthol in mainstream smoke in the total particulate matter. Consistent with the results reported by Riehl and coworkers (1972), the per puff activity of [14C]menthol in total particulate matter of mainstream smoke increased linearly as puff count increased from puffs 2 through 8 (Jenkins, Jr. et al., 1970). Nearly all (98.9%) of the total mainstream activity of 14C smoke product was identified as [14C]menthol. Of the remaining 14C activity in mainstream smoke, 0.1% was in the form of carbon dioxide, 0.2% in menthane, 0.4% in menthone (an impurity in the source of menthol) and 0.3% in unidentified constituents (Jenkins, Jr. et al., 1970).

6.4.3 Effect of Menthol on Smoke Chemistry and Physics

Menthol changes smoke chemistry. Application of menthol at 0.12% to 1.31% wt/wt to cigarettes had no effect on yields of tar and nicotine in the total particulate matter of mainstream smoke (Perfetti & Gordin, 1985). However, the addition of 1.8% menthol in a mixture with cocoa shells, licorice extract and corn syrup decreased nicotine yield compared to a control cigarette containing the same tobacco blend (35% Bright, 23% Burley, 15% Oriental, 27% reconstituted) (Rustemeier et al., 2002). The added mixture also increased tar significantly by at least 20% and total particulate matter by at least 16%. Whether these changes resulted from menthol, another ingredient, or alterations in the amount of tobacco is unclear.

6.4.4 Biological Activities of Menthol in Cigarette Smoke

6.4.4.1 Cell cultures and tissues

Cigarette smoke has genotoxic effects. For example, cigarette smoke condensate and smoke bubbled through buffered saline solution increases sister chromatid exchange in Chinese hamster ovary cells (1989). Several investigators have failed to find alterations in the genotoxicity of cigarette smoke induced by menthol. In a preliminary report, no significant differences occurred in Ames, sister chromatid exchange, or cytotoxicity of mainstream smoke condensate from mentholated and nonmentholated cigarettes (Bombick et al., 2001). The mutagenic response of Salmonella strains following exposure to total particulate matter collected from cigarette smoke did not differ between results from cigarettes with menthol (1.8%), added as a mixture in combination with cocoa shells, licorice extract and corn syrup, compared to results from a control cigarette containing the same tobacco blend (35% Bright, 23% Burley, 15% Oriental, 27% reconstituted) but without added ingredients (Roemer et al., 2002). Similarly, the cytotoxic response of mouse embryonic cells following exposure to gas phase and total particulate matter
collected from cigarettes did not differ between results from cigarettes with menthol (1.8%), added as a mixture compared to results from a control cigarette without added ingredients (Roemer et al., 2002).

Using human epithelial tissue within 24 h of excision and rabbit and rat cross-sectional tracheal rings within 1 h of extraction, Rakieten and coworkers (1952) observed microscopically that l-menthol (0.4 mg/cc) had little effect on epithelial ciliary activity. Effects on activity of respiratory cilia did not differ between exposures to mentholated and nonmentholated cigarette smoke collected in Locke-Ringer’s solution.

6.4.4.2 Animal models

Gaworski and coworkers (1997) conducted an animal study to determine whether menthol in cigarettes would affect respiratory tissues. Rats exposed to mentholated cigarette smoke did not differ in weight gained at any smoke concentration (target smoke particulate matter of 200, 600 and 1200 mg/m³) compared to rats exposed to non-mentholated cigarette smoke. In contrast, an exposure-related increase of 26% and 50% occurred in the incidence of clear nasal discharge for the non-mentholated group exposed to target smoke total particulate matter concentrations of 600 and 1200 mg/m³. This effect was not observed in rats exposed to mentholated cigarette smoke.

Vanscheeuwijck and coworkers (2002) generated mainstream smoke, using ISO protocols, from cigarettes with menthol (1.8%), added as a mixture in combination with cocoa shells, licorice extract and corn syrup, and control cigarettes containing the same tobacco blend (35% Bright, 23% Burley, 15% Oriental, 27% reconstituted) but without added ingredients. They diluted the smoke with fresh air at a rate of 1:150 to 1:170 depending of the yield of total particulate matter. Sprague-Dawley rats were exposed (nose only) to either smoke from the menthol mixture (four groups; two each male and female) or control smoke (two groups; one each male and female) at a concentration of 150 µg/L for 6 h/d, 7 d/wk for 90 days. No differences were evident for respiratory parameters, blood carboxyhemoglobin or urinary metabolites of nicotine between the menthol mixture and control groups. No significant differences occurred in ophthalmologic or hematologic observations, except for one group of female rats exposed to the menthol mixture that had lower neutrophil counts compared to the female control rats. Absolute and relative weights of the thymus were significantly greater for both male groups and one female group exposed to the menthol mixture compared to control groups. Rats exposed to the menthol mixture did not have an increased incidence of histopathology in nasal, laryngeal, tracheal, bronchial or lung tissues compared to control rats.

Vanscheeuwijck and coworkers (2002) did find that male rats in the menthol mixture group had significantly increased blood concentrations of total cholesterol and serum
urea. Other studies reported decreased serum cholesterol among non-mentholated cigarette smoke-exposed female rats relative to air controls (Heck et al., 2002) and rabbits injected with nicotine-free cigarette smoke extract (Yamaguchi et al., 2000). Similarly in humans, children with long-term passive cigarette smoke exposure have lower high density lipoprotein cholesterol concentrations suggesting the possibility that cigarette smoke exposure may negatively affect cholesterol (Moskowitz et al., 1999). Menthol inhibits lecithin-cholesterol acyltransferase activity (Cooney et al., 1984) and 3-hydroxy-3-methylglutaryl coenzyme A reductase activity (Clegg et al., 1982).

6.4.4.3 Absorption, deposition, metabolism and elimination of smoke constituents

Under experimental conditions, menthol is known to increase dermal drug permeation (Beutner et al., 1943; Giannakou et al., 1998; Heng, 1987; Kommuru et al., 1999; Lu et al., 1992; Morimoto et al., 2002; Shojaei et al., 1999; U.S. Environmental Protection Agency, 2002; Zanker et al., 1978). It has been proposed that menthol may increase the risk of adverse health effects from smoking by increasing uptake of cigarette tobacco smoke substances (Kobayashi et al., 1994; Miller et al., 1994).

MacDougall and others (2003) investigated the effect of menthol on nicotine metabolism. Incubation of liver microsomal fractions with menthol was found to inhibit nicotine oxidation to cotinine. This effect of menthol on nicotine metabolism could lead to an increase in risk of adverse health effects for menthol cigarette smokers (MacDougall et al., 2003). Metabolism of the cigarette flavoring ingredient, coumarin, by coumarin 7-hydroxylase, was also inhibited in the presence of menthol. Additional research on the effects of menthol in cigarette smoke on the deposition, clearance and absorption of other smoke constituents is needed.

6.4.4.4 Biomarkers of exposure

Gaworski and coworkers (1997) measured several biomarkers of exposure in rats exposed to menthol in cigarette smoke. Groups of male and female rats were exposed (nose only) to filtered air (control) or smoke from American style filter cigarettes, with or without 5000 ppm of synthetic l-menthol, at target mainstream smoke total particulate matter concentrations of 200, 600, or 1200 mg/m³. The frequency of exposure was 30 minutes, twice per day, five days per week for 13 weeks, followed by a six-week non-exposure recovery period. Serum nicotine and cotinine concentrations did not differ between rats exposed to the smoke from non-mentholated cigarettes and mentholated cigarettes except for a lower cotinine concentration in rats exposed to mentholated smoke at the 200 mg/m³ smoke concentration. In their animal model study, Gaworski and coworkers (1997) used smoke concentrations that correspond to v/v dilutions of less than 5% of the mainstream smoke. In contrast, cigarettes smoked by humans produce a
concentrated smoke. The FTC tar yields with total volumes of 10 puffs at 35 ml each yield 40,000-60,000 mg/m³ for mainstream smoke. The menthol concentration in typical American blend cigarettes can be more than three times higher than in this study.

Lugton and coworkers (1978) measured conjugated l-menthol in urine samples from five smokers who consumed 18-23 mentholated cigarettes over an eight-hour period. Conjugated l-menthol was not detected in urine samples collected prior to the smoking period. Maximal excretion of conjugated l-menthol occurred between the fourth hour of smoking and the fourth hour post-exposure for four of five subjects.

Miller and coworkers (1994) injected commercially available cigarettes (1992 FTC yield: 1.1 mg nicotine, 16 mg tar, 14 mg carbon monoxide) with 0 mg, 4 mg, or 8 mg of menthol. They provided these experimental cigarettes to smokers in random order, for three controlled-exposure sessions, conducted one week apart. Subjects were African American male smokers (n = 12) participating in a substance abuse program. Six subjects identified themselves as smoking mainly mentholated cigarettes and the other six subjects identified themselves as smoking mainly regular cigarettes. Following an overnight abstinence from cigarettes, confirmed by a 70% decline in expired carbon monoxide level, subjects drew puffs at 30-second intervals from an experimental cigarette until they inhaled a total of 600 mL of smoke. Breath samples were collected, the smoking protocol was repeated, and final breath samples were collected. On average, subjects had to draw 18 puffs to inhale 1,200 mL of smoke. The change in expired carbon monoxide from before to after the 1,200 mL total inhalation volume, increased linearly as menthol dose increased (p < 0.01). Subjects who preferred mentholated cigarettes at baseline had a larger increase in expired carbon monoxide, (7.6 ppm) vs. subjects that described themselves as having a preference for regular cigarettes (5.6 ppm).

Ahijevych and coworkers (2002) examined the association of menthol with some exposure parameters in women. In contrast to the report by Miller and coworkers (1994), subjects who preferred nonmentholated cigarettes at baseline had a greater increase in expired carbon monoxide than subjects smoking mentholated cigarettes. Baseline plasma nicotine and cotinine concentrations did not differ significantly between smokers of mentholated and nonmentholated cigarettes. However, a more recent study by Ahijevych & Parsley (1999) contradicted these findings and indicated that women smoking menthol cigarettes had significantly higher blood cotinine concentrations than women smoking nonmentholated cigarettes. Results were not adjusted or controlled for nicotine intake. The more recent report also differed in women smoking mentholated cigarettes who had significantly larger puff volumes than women smoking nonmentholated cigarettes. Clark and coworkers (1996) found higher blood cotinine concentrations among menthol cigarette smokers (n = 76) than nonmenthol cigarette smokers (n = 85) after adjusting for ethnicity,
cigarettes smoked per day, and the mean amount of each cigarette smoked. Menthol smokers may smoke cigarettes with higher nicotine yields or smoke cigarettes to obtain greater absolute yields of nicotine.

6.4.4.5 Smoking behavior

African-American smokers prefer mentholated cigarettes. Among 1424 adult smokers in four states, 89% of African Americans reported smoking mentholated cigarettes compared to 30% of Caucasian smokers (Wagenknecht et al., 1990). Benowitz and coworkers (1999) reported striking racial differences among smokers in San Francisco, where 76% of African Americans (n = 51) smoked mentholated cigarettes compared to 9% of non-Latino white smokers (n = 51). Similarly, Clark and coworkers (1996) reported that 83% of African American smokers used mentholated cigarettes compared with 23% of the non-Hispanic white smokers.

Menthol in cigarettes influences smoking behavior. Nil and Battig (1989) recruited 15 smokers (11 women) in Sweden to measure several parameters of smoking behavior. Each subject smoked seven different cigarettes: the subject’s own preferred brand and six others, representing three types of cigarette (mentholated, dark tobacco, blond tobacco) and two levels of yield (high, low). Smoking sessions were conducted one week apart for seven weeks. When subjects smoked in their own manner, changes in heart rate from pre to post smoking were as high or higher for mentholated cigarettes than for other cigarettes. In contrast, post puff inspiration time was as low or lower for mentholated cigarettes than for other cigarettes. Likewise, as subjects smoked in their own manner, they drew fewer puffs per cigarette from mentholated, high yield cigarettes than from all other cigarettes except dark tobacco, high yield cigarettes. Correspondingly, the subjects had the longest puff intervals when they smoked mentholated, high yield cigarettes than when they smoked all other cigarettes except dark tobacco, high yield cigarettes.

McCarthy and coworkers (1995) conducted a crossover design study with 29 male smokers who were in treatment for substance abuse. At baseline, smokers who preferred mentholated cigarettes (n = 11) had a higher average heart rate than smokers who preferred nonmentholated cigarettes (n = 18), 80 vs. 69 beats per minute, respectively. Two smoking sessions were conducted one week apart, using one type of commercially available cigarette at each session, mentholated or nonmentholated. Both types of cigarettes had 1991 FTC yields of 1.2 mg nicotine and 17 mg tar, but differed slightly in carbon dioxide yield (mentholated: 17 mg, nonmentholated: 15 mg). The mean puff volume of subjects smoking mentholated cigarettes was 7.5 mL less than the mean puff volume of subjects smoking nonmentholated cigarettes. Smokers of mentholated cigarettes took an average of four fewer puffs per cigarette. Therefore, the total puff volume smoked per mentholated cigarette was 39% less than that smoked per nonmentholated cigarette (p = 0.001).
A later report by the same research group described a similar crossover design with 20 male smokers (Jarvik et al., 1994). Each type of commercially available cigarette tested had a reported yield of 1.2 mg nicotine, 15 mg carbon monoxide, and 16 mg tar. Subjects drew smaller mean puff volumes and less total puffs from mentholated cigarettes. However, no significant differences for cigarette types were reported for peak puff flow, puff duration, puff interval, lung retention time or yield of total particulate matter.

6.4.4.6 Other human experimental studies

Pritchard and coworkers (1999) measured heart rate and encephalographic (EEG) activity in 22 male smokers, 12 of whom were regular smokers of menthol cigarette (8 African Americans, 4 Caucasians) and 10 of whom were regular smokers of non-menthol cigarettes (Caucasian). Each subject smoked two denicotinized test cigarettes (yield: 0.06 mg nicotine, 8 mg tar, ~8.5 mg carbon monoxide), one of which contained 4.1 mg menthol. The pre-to post-smoking increase in heart rate was greater for regular menthol cigarette smokers than for regular non-menthol cigarette smokers. The dominant alpha frequency was more than 0.7 Hz slower for regular menthol cigarette smokers than for regular non-menthol cigarette smokers. Regular non-menthol cigarette smokers showed a marked difference in the effect of smoking menthol versus non-menthol test cigarettes on eyes-closed beta1 power EEG activity. In contrast, menthol smokers showed a consistent slight increase in beta1 activity after smoking menthol and nonmenthol cigarettes. At locus Pz, menthol cigarette smoking led to a lowering of beta2 power. Beta2 power was unchanged by non-menthol cigarette smoking. The groups were not racially balanced.

6.4.4.7 Other human observational studies

In a case report, a 58 year-old woman, who had smoked cigarettes for 30 years, developed digestive and nervous disorders (vomiting, irritability, unsteadiness, insomnia, speech disorders, tremors, confusion) and slowed heart rate three months after switching to mentholated cigarettes. After the switch, she had rapidly increased consumption to 80 cigarettes per day. Her symptoms disappeared seventeen days after switching back to a non-mentholated brand of cigarette. Her physician challenged her with one gram of menthol, three times daily. Symptoms returned on the third day and increased in severity by day seven, at which time the apparent oral challenge was withdrawn and symptoms abated (Luke, 1962).

TNO BIBRA International (1990) compiled and reviewed five case reports of smokers of mentholated cigarettes who experienced hives, dermatitis or skin rash that cleared after restricting exposure to menthol. At least two of these smokers also used other mentholated products. Clinical conditions reappeared when pa-
tients were challenged with a mentholated product (dermal, oral or smoking cigarettes).

Based on analysis of the literature, Garten and Falkner (U.S. Congress, 2002) propose that mentholation of cigarettes decreases smokers’ ability to detect early symptoms of respiratory disease, such as shortness of breath and chronic cough. Menthol has decongestant (Eccles & Jones, 1983), antitussive (Laude et al., 1994; Morice et al., 1994), expectorant (Boyd & Sheppard, 1969), antibacterial (Moleyar & Narasimham, 1992; Pattnaik et al., 1997) and anti-allergic activity (Arakawa et al., 1992) which may obscure warning symptoms of disease. The delay in detection of symptoms by menthol cigarette smokers may contribute to a delay in seeking medical care and worsening of health outcomes (U.S. Congress, 2002).

In a case control study, Hebert and Kabat (1989) examined the relationship between currently smoking mentholated cigarettes and risk of esophageal cancer, using data collected between 1969 and 1984 in nine U.S. cities, part of the data collected in the American Health Foundation study. Control smokers were matched by hospital, gender and age. The study suggested that women, but not men who smoked mentholated cigarettes, had an elevated prevalence of esophageal cancer.

In another case-control study, Kabat and Hebert (1991) examined the relationship between smoking mentholated cigarettes and prevalence of lung cancer using data collected between 1985 and 1990 at eight hospitals in four U.S. cities. They matched control smokers by hospital, age, gender, race/ethnicity and date of interview. The results did not support an association between the use of mentholated cigarettes and lung cancer in either sex. In a third study, these authors found that men who ever smoked mentholated cigarettes exhibited a positive association with cancer of the pharynx after adjusting for age, education, filter use, and ethnicity (odds ratio 1.7, 95% CI: 0.8-3.4) (Kabat & Hebert, 1994).

Similarly, Friedman and coworkers (1998) assessed the incidence of non-lung cancers among smokers of mentholated and nonmentholated cigarettes in a cohort study of members of the Northern California Kaiser Permanente Medical Care program from 1979 to 1985 who had smoked for 20 or more years. The age-adjusted rate ratio of cancer cases for use of mentholated compared to nonmentholated cigarettes was 1.06 or less for all cancers (mouth, pharynx, larynx, esophagus, pancreas, cervix, urothelium) except renal adenocarcinoma in men (1.28, 95% CI: 0.39, 4.15), 4 cases in 1,575 male mentholated cigarette smokers and 9 cases in 4,182 male nonmentholated cigarette smokers. The incidence of non-lung cancers did not increase among long-term smokers who preferred mentholated cigarettes compared to smokers who preferred nonmentholated cigarettes.

Kabat and Hebert (1991) examined the relationship between smoking mentholated cigarettes and risk of lung cancer using data from 2,368 smokers collected between 1985 and 1990. Control smokers were matched by hospital, age, gender, ethnicity
and date of interview. The adjusted odds ratio for risk of lung cancer was not significantly increased for either short-term (1 to 14 years) or long-term menthol use (15 or more years).

Sidney and coworkers (1995) examined the risk of lung cancer and use of mentholated cigarettes among smoking members of the Northern California Kaiser Permanente Medical Care program from 1979 to 1985. The duration of smoking mentholated cigarettes was directly related to lung cancer in men (p = 0.02), but not women. Among men who were long-term smokers, mentholated cigarette use was associated with a statistically significant, 45% increase in the incidence of lung cancer relative to those who smoked nonmentholated cigarettes. In contrast, smoking mentholated cigarettes did not significantly alter the risk of lung cancer in women who were long-term smokers.

Carpenter and coworkers (1999) examined the association between smoking mentholated cigarettes and lung cancer risk by comparing cases of lung cancer in smokers from 35 hospitals with population control smokers in Los Angeles County. Exclusive menthol smokers were not at increased risk of lung cancer when compared to exclusive non-menthol cigarette smokers. The rates for women were not statistically distinguishable from those for men, but were consistent with the findings of Sidney and coworkers (1995) discussed earlier. There was a significant (p=0.04) linear trend for decreased lung cancer rate among women as pack-years of mentholated cigarette smoking increased compared to women exclusively smoking nonmentholated cigarettes.

Lee (2001) reanalyzed epidemiological data from three U.S. studies (Carpenter et al., 1999; Kabat & Hebert, 1991; Sidney et al., 1995) and concluded that the data were inconsistent for a difference in the risk of lung cancer for those who regularly smoked mentholated cigarettes compared to those who smoked nonmentholated cigarettes. His meta-analysis showed a decreased relative prevalence in women, 0.70 (95% CI: 0.52-0.95), but not men, 1.23 (0.88-1.72).

Brooks and others (2003) assessed the role of smoking mentholated cigarettes on the risk of developing lung cancer by analyzing data from the Slone Epidemiological Center Case Control Surveillance Study. They examined the smoking histories of 643 cases (cigarette smokers for at least 20 years with no history of any form of cancer before diagnosis of lung cancer within a year of current hospitalization) and 4,110 controls (individuals hospitalized for conditions not regarded as attributable to cigarette smoking) and used logistic regression to determine the relative risk of lung cancer as it relates to the number of years that an individual smoked. Their results indicate no increased risk of developing lung cancer from smoking menthol cigarettes as compared to smoking cigarettes without menthol and no apparent influence of race (Black or White) or sex on risk of developing lung cancer due to
smoking menthol cigarettes. The interpretation of these results is complicated by the investigators’ assumption that subjects for whom brand-smoking histories were incomplete smoked non-menthol cigarettes prior to 1956.

### 6.4.5 Summary of test data about menthol

Menthol is commonly added to cigarettes. Menthol typically comprises approximately 2% of the weight of a cigarette. Menthol does not pyrolyse appreciably during smoking, but transfers into the smoke. Intact menthol is the primary substance transferred in mainstream smoke. Menthol can change smoke chemistry. Menthol in cigarette smoke is absorbed into the blood stream and metabolized. Smokers of mentholated cigarettes have higher blood cotinine concentrations, increased puff volumes, shortened post puff inspiration times, and decreased puff numbers per cigarette. The exposure-response relationships for these effects are poorly known.

### 6.5 SUMMARY

Except for observational studies of smokers, the tests and testing programs traditionally used in toxicology can most usefully be adapted for the review of ingredients added to cigarettes to understand metabolic effects and detect novel toxic effects. Application of traditional testing programs to ingredients that do not transfer in smoke seems irrational and wasteful. Even for substances that transfer in smoke, dosimetry estimates and scientific judgment will come into consideration in making decisions about subsequent testing for health effects. For example, some ingredients added to cigarettes are complex natural products. Some proportion of the mass of such products likely will transfer in smoke. Determining the proportion of mass sufficient to merit subsequent inhalation toxicology testing will best be left to case-by-case decision-making. Identification of the transferred products and prior knowledge will strongly influence decisions about subsequent testing. LSRO will review this subject in the Phase Two report of this project.

It is a different matter to determine the maximum amount of an ingredient added to a cigarette that does not change stated parameters of, for example, smoke chemistry, or to determine the maximum amount that does not change the parameter by more than a predetermined amount. The judgment that neither a substance nor its pyrolysis products transfers in smoke in biologically meaningful amounts can mostly be made without reference to cell culture and animal model information.

The principle that multiple, independent tests do not necessarily provide better quality information than a single test, will prove crucial to the construction of LSRO’s approach to feasibility. It justifies the notion that a few well-chosen tests will potentially provide more useful data about the relative risk of an ingredient added to cigarettes than an extensive list of tests.
A case study of menthol proved particularly helpful to LSRO in assessing the feasibility of testing ingredients added to cigarettes, although the review did not offer the in-depth perspective of a more extensive and comprehensive examination suitable for regulatory purposes. In addition, the review did not aim to recommend a limit on the maximum quantity of menthol added to a cigarette. However, the review did help reveal that testing of ingredients is feasible.
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7.6 SUMMARY
7

RESEARCH NEEDS

7.1 INTENT & PURPOSE

To assess the feasibility of determining the relative risk of smoking cigarettes with added ingredients as compared to smoking cigarettes without added ingredients LSRO extensively reviewed the relevant scientific literature. Specifically, LSRO staff evaluated data concerning cigarette smoke, human exposures to cigarette smoke, the adverse health effects of smoking, and issues about the methods used to study cigarette smoke. In addition, LSRO studied menthol, an example of an added ingredient. The overall review revealed areas where additional data would help an assessment of the feasibility of testing whether added ingredients increase the relative risk of adverse health effects of smokers. In this chapter, LSRO identifies areas of research that warrant further scientific investigation.

7.2 THE CHEMISTRY AND PHYSICS OF SMOKE

7.2.1 Smoke Chemistry

LSRO adopted a criterion of “no detectable change,” as an initial basis for elimination of potential relative risks of adverse health effects associated with ingredients added to cigarettes. Molecules of an ingredient in a cigarette will encounter diverse circumstances on ignition of a cigarette; they may transfer intact with smoke, convert into one or more other substances, or undergo a combination of chemical and physical processes. As such, the burning of added ingredients in cigarettes could generate chemicals absent from the smoke of cigarettes that lack or contain lesser amounts of the added ingredient. Other potential effects of the inclusion of added ingredients include increases or decreases in the amounts of one or more chemical components of the smoke matrix and multiple, diverse effects on smoke substances, such as alteration in lung absorption. Thus, the inclusion of added ingredients in cigarettes could alter the exposure of smokers to smoke chemicals and change the morbidity and mortality associated with smoking cigarettes.

LSRO has suggested an initial step in the assessment of the relative risks of adverse health effects imparted by ingredients added to cigarettes of comparing, under standardized conditions, the chemical composition of smoke from reference cigarettes to smoke from cigarettes approximately identical to the reference
cigarette, except for the presence or absence of an ingredient or a defined mixture of added ingredients. Testing a range of concentrations of the added ingredient(s), including the concentration of the ingredient used in commercial cigarettes and lower and higher concentrations of the added ingredient will be desirable. Any significant change detected in smoke quality or quantity arising from inclusion of an added ingredient, or mixture of ingredients, might imply a change in risk of adverse health effects of smoking cigarettes. The meaning of change is a matter for scientific evaluation and interpretation. However, the information derived from relative chemical analyses of smoke will have vital importance in setting upper limits on the amounts of added ingredients per cigarette.

The question of whether an added ingredient transfers into smoke and how much of the ingredient transfers, also will contribute important insight about the identity of smoke substances and the exposure of smokers to these substances. An understanding of the process is of importance since knowledge about what transfers in smoke may be applied to understanding relevant exposure levels of smoke substances. Boiling point, vapor pressure, and volatility of smoke substances are useful for predicting what will transfer in smoke, however, we lack complete understanding of the factors that determine what transfers in smoke.

### 7.2.1.1 Analytical Methods

#### 7.2.1.1.1 Pyrolysis

Cigarette smoke is a complex mixture of approximately 4,800 different compounds (Green & Rodgman, 1996). Diverse approaches have been employed in an attempt to elucidate the relationship between cigarette ingredients and substances in smoke. Hobbs (1957) predicted a low concentration of oxygen within the burning zone of a cigarette, which was later confirmed by Newsome and Keith (1965). Based on this information, pyrolysis studies of pure added ingredients in cigarettes and tobacco fractions were proposed to study the relationship between specific ingredients in cigarettes and smoke substances. Other studies, including those of Wynder and Hoffmann, include pyrolysis studies of added ingredients in inert atmospheres (Wynder & Hoffmann, 1964; Wynder & Hoffmann, 1967). Criticism of this method included the use of an oxygen-free atmosphere for pyrolysis, which does not exist under normal smoking conditions, as well as the dependence of oxygen concentration on the amount of diluting air and paper porosity (Rodgman et al., 2000).

Pyrolysis of leaf extract has also been examined (Schlotzhauer et al., 1976). Although these studies may identify potential contributions of some chemicals to compounds in smoke, pyrolysis is not the only process that occurs in a burning cigarette (Schmeltz et al., 1978). Changing smoke generating conditions affects the yields of smoke substances. Bell and coworkers (1966) suggested that the pyrolysis of certain carbohydrates in air generates higher amounts of phenols than are generated in a nitrogen atmosphere. Brunnemann and Hoffmann (1982)
proposed that the fate of chemicals should be determined in the context in which cigarette smoking occurs.

Pyrolysis studies of ingredients added to cigarettes provide information about the relationship between added ingredients and cigarette smoke substances. The identification and measurement of substances in smoke is important for making decisions about which smoke substances are important for testing.

Some added ingredients may not transfer into smoke, but could disturb the smoke matrix and affect the dose of smoke substances to which the smoker is exposed as well as the toxicity of the smoke. The presence of an indicator component that accurately reflects dosimetry and toxicity of other substances in the mixture that are applicable to the health effect of interest is a useful tool (Fleming et al., 1994). The ability to detect changes in the smoke matrix would be extremely useful for assessing the feasibility of studying the relative risk of adverse health effect of smoking cigarettes with added ingredients as opposed to smoking cigarettes without the added ingredient. If scientists could identify an indicator component for changes in the smoke matrix, we would better understand whether the smoke matrix changes when a specific added ingredient is present. If any particular substance is a better signal of changes in the chemical composition in the smoke than any other, LSRO is not aware of it. Other than convenience and signal-to-noise ratio, a scientific rationale to select chemical markers for routine analysis is not apparent. One approach to prioritizing is to select chemical substances that have high concentrations in smoke e.g. acetaldehyde and 1,3-butadiene in the gas phase. Additional research might provide better candidate substances to signal changes in the chemical composition of cigarette smoke. Information about the best chemicals to analyze as an index of change in the smoke matrix would be informative for determining feasibility.

**7.2.1.1.2 Isotopic labeling of substances in cigarettes**

As noted previously, LSRO could not find evidence that pyrolysis of isolated added ingredients will provide information about interactions of added ingredients and their pyrolysis products in cigarette smoke. Growing tobacco plants in a [14C] carbon dioxide atmosphere and performing chemical analyses of the smoke yielded from cigarettes containing the radioactive label, provides an alternate approach to pyrolysis (Schmeltz et al., 1978). This process reflects, more accurately, the contributions of specific added ingredients to the formation of smoke chemicals and is useful for identifying degradation products at low levels (Jenkins, Jr. et al., 1970; Schmeltz & Schlotzhauer, 1968). Syringe-spiking cigarettes with [14C] carbon-labeled cigarette ingredients, spraying with radioactive compound, and sheetcasting-blending are other approaches to incorporating radioactive label into cigarettes (Jenkins, Jr. et al., 1970; Jenkins, Jr. et al., 1977). Challenges in conducting radioisotope analysis of smoke constituents include assuring that the chemicals
demonstrate the requisite activity and are labeled in multiple positions (Schmeltz et al., 1978). The use of stable isotopes is another approach. Insufficient baseline data exist to document the performance of mass spectrometer detection combined with chromatographic processing of smoke. It may be that introduction of a stable isotopically-labeled marker substance into a cigarette will simultaneously provide adequate data to evaluate the transfer of an added ingredient and changes in smoke composition during cigarette combustion. Knowledge of the fate of cigarette ingredients in an intact cigarette provides more pertinent information about the chemical mixture that comprises smoke and new smoke substances that may form during the burning of a cigarette, relevant to the feasibility of testing for the relative health risk of added ingredients.

7.2.1.2 Standardization of analytical methods

The Federal Trade Commission (FTC) standards for smoking machines were designed in 1967 to compare mainstream smoke yields of nicotine and tar (Federal Trade Commission, 1967a; Federal Trade Commission, 1967b), and later carbon monoxide (Federal Trade Commission, 1979) from different cigarettes smoked under identical standardized conditions. Subsequent to the development of these standards, other standards for smoke generating machines have been introduced. In 1991, the International Organization for Standardization (ISO) harmonized then existing standards, which Canada and the United Kingdom adopted (International Organization for Standardization, 2000; International Organization for Standardization, 2002). In the United States and Japan, the FTC standard still is used, while some European countries use CORESTA standards (CORESTA, 1994). For purposes of testing ingredients added to cigarettes, standardization will be useful for controlling conditions and limiting variations in measurements. One approach to generating comparable data is to use the FTC standard of smoking cigarettes, because extensive data on cigarette smoke chemistry generated using the FTC methods are presently available. The use of such an established technique to produce smoke is of value since the yields of some chemicals in cigarette smoke have not been measured with validated techniques (Rodgman & Green, 2002). Standardization of techniques could also help standardize terminology. For example, the FTC and CORESTA methods define tar differently (CORESTA, 1991c; Pillsbury et al., 1969). Standardization of techniques and terminology could provide more comparable results, which will assist the interpretation of data, thus assisting decisions about feasibility.

Assessment of existing methods to detect and quantify chemical substances in smoke might allow the determination of their usefulness at the concentrations found in smoke. Thus, the process of validation of testing methods currently in use and determining new approaches applying sophisticated methodologies and instrumentation would advance the study of smoke chemistry. There are, however, limitations on the applicability of smoke chemistry studies. Because the specific
substances responsible for human morbidity and mortality remain unknown, side-by-side chemical analysis of cigarettes with and without an ingredient cannot yield information directly relevant to human morbidity and mortality. Instead, such studies show that a kind of cigarette generates smoke similar to the cigarettes previously consumed by cohorts in published epidemiology studies.

7.2.1.2.1 Reference tobaccos

Reference cigarettes are essential controls for testing ingredients with and without ingredients. Tar and nicotine yields of older reference cigarettes are not reflective of today’s cigarettes (Baker, 1999; World Health Organization, 1986). The use of standardized reference tobaccos would generate data on yields of chemicals in cigarettes currently being smoked. Consistent and definitive documentation of the use of reference cigarettes in experiments would provide useful information for an assessment of feasibility (Baker, 1999; World Health Organization, 1986). Information about the source of the tobacco crop used in the cigarette, the quantity of reconstituted tobacco, as well as its processing, housing, packaging and transport would provide researchers with useful detail. The specification of the concentration and technical grade of the added ingredient used is a useful approach (CORESTA, 1991b; World Health Organization, 1986).

7.2.1.2.2 Identification of smoke substances

Various chemical substances in smoke are thought to be toxicants, however, no standardized method exists for the identification of the majority of these chemicals (Rodgman & Green, 2002). In addition, other groups have not verified the presence and concentrations of some putative toxicants. Rodgman and Green (2002) proposed that some substances may be present as artifacts. Rodgman and Green (2002) described a process to develop a more complete information base, by confirming the presence of the chemical in smoke, recording toxic properties, and evaluating of the process used to characterize amount of the substance found in smoke. However, LSRO knows of no test of toxic properties that is predictive of the human health effects observed in epidemiological studies.

7.2.2 Smoke Physics

Cigarette smoke has been defined as an “aerosol of aqueous droplets which contain dissolved and suspended substances dispersed in a mixture of vapors and gases, including air” (Davies, 1988). The microenvironment, and processes such as nucleation and particle growth, influence smoke formation (UK Department of Health, 1998). Cigarette smoke aerosols exhibit cloud-like behavior, which may result in different patterns of movement than are predicted for the individual particles that make up the cloud (Chen & Yeh, 1990). Such differences in patterns of movement will affect lung deposition. The physics of smoke affects deposition of
cigarette smoke aerosols in the trachea and lung bifurcation regions (Chen & Yeh, 1990).

As smoke ages, smoke particle size increases and particle concentration decreases (Morimoto et al., 2002). Knowledge of the substances contacting smokers’ lungs will provide insight into exposure. The relationship between chemical variability and the size of a smoke particle has not been fully described (McRae, 1990). Whether the presence of added ingredient affects these processes is not known. Light scattering studies (or other measurements relevant to particle sizes) of smoke (Ingebrethsen, 1984) generated from cigarettes with and without added ingredients would contribute to knowledge about aerosol dynamics and to decisions about feasibility.

7.2.3 Reduction of Variables

The process of human cigarette smoking is complex. One research approach to the chemistry and physics of cigarette smoke would be to simplify the process by removing many variables from study. Investigators could pack different tobaccos at different densities in impermeable tubes, such as stainless steel tubes. They could send defined gases through the tubes at different rates and temperatures, such that the gases sustain combustion. Scientists could study the chemical and physical characteristics of the smoke, either at specific time points or collectively through measurements of the collected total smoke. Similarly, the spectrum of light absorption might provide instantaneous estimates of changes in chemical composition. The application of such techniques might be useful for improving understanding about the process of smoke formation. An approach to studying pyrosynthesis is to supply a potentially reactive substance in the gas mixture to a tobacco column undergoing combustion. Reaction products incorporating this substance would essentially trap pyrosynthesis in progress.

Given a better understanding of how variables, such as oxygen tension, temperature, flow rate, packing density, and kind of tobacco affect the chemical and physical composition of smoke; the scientific community would be better positioned to understand the more complex phenomenon of human smoking. Knowledge about this would provide additional understanding about the relationship between chemists’ test cigarette smoke and relate it to human exposure in order to make predictions about health effects.

7.3 SMOKE EXPOSURE

Cigarette smoke exposure refers to the external relationship between tobacco smoke and the body (Shields, 2002). Changes in exposures to chemical substances in smoke change adverse health effects, most obvious in smoking cessation, where
smoking intensity (cigarettes consumed per unit time) and smoking duration (years of smoking), both change. However, more subtle differences reflecting changes in smoke composition remain elusive.

7.3.1 Smoking Behavior

7.3.1.1 Smoking initiation and maintenance

Many biological factors have been implicated as contributory to the initiation of a smoking habit. Socio-cultural factors, including attitudes of individuals and their peers toward smoking and smoking behaviors of individuals in the immediate environment, may also play a role in smoking initiation (Rennard & Daughton, 2002). Fowles (2001) suggested that some added ingredients, such as licorice, sugars and artificial sweeteners, and glycerol and methylglycerol, which alleviate throat irritation caused by cigarette smoke may increase the appeal of cigarettes. Menthol produces a cooling effect, which also may alleviate the burning sensation experienced during smoking (Miller et al., 1994). Whether the presence of an added ingredient could influence the decision to initiate smoking remains unknown and elusive. Studying smoking initiation could contribute to an understanding of feasibility of assessing the relative risk of added ingredients.

According to Levin and Rose (1995), some smokers perceive an improvement in brain function and an energy surge from smoking a cigarette. The expectation of such effects of smoking may influence the development of a smoking habit in non-habitual smokers. The role of anticipation of effects, and whether added ingredients contribute to the anticipation of smoking, are unknown. LSRO would like to know whether anticipation changes smoking behavior in a measurable way, such as consumption of cigarettes or biomarker levels.

Surveys of youth from diverse racial and ethnic backgrounds, who initiate cigarette smoking, would be beneficial for dissecting the process of initiation and maintenance of smoking habit (Ahijevych, 1999). If ingredients added to cigarettes are shown to be a factor in the initiation and maintenance of smoking, LSRO will want to revisit this topic.

The role of genetics in smoking initiation is not fully described (Minnesota Department of Health, 2003). The rate of nicotine metabolism may affect the initiation and maintenance of cigarette smoking (Ahijevych, 1999). The roles of polymorphisms in dopamine b-hydroxylase, monoamine oxidase A and B, and other enzymes in the development and maintenance of a smoking habit are discussed later in this chapter.
7.3.1.2 Smoking cessation

Little is known about the physiological, cognitive, behavioral, and psychological effects of withdrawal from smoking cigarettes with additives. It will be important to determine whether the presence of added ingredients affects the ability of individuals to cease smoking. According to Sommese et al. (1995), many studies report psychological, and/or physiological responses to nicotine withdrawal, including a decrease in carboxyhemoglobin, digit recall, serial addition/subtraction, and job satisfaction; and an increase in blood pressure, depression, absenteeism, caloric intake, craving, aggressiveness, confusion, and impulsivity. Whether any added ingredient alters responses to cigarette withdrawal and successfully quitting smoking remains unknown.

7.3.1.3 Cigarette brand choice

In a review of compensation and smoking, Scherer (1999) noted that smokers can compensate for smoke taste, cigarette smoke irritation, the draw resistance of the cigarette, and yields of nicotine and tar. Studies of the effects of nicotine yield on cigarette smoking report conflicting results. If the nicotine yield does not change cigarette smoking, LSRO might revise its decisions about feasibility. Thus, changes in nicotine yields of smoke from cigarettes with and without added ingredients will prove useful to know. In addition, the role of the presence of added ingredients on the decision to smoke a specific brand of cigarette, or to switch cigarette brand has not been fully explored, although LSRO has no insight into better research methods to pursue these topics. However, changes in behavior could increase the exposures of smokers to toxicants.

7.3.2 Smoke Inhalation and Deposition

7.3.2.1 Smoke inhalation

The risk of adverse health effects of inhalation exposure to unpyrolyzed and pyrolyzed added ingredients would be useful to know. LSRO endorses approaching this problem by conducting a case-by-case by study of added ingredients. Davies (1988) describes a relationship between the amount of smoke inhaled and the intakes of carbon dioxide and particles, which affect the physiological and psychological responses to smoking. Human body temperature may affect the surrounding ambient air, which influences the inhaled fraction of airborne particles (Ogden & Birkett, 1975). Reliable analytical methods for the determination of the bioavailability of components of the smoke and levels of metabolites in tissues would contribute to an understanding of the relationship between whole smoke and inhalation. A better baseline of data about smoke inhalation would contribute more information to decisions about the feasibility.
7.3.2.2 Smoke deposition

Davies (1988) defines the deposited fraction of an aerosol as the fraction that, during steady breathing, does not exit the respiratory tract on exhalation. The deposited doses of smoke components determine the risk of adverse health effects of smoking cigarettes. Factors that affect the deposition of inhaled aerosols include the physical and electrical characteristics of aerosol particles, the structure of the respiratory system, and the way that the smoker breathes (Martonen et al., 2000). Particles are deposited via three primary mechanisms: inertial impaction, sedimentation and diffusion (McKemy et al., 2002). Multiple theoretical models have been developed in an attempt to predict the deposition pattern of inhaled cigarette smoke. However, these models have not been validated. Factors such as age, anatomical anomalies, the physics of airflow, hygroscopicity, and aerosol charge, have not be addressed satisfactorily (Phalen & Oldham, 2001). Identification of the effective dose and its relationship to individual features, such as body size, have not been fully explained (Phalen & Oldham, 2001). These data gaps are relevant to a decision about feasibility, since better information might provide some insight into the susceptibility of specific groups of individuals in the population to diseases associated with cigarette smoking. Studies of factors contributing to locations of deposition in human airways and explanations of how exposure leads to long-term tissue pathology would be useful. Better information about how to study deposition could also affect a decision about feasibility of assessing relative risk of adverse health of smoking cigarettes with and without added ingredients.

The mechanisms of smoke uptake into lung tissue have not been characterized. Although nicotine may be absorbed by more than one mechanism, the predominant mechanism of absorption is not known, nor is the influence of added ingredients on nicotine absorption known. Gray and Kozlowski (2003) propose banning ingredients which increase nicotine bioavailability. Information about the molecular structure and function of nicotinic receptors could provide insights about potential interactions between added ingredients and nicotine receptors (Society for Research on Nicotine and Tobacco, 2002).

Heeschen and coworkers (2001) showed that nicotine promotes growth of atherosclerotic plaques, decreases apoptosis of endothelial cells, and enhances development of capillary networks. The overall effects of tobacco smoke may differ from the effect of nicotine in isolation. Detailed characterization of the physiological actions of nicotine and the effects that nicotine may elicit on behavior and cognition would provide useful information.

Proposed mechanisms of action of one added ingredient, menthol, include altering the effect of known carcinogens and an effect on smoking behavior (McCarthy et al., 1995). Jarvik and coworkers reported that smoking mentholated cigarettes
involved smaller puff volumes and decreased numbers of puffs as compared to smoking non-mentholated cigarettes (Jarvik et al., 1994). In contrast, McCarthy and others (McCarthy et al., 1995) found increased number of puffs and higher puff volumes when smoking mentholated cigarettes as opposed to smoking non-menthol cigarettes. Smokers of mentholated cigarettes have higher blood levels of carboxyhemoglobin and greater expired carbon monoxide levels than smokers of non-mentholated cigarettes, although retention times are similar for all smokers (Jarvik et al., 1994). The mechanism by menthol achieves increased levels of carboxyhemoglobin has not been fully described (Jarvik et al., 1994).

Thus, whether menthol affects the deposition, absorption and clearance of smoke components remains to be determined. Research questions to be addressed include whether menthol in the presence of heat increases “penetration” of smoke constituents, exposure to toxic components of cigarette or other alterations of biological activity. Neuropharmacological tests of how added ingredients might impact the pharmacology of addiction might be applied (Fowles, 2001).

7.3.2.3 Biomarkers

A biomarker or biological marker has been described as “a measure that is used as an indicator of normal biologic processes, pathogenic processes or pharmacological responses to therapeutic intervention” (De Gruttola et al., 2001). The three categories of biomarkers include biomarkers of exposure, biomarkers of biologically effective dose, and biomarkers of potential harm (Institute of Medicine, 2001).

General principles for use of biomarkers have been outlined (Shields, 2002). Important processes include biomarker validation and evaluation for sensitivity and specificity at realistic exposure concentrations and at lower amounts. In addition, testing of the biomarker in different populations and noting the variability of data on multiple testing of an individual, between individuals in the same laboratory and between different laboratories also is important. Identification of alternative sources of biomarker can clarify the relationship between exposure and biomarkers.

Other research needs are the identification of early indicators of disease and the validation of biomarkers of tobacco exposure (Shields, 2002). Mao and coworkers (Mao et al., 1997) reported loss of heterozygosity mutations at the 3p14, 9p21 and 17p13 loci in the lung tissue of smokers that are asymptomatic for lung cancer. These genetic loci code for FHIT (fragile histidine triad gene), p16/CDKN2 and p53 tumor suppressor genes, respectively. The investigators propose the identification of clonal genetic markers to separate out individuals most likely to develop lung cancer (Mao et al., 1997). A biomarker that fails to detect substances in cigarette smokers during nicotine replacement therapy, will validate smoke exposure (McKemy et al., 2002).
DNA adducts may indicate biologically effective doses of smoke in lung tissue (Spitz et al., 1999). According to Wiencke and others (Wiencke et al., 1999), DNA adducts are affected by the intensity of exposure and time elapsed since exposure to tobacco smoke. Detoxification and the disappearance of adducts can result from DNA repair or cell turnover. DNA adduct formation, however, is not sufficient for commencement of carcinogenesis or mutagenesis (Kramer, 1998). Adducts to proteins, such as hemoglobin, may also be useful biomarkers of biologically effective dose. Kramer (1998) describes the advantages of using protein adducts, which include their long half-life (120 days) and dose dependence of binding.

7.4 ADVERSE HEALTH EFFECTS OF SMOKE

7.4.1 Premature Mortality

Epidemiological studies have identified cardiovascular diseases, cancer and chronic obstructive pulmonary disorder (COPD) as the primary causes of death associated with cigarette smoking. The mechanisms by which chemical substances in smoke contribute to the development and progression of these diseases are not known. Furthermore, the identity of the smoke chemicals most toxic to humans are not known. Additional research to elucidate the mechanisms of pathogenesis of these diseases will aid the development of predictive models.

Statistically, greater accuracy resides in enumerating all of the excess deaths associated with some exposure, instead of some portion of these deaths associated with a cause of death. In addition, better quality data are available from the interpretation that a person has died, than from the cause of death, which requires an inference based on symptoms and may require an autopsy. For these reasons, the emphasis placed on specific causes of death over premature mortality in epidemiological studies of cigarette smoking is difficult to understand. An important research priority is to ensure that supporting mortality data are made available in published articles. If tabulations of, for example, lung cancer data are the only available information, LSRO will review them, but these data will be inferior to the mortality data from which investigators derived the lung cancer data.

LSRO will continue to seek insights into the health impact of non-tobacco ingredients added to cigarettes. Thus, comparative studies of health statistics of smoking from countries where the cigarettes consumed have, or do not have additives will remain a high priority research topic. LSRO learned much from comparisons of health outcomes from smoking filtered and unfiltered cigarettes (Lee, 2001).
7.4.2 Causes of Mortality

7.4.2.1 Cardiovascular disease

Genetic and environmental factors influence cardiovascular disease. Elucidation of the molecular and cellular events underlying various cardiovascular diseases would lead to a better understanding of the role of smoke chemicals, and possibly added ingredients, in development of these diseases. Cigarette smoke increases the rate of progression of arteriosclerosis (Waters et al., 1996). Various mechanisms have been proposed for cigarette smoke associated induction of stroke, including increased platelet aggregability and reduced blood flow by arterial vasoconstriction (Rogers et al., 1985).

7.4.2.2 Chronic obstructive pulmonary disease (COPD)

COPD is characterized by airflow limitation that is progressive, difficult to reverse, and associated with atypical lung inflammation (Turato et al., 2001). Perhaps fifteen percent of cigarette smokers develop “clinically significant” obstruction of their airways (Fletcher & Peto, 1977; Sethi & Rochester, 2000). Genetic susceptibility, environmental and host factors are thought to contribute to the development of COPD (Sethi & Rochester, 2000). Deficiency of alpha-1-antitrypsin (α-1AT) is a known risk factor for COPD (Eriksson, 1965). Identification of other risk factors of COPD is in the early stages (Higham et al., 2000; Patuzzo et al., 2000). Taylor and coworkers (1986) proposed that an imbalance of oxidants and antioxidants contributes to the development of COPD. The process includes antiprotease inactivation (Janoff, 1985; Laurell & Eriksson, 1963), inflammation (Bosken et al., 1992), infection, and activation of transcription factors (Collins, 1993; Schreck et al., 1991). How the inflammation associated with COPD begins and maintains, is not known (Shapiro, 2002). The roles of bacteria in the inflammation and the mechanisms by which smoke chemicals give rise to COPD have not been fully described and remain a research need. Whether added ingredients contribute to smokers’ outcomes in relation to COPD is not known.

7.4.2.3 Cancer

7.4.2.3.1 Lung cancer

Various groups have reported a trend of increased prevalence of adenocarcinoma with respect to squamous cell carcinoma in smokers (Travis et al., 1995; Larson, 1960). Additional research is needed to determine whether this apparent shift in incidence is due to advances in technology used for disease diagnosis, changes in the classification of the diseases, or a shift of carcinogenesis. Observed changes in relative incidence of cancers could result from an increase in depth of inhalation of smoke by smokers of low yield cigarettes (Levi et al., 1997; Thun et al., 1997). The use of added ingredients has changed during the same time frame. So, a change in relative incidence might relate to an added ingredient, instead of a low
yield structure. If on evaluating the data, this trend reflects disease incidence, the potential effects of added ingredients on the shift from squamous cell and small cell carcinoma to adenocarcinoma and bronchioalveolar carcinoma will need to be determined.

7.4.2.3.2 Other cancers
Smokers are more likely to have neoplastic diseases (Read, 1984; Shaw & Milton, 1981). Cigarette smoking is associated with cancers of the mouth, esophagus, kidney, pharynx, larynx, bladder and pancreas (World Health Organization, 1986). Additional research is needed in order to determine whether cigarette smoking underlies other diseases, such as breast cancer and leukemia (University of California Office of the President, 2000). Whether there are other carcinogenic effects that have not yet been described is unknown.

7.4.3 Immune System Effects
Studies suggest that deleterious effects of cigarette smoking, which ultimately manifest as multiple pathologies, may relate, in part, to the suppression of the immune system by chemical substances in cigarette smoke (Holt & Keast, 1977). Cigarette smokers reportedly are more likely to contract influenza and mount a weaker antibody response to the virus (Aronson et al., 1982; Finklea et al., 1969; Kark et al., 1982). Levels of many immunoglobulins are suppressed in smokers (Andersen et al., 1982; Gerrard et al., 1980).

Specifically, nicotine has been identified as a candidate suppressor of the immune system (Geng et al., 1996). Immune suppression may be achieved via multiple mechanisms, including inhibition of T-cell function (Geng et al., 1996) or increasing adrenocorticotropic hormone (ACTH) and catecholamines (Sopori & Kozak, 1998). These results require independent validation. Additional research on the mechanisms underlying suppression of the immune system might be useful (Sopori & Kozak, 1998). Further understanding of a mechanism by which cigarette smoke suppresses the immune system would provide a potential bioassay to evaluate whether an added ingredient causes immunosuppression.

7.4.4 Free Radicals
Halliwell and Gutteridge (1999) defined free radicals as “chemical species capable of independent existence that contain one or more unpaired electrons.” A minimum of four classes of free radicals occur in cigarette smoke, as detected with electron spin resonance (Pryor et al., 1983b). Free radicals in tar may be transported in respiratory tract lining fluids (Hatch, 1992; Mudway & Kelly, 1998; Pryor, 1992b; Cross et al., 1994), which contain antioxidants, providing an initial defense system (Cross et al., 1997). Classes of antioxidants found in respiratory tract lining fluids include low molecular mass antioxidants, metal binding proteins, antioxidant enzymes,
sacrificial reactive proteins, and unsaturated lipids (Davis, 1998). Free radicals may interact with lung tissue (Church & Pryor, 1985; Pryor et al., 1983b; Pryor et al., 1983a; Pryor, 1997; Stone et al., 1994) and cause DNA damage (Church & Pryor, 1985; Pryor et al., 1983a; Stone et al., 1994; Leanderson & Tagesson, 1990; Lehr et al., 1994; Pryor, 1987; Eiserich et al., 1995). An imbalance of oxidants and antioxidants may contribute to the development of COPD (Taylor et al., 1986), and free radicals have been implicated in the development of cardiovascular diseases (Maxwell & Greig, 2001). Information about the nature and the mechanisms by which free radicals contribute to the development of various diseases associated with cigarette smoking would be desirable. Whether added ingredients in cigarettes contribute to the production and activity of free radicals has not yet been described.

7.4.5 Risk Factors

The State of California has listed as a research initiative the study of tobacco smoking initiation and mechanisms of development of adverse health effects—how factors such as age, ethnicity, race or gender modify these effects (University of California Office of the President, 2000).

7.4.5.1 Genetics

Various groups report that genes influence smoking behaviors (Carmelli et al., 1992; Edwards et al., 1995; True et al., 1997). Genes potentially affect smoking initiation, addiction, metabolism, host repair, and disease defense mechanisms (Thun et al., 2002). This topic has recently been reviewed (Batra et al., 2003). Polymorphisms in cytochrome P450 enzymes, the major enzymes involved in the metabolism of nicotine to cotinine, affect nicotine metabolism and thus, may ultimately change smoking behaviors (Pianezza et al., 1998). Null alleles of the CYP2A6 enzyme, the primary enzyme involved in nicotine metabolism, may confer protection from development of smoking habit. Smokers with CYP2A6*2 and CYP2A6*3 alleles, which have reduced activity, are less likely to be tobacco-dependent, smoke fewer cigarettes smoked per day (Messina et al., 1997; Nakajima et al., 1996; Pianezza et al., 1998), and are better able to quit smoking (Batra et al., 2003; Tyndale & Sellers, 2001).

Persons with some CYP2D6 polymorphisms may be categorized as poor metabolizers (Alvan et al., 1990; Daly et al., 1996), extensive metabolizers, or ultrarrapid metabolizers of nicotine (Lovlie et al., 1996). This gene may play a role in smoking behaviors after development of a smoking habit (Boustead et al., 1997), as opposed to the initiation of a smoking habit. The role of the CYP2D6 allele and risk of adverse health effects of cigarette smoking remains to be determined (Bouchardy et al., 1996; London et al., 1997; Tefre et al., 1994).
Other genes involved in nicotine metabolism including monoamine oxidases A and B (Costa-Mallen et al., 2000; Fowler et al., 1996a; Fowler et al., 1996b) and dopamine B-hydroxylase (McKinney et al., 2000) may also play a role in smoking behavior. Studies examining the roles of tyrosine hydroxylase (Lerman et al., 1997) and catechol-O-methyltransferase do not show a relationship with smoking behavior (McKinney et al., 2000).

The dopaminergic reward pathways of the brain are stimulated by nicotine (Clarke, 1990; Tefre et al., 1994). Dopamine D1, D2 and D4 receptors genes have been implicated in smoking behavior. Some studies suggest that the Dopamine D2 receptor gene (DRD2) DRD2A1 allele is associated with fewer dopamine receptors (Thompson et al., 1997) and increased likelihood of being a smoker (Noble et al., 1994), while others have not found such an association (Bierut et al., 2000; Singleton et al., 1998). The role of dopamine receptors in the development of a dependence on nicotine has not been resolved.

The SLC6A3 gene codes for the dopamine transporter proteins. Lerman and others (1999) showed that individuals that express the 9 tandem repeat allele (SLCA3-9) were older at initiation of a smoking habit, had an increased likelihood of quitting smoking, and exhibited longer periods of abstinence from smoking. Data from another study conflicts with this result (Jorm et al., 2000). Sabol and others (1999) supported the association between this gene and propensity for longer periods of smoking abstinence but did not support a role for this gene in smoking initiation (Batra et al., 2003). Elucidation of the regulatory mechanisms of brain nicotinic receptors would be useful for determining mechanisms of nicotine addiction. Whether inclusion of added ingredients influences the mechanism of nicotine dependence is an area of research that has not been fully explored.

Since nicotine increases brain serotonin release (Mihaiescu et al., 1998; Ribeiro et al., 1993) it has been proposed that serotonin receptor and transporter genes may also be involved in smoking behavior. The roles of these as well as acetylcholine, adrenergic, mu opioid, and cannabinoid receptors on smoking behavior have not been fully characterized (Arinami et al., 2000). Whether the presence of added ingredients in cigarettes modifies these pathways has not been determined.

### 7.4.5.2 Race/Ethnicity

A more complete understanding of factors associated with populations that affect risk of developing smoking related disease could lend insight into how added ingredients may impact risk and outcomes of smoking related diseases. According to Burchard and coworkers, (2003) studies of the roles of race and ethnicity in disease susceptibility may be complicated by genetic admixture, which is the occurrence within a population of individuals who have multiple racial or ethnic
origins. However, race and ethnicity are descriptors that could help to identify differential susceptibility to disease and disease outcomes between groups.

A large disparity exists in preference for menthol cigarettes between black and white individuals. Between 70 and 80% of African American smokers smoke menthol cigarettes, whereas approximately 25% of Caucasian smokers smoke menthol cigarettes (Cummings et al., 1987; Orleans et al., 1989; Sidney et al., 1995). Black male smokers have a higher rate of morbidity from cancers associated with smoking than do white male smokers (Day et al., 1993; Harris et al., 1993; Satariano & Swanson, 1988; Teter et al., 2002; Stotts et al., 1991). Black men initiate smoking at later ages than white men (Rogers & Crank, 1988), and black male smokers reportedly smoke fewer cigarettes per day than white male smokers (Novotny et al., 1988). Black smokers have higher levels of cotinine than white smokers (Ahijevych et al., 1996; Ahijevych & Parsley, 1999; Benowitz et al., 1999). The rate of cotinine clearance in African American smokers (Perez-Stable et al., 1998) and Chinese-American smokers (Benowitz et al., 2002b) is significantly lower than the rate of cotinine clearance in Caucasian smokers. Several investigators found that black persons carry an allele of the CYP1A1 gene associated with lung adenocarcinoma (Cosma et al., 1993; Crofts et al., 1993; London et al., 1999; Shields et al., 1993; Taioli et al., 1998). Other differences could result from differences in smoking behavior or exposure to smoke in the home or work environment (McCarthy et al., 1992).

The risk of morbidity and mortality from COPD is higher for white smokers than for black smokers (Coultas et al., 1994). However, the mechanism(s) underlying this phenomenon have not been explained (Sellers, 1998). Several metabolic enzymes exhibit differences in gene frequencies across ethnic groups. As reviewed in Sellers (1998), UDP glucoronol transferase enzymes, which conjugate and inactivate carcinogens (Richie, Jr. et al., 1997), glutathione transferase enzymes which inactivate and remove reactive electrophilic intermediates (Strange & Fryer, 1999), cytochrome P450 enzymes CYP2E1, and CYP2D6 could deactivate chemical substances in cigarette smoke and influence the risk of smokers for developing various smoking related diseases (Sellers, 1998). The Tobacco Use Needs Assessment program of the state of Arizona provides statistics on tobacco use according to geographic area and population (University of Arizona College of Public Health, 2002). Knowledge about the contribution of race and ethnicity to differences in smoking behavior and exposure to differences in rates of morbidity and mortality from smoking related diseases could enhance feasibility of testing for the health effects of ingredients added to cigarettes.
7.4.5.3 Gender

Several lines of research suggest that women have a higher risk of developing lung cancer than do men who smoke (Brownson et al., 1992; Harris et al., 1993; Lubin & Blot, 1984; McDuffie et al., 1987; McDuffie et al., 1991; Osann et al., 1993; Risch et al., 1993; Zang & Wynder, 1992). However, other studies do not report such an increased risk (Thun et al., 2000). Data from Wei and coworkers (1993; 2000) suggests that women have decreased ability to repair DNA damage. This group proposes that a reduced ability to correct DNA damage is associated with a greater risk for lung cancer.

Various physiological, psychological and behavioral factors may contribute to an individual’s smoking behavior. Creighton and Lewis (1978) and Battig et al. (1982) show that smoking behavior differs between men and women. Both groups found higher puff frequencies and smaller puff volumes in women. Men and women also differ in their responses to the removal of olfactory sensory cues during smoking (Perkins et al., 2001).

As reviewed by Gritz et al. (1996), gender differences in nicotine sensitivity, tolerance, dependence and withdrawal may affect smoking cessation. Although other studies revealed no differences in effectiveness of pharmacological smoking cessation aids (Fortmann & Killen, 1995; Hurt et al., 1994). Female biological processes such as menstruation, menopause, and pregnancy may also affect the ability to cease smoking (Gritz et al., 1996).

According to Grunberg et al. (1991), nicotine metabolism occurs faster in men than in women. In addition, nicotine has a stronger influence on body weight and food and water intake in women than in men (Grunberg et al., 1986).

Kim and Hu (1998) reported that the efficiency of deposition of aerosol particles is generally higher in women, however this has not yet been fully described (Phalen & Oldham, 2001). More research is needed to understand the effects of gender on smoking behavior and exposure and adverse health effects of smoking. Gender differences could exist in the response to ingredients added to cigarettes.

7.4.5.4 Age

Approaches to understanding deposition of aerosol particles included models that often failed to account for the role of aging on particle deposition efficiencies. As individuals age, the size of distal airways may increase, tissue elasticity may be altered, and the body may experience deterioration (American Heart Association, 2001). The effect of added ingredients on deposition may vary depending on the age of the smoker. In addition, Wei and colleagues (1993; 1998) report that as
individuals age, the ability for DNA repair decreases, however, later studies by the same group (Wei et al., 2000) did not support this finding. Consideration of age in modeling deposition may be valuable.

### 7.4.5.5 Culture

Some data suggests that culture impacts the way that cigarettes are smoked (Schwartz et al., 1961; Hammond, 1959). The State of California has developed research initiatives to investigate the contributions of family and environment on smoking behavior (University of California Office of the President, 2000). Studies have demonstrated that children with siblings and friends who smoke are more likely to adopt the habit of smoking (Alexander et al., 1983).

### 7.4.5.6 Diet and nutrition

The role of diet and nutrition in the development of diseases associated with smoking cigarettes has not been completely described. Epidemiological studies have shown that diets rich in fruits and vegetables associate with a decreased risk of lung cancer (reviewed in (Ziegler et al., 1996)). Khaw and others (2001) identified an inverse relationship between the plasma concentration of vitamin C and risk of death from cardiovascular disease. Elinder and coworkers (1994) showed that polyphenolic flavonoids may confer protection from cardiovascular disease (Kromhout et al., 1995; Hertog et al., 1993). Other studies, however, do not support a protective effect of antioxidants (Elinder & Walldius, 1994). Maxwell and Grieg (2001) propose conducting long-term prospective randomized controlled trials in order to investigate advantages of increases in antioxidants.

Wynder et al. (1987) suggested that fat intake could account for difference in lung cancer between United States and Japan. Fat may affect the function of some lung oxidases and lead to additional conversion of carcinogenic form of compounds (Wynder et al., 1987). It has been suggested that obesity (Alavanja et al., 1996) and a diet rich in fat (De Stefani et al., 2002) are risk factors for adenocarcinoma of the lung. Whether diet and body weight could influence the effect of ingredients added to cigarettes is not known.

### 7.4.6 Individual variation in health effects and behavior

There is a wide intra-individual variation in uptake of inhaled substances. Physiologically based pharmacokinetic simulation models using experimental animal and human data have shown that physiologic factors, such as alveolar ventilation, substantially impact the kinetic behavior of potentially carcinogenic inhaled substances (Lin et al., 2001). In addition, rates of metabolism and clearance of chemicals from the body are known to vary. Exposure time to nicotine is affected by the rate of nicotine metabolism and clearance from the body. Factors that affect an individual’s exposure to compounds and health outcomes due to exposure
have not been fully described. Ingredients added to cigarettes may influence the uptake of substances already present.

7.5 TESTING ISSUES AND METHODS

7.5.1 Animal Models

There are multiple tests using animal models that may be applied to the study of health effects of smoking cigarettes. However, whether they predict the development of human diseases, particularly the human diseases associated with smoking, is an important consideration. Validated animal models of smoking-related diseases will prove useful for a determination of the effects of added ingredients on the risk of cigarette smoking related diseases.

Various animal models have been used to study the biological effects of inhalation exposure to cigarette smoke. In addition to the thousands of other substances in cigarette smoke, animals are exposed to carbon monoxide. The affinity of carbon monoxide for hemoglobin is 240 times that of oxygen for hemoglobin (Douglas et al., 1912). Carbon monoxide uptake in rats is faster than the predicted rate of CO uptake in man (Silbaugh & Horvath, 1982). To decrease the probability of rodent carbon monoxide related poisoning, investigators dilute the cigarette smoke to which the animals are exposed and allow intermittent exposure to cigarette smoke.

7.5.2 Clinical Studies (behavior)

The effects of menthol in cigarettes has undergone some investigation (Caskey et al., 1993; Porchet et al., 1987; Tsujimoto et al., 1975). However, the effects of many other added ingredients on smoking behavior are not known. Added ingredients could cause multiple changes in smoking behavior. They could affect smoking topography, the number of cigarettes smoked per day, the amount of the cigarette smoked and choice of cigarette smoked. Investigators could assess many parameters such as the number of puffs per cigarette, puff duration, puff volume, peak puff flow, inter-puff interval, total smoking time, and total inhalation volume to describe how a cigarette is smoked and measure the variation within and among smokers. Measurement of these parameters with paired, otherwise identical cigarettes, with and without an added ingredient, are not likely to prove useful in understanding the effect of added ingredients on smoking behavior, because it is not readily apparent how to integrate these parameters into a measure of behavior. Measurement of the number of cigarettes smoked and of a biomarker of smoking behavior will lead to an integrated measure. Conducting of such behavioral studies is a high priority for LSRO, in the sense that additional data about such studies would make decisions about the feasibility of testing easier.
7.5.3 Analysis of Data

Many epidemiological studies have explored information about the health effects of cigarette smoking. Additional analysis of these surveys and other types of data may help to determine how cigarette smoking impacts relative risk of adverse health effects of cigarette smoking and to determine how age, gender, race, and ethnicity affect that risk (University of California Office of the President, 2000). cDNA microarray expression profiling and proteomic techniques may prove useful characterizing individuals risk of morbidity and mortality from cigarette smoking related diseases.

7.6 SUMMARY

LSRO and its advisors have identified areas where additional research would improve decision-making about the feasibility of determining the relative risks of smoking cigarettes with added ingredients as compared to smoking cigarettes without added ingredients. Additional research is needed to understand the chemistry of cigarette smoke. The relationship between substances in the cigarette and smoke from the cigarette could be used as a basis to assess the effects of the added ingredient on the smoke matrix. Additional validated, standardized techniques for smoke chemical detection will aid this process. Information about the physics of cigarette smoke would serve as a basis for determining whether added ingredients affect exposure levels of individuals to cigarette smoke. Research is needed to elucidate the biological and social factors that elicit changes in smoking behavior. Consistent use and further development of biomarkers of exposure and effect will help to provide insight into health effects of smoking cigarettes with added ingredients. Improved understanding of the mechanisms of disease associated with cigarette smoking would provide insight as to whether added ingredients contribute to these diseases. How risk factors such as gender, race or ethnicity, culture, gender, age and diet influence risk of modify health risks will also contribute to such analyses. Clinical studies of the effects of added ingredients on exposure to smoke, as mediated by smoking behavior, will also be useful to making decisions about the feasibility of testing for the relative risk of adverse health outcomes from smoking cigarettes with added ingredients as compared to smoking cigarettes without added ingredients.
CONCLUSIONS

OUTLINE

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8.4 PRIORITY SETTING
8.1 PRIMARY CONCLUSIONS

LSRO recommends that manufacturers test ingredients added to cigarettes. Such testing is both feasible and worthwhile as discussed below. (See Appendix G). Test data will inform judgments about the risks of cigarettes containing these ingredients, relative to similar cigarettes without the same ingredients. Some of the assumptions that support this recommendation merit expression.

Absolute safety, defined as the absence of risk, is unattainable. The term, “safe,” as used in most product safety testing, comes closest to the criterion of “reasonable certainty of no harm,” as used at the U.S. Food and Drug Administration (FDA). When most experts refer to a product as “safe,” they mean something operational, for example, that a food additive has undergone FDA review and passed.

In this report, LSRO has used a different approach from FDA’s, looking at the effects of ingredients added to cigarettes for relative risk, defined as the risk of exposure to a cigarette with the ingredient, relative to the risk of exposure to a cigarette without the ingredient. Employing relative risk created many advantages in LSRO’s review. A relative risk approach diverges from traditional product safety testing. An “unchanged relative risk” does not imply that the underlying activity, cigarette smoking, is safer or that addition of the ingredient has changed the risk of the underlying activity.

The feasibility of testing for relative risk depends crucially on testing paired cigarettes that differ only in the presence or absence of an ingredient or mixture of ingredients. Traditional safety testing would test the ingredient in isolation. Instead, LSRO recommends testing an ingredient within cigarettes during the act of smoking. Should experimental pairs of cigarettes, with and without an added ingredient, prove impossible to manufacture or unworkable in testing, the approach taken here will not apply.

The feasibility of testing ingredients added to cigarettes also depends on practical decision-making. For example, initiation of smoking is a public health concern. However, direct study of the effects of ingredients added to cigarettes on initiation of smoking, by presenting non-smokers with cigarettes with and without the additive, is unethical.
The conclusions of this Phase One report could apply to new or existing ingredients currently added to cigarettes, not only to the existing ingredients described in Appendix D. However, LSRO’s emphasis has been to review ingredients already in use first. (See Section 8.4) LSRO’s Phase Two report will consider the scientific criteria to be applied to the evaluation of ingredients and will address whether certain ingredients should obtain expedited review. The case studies and discussions within this report revolve around substances already in use.

Potentially, new ingredients and/or mixtures of ingredients may result in effects not previously associated with cigarette smoking. Thus, the introduction of novel ingredients may require more robust or extensive testing and monitoring than considered in this report. Importantly, LSRO has operationally excluded from this report considerations pertaining to issues whereby an additive could potentially decrease the overall intrinsic toxicity of cigarette smoke.

In this report, LSRO’s conclusions have five underpinnings: the matrix of chemical substances in cigarette smoke, the pyrolysis and transfer of ingredients during smoking, the concept of no detectable change in overall biological toxicity, the notion of human smoking behaviors, and a case-by-case evaluation using the sum of base knowledge and newly generated knowledge. The subsections below review each underpinning. Each has scientific justification.

8.2 SCIENTIFIC RATIONALE

8.2.1 A Matrix of Chemical Substances in Cigarette Smoke

Scientific data, reviewed in Chapter 3 of this report, establish that cigarette smoke is a complex mixture of many chemical substances, which changes with time. Throughout this report, LSRO has referred to the “chemical matrix” or the “smoke matrix,” as a shorthand way of communicating this concept. Many research studies have attempted to relate the biological toxicities of individual smoke constituents to human health outcomes. The relative lack of overall success in this endeavor has persuaded LSRO to regard human health effects in relation to cigarette smoke as a whole.

When comparatively testing otherwise identical cigarettes, the added ingredient should alter the baseline smoke matrix as little as is possible. Animal tests are not sensitive measures of change. Smoking machines, which create experimental cigarette smoke, do not necessarily reproduce the volume of inhaled smoke delivered to the human lung. They do represent a fair technology for examining comparative toxicities of different cigarettes smoked in a similar manner. The experimental
smoke can be regarded as a physico-chemical surrogate for human-generated
smoke.

8.2.2 Pyrolysis and Transfer

The scientific data in Chapter 3 of this report review the evidence that some
ingredients pyrolyze during cigarette combustion, even to complete elimination.
Similarly, Chapter 4 emphasizes that an ingredient, or pyrolysis products of the
ingredient, can transfer into a smoker’s lungs along with the smoke. The current
state-of-the-science is such that exclusively testing ingredients outside of the smoke
matrix does not provide sufficient information to predict the behavior of the ingredient
within the smoke matrix.

The potentials for pyrolysis and for transfer of an ingredient within a smoke matrix
are crucial elements in LSRO’s approach to the relative risks of different amounts
of the ingredient in a cigarette. Testing unpyrolyzed ingredients in isolation,
particularly by different routes of administration, will not provide information about
potential pyrolysis products. Some ingredients and pyrolysis products of ingredients
will undergo complete degradation during cigarette combustion and will not reach
the smoker’s respiratory tract. Other ingredients, like menthol, will resist pyrolysis,
distilling, condensing and redistributing down the tobacco column, transferring nearly
completely intact.

Scientific methods and technology are readily available to generate data about the
effects of an ingredient on smoke chemistry. Thus, tests to resolve questions
about pyrolysis and ingredient transfer to smoke are feasible.

When an ingredient added to cigarettes, or a pyrolysis product of the ingredient
added to cigarettes, transfers in the smoke, traditional methods of inhalation
toxicology can be brought to bear to obtain dose-effect data and exclude an increase
in risk superimposed on the known chronic adverse health effects of smoking.
Because scientific methods and technology exist to meet these concerns, testing
ingredients added to cigarettes to understand the relative risks of those ingredients
when added to cigarettes is feasible.

To decrease the risk that an ingredient added to cigarettes might increase a known
adverse health effect associated with cigarette smoking, it will be necessary to
understand better the chemistry, exposure, and adverse health effects associated
with cigarette smoking. In addition to excluding an additional health effect, one
associated with the added ingredient, a concern exists that the additive might change
the chemistry of the smoke matrix in such a way that the known, chronic adverse
health effects of smoking might change. A change could come about in either of
two ways: (1) the ingredients added to cigarettes, or pyrolysis products of the
ingredients added to cigarettes, might change the toxicity of the inhaled smoke or
(2) the ingredients added to cigarettes, or a pyrolysis product of the ingredients added to cigarettes, might change smoking behavior so as to result in greater initiation or either of these possibilities also might occur in the absence of transfer of the added ingredient and its pyrolysis products during inhalation of a plume of hot smoke.

To minimize the risk of adding new adverse health effects to the existing adverse health effects associated with cigarette smoking, research data will have to reveal the effects of an ingredient added to cigarettes on smoke chemistry, because one of the prominent concerns relates to potential pyrolysis products of the ingredients added to cigarettes, not only to the added substance *per se*.

While science-based testing can achieve, or at least approach, many of the above objectives, application of a set of standardized safety tests, such as those used by FDA’s food additives program or EPA’s pesticides program, could not. Testing a purified substance could miss a novel health effect associated with a pyrolysis product. If the purified, isolated substance is pyrolyzed separately, the problem remains of demonstrating that these pyrolysis products have some identity with those formed in the cigarette smoke matrix. Testing in experimental animals is insensitive, relative to testing with techniques of analytical chemistry and physics.

Existing recommended, standardized programs for testing toxic effects, *i.e.*, food additive methods, are not directly transferable to testing cigarette additives. Gastrointestinal and cutaneous pathways differ markedly from the inhalation pathway. In addition, the effects of a chemical substance may not resemble the effects of its pyrolysis product(s). Therefore, existing test guidelines may have relevance, but they will require modification. Any valid, useful test must comply in principle with the testing principles in Chapter 6 of this report.

Data about the pyrolytic fate of each ingredient added to cigarettes under smoking conditions will be uniformly desirable for all ingredients. In addition to understanding whether an added ingredient shifts the chemical composition of other substances in the smoke matrix, test data must be available to understand the relative risks, if any, of the effects of inhalation exposure to an unpyrolyzed ingredient.

### 8.2.3 No Detectable Change

A “no detectable change” approach interprets test data and other health effects information to specify an upper limit on the amount of an ingredient added to a cigarette. The upper limit relates to test signals, which depend on sampling and test methods. The approach reduces the amount of an ingredient per cigarette below a level where change is detected in any of a group of tests. The tests should be balanced among the kinds of tests (physical, chemical, biological, and clinical) that generate information about potential human health effects. At some upper
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limit of the amount of ingredient per cigarette, if tests do not detect a difference between smoke from a cigarette containing an ingredient and smoke from an otherwise identical cigarette without the ingredient, no a priori reason will exist to suspect a differential health effect.

Other approaches exist to designating an upper limit on the amount of an ingredient to add per cigarette. As an example, a manufacturer can limit the amount of an ingredient by comparison to a highly toxic substance. If the maximum dose acquired by smoking a cigarette falls below a biological cut-off or limit for a highly toxic substance, as established by other means, the manufacturer could regard this maximum amount as acceptable.

Any inference based on testing of an ingredient for pyrolysis or for transfer will depend crucially on the hypothesis that changes in test cigarette tobacco will not alter the response relationship between the amount of ingredient and test signal for either pyrolysis or transfer. If the test cigarette contained Burley tobacco and the experimenter changes to Maryland tobacco, test signals will change in relation to the same amounts of ingredient added. If a change in the tobacco substrate alters the nature or magnitude of changes in the smoke matrix, the logic of testing to detect a point of no detectable change will still hold, as long as the change in the tobacco substrate does not change the ingredient-signal relationship. Similarly, differential changes in the levels of substance in the smoke, given a change in the tobacco substrate, would not necessarily invalidate experiments to set the maximum level of ingredients added to cigarettes per cigarette. The tobacco substrate would need to shift the relationship between the amount of an ingredient added and the test signal.

If testing revealed no incremental chemical or biological effect, a new test, previously not used, might detect a difference. Even if LSRO obtained data from every known test, a new test might arise, which could detect a difference. Like all areas of science, LSRO’s inference will relate to the state-of-the-art applicable technologies existing at some point in time. A test also can generate a false negative result, a finding of no effect when one exists. Employing a battery of tests, instead of a single test, will reduce the probability that a false negative result will influence estimation of the upper limit. Each test has characteristic statistical limitations, such that retesting with more samples could usually detect a difference, not previously noted. Testing with reliable methods that have good, although presently unspecified, signal-to-noise characteristics will minimize the possibility of underestimating the upper limit for an ingredient not to change smoke chemistry. In this regard, selection of specific tests, which LSRO will describe in its Phase Two report, Scientific Criteria, will become important.

Most ingredients are added to cigarettes in relatively small amounts, for example, in parts per million. The ability to detect a change above background noise is

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potentially meaningful, because exceeding background noise could mean that amount of an added ingredient altered a biological response. Specific cigarette brands also contain unique mixtures of added ingredients, and the components of these mixtures may interact with one another. Therefore, an ingredient in a mixture should be evaluated in the brand of cigarette to which it will be added and marketed.

For existing ingredients added to cigarettes, LSRO will seek useful test data to make decisions about two objectives: (1) not increasing any of the well-known adverse health effects associated with cigarette smoking, and (2) not adding a new, different adverse health effect. Detection of a difference induced by an ingredient in smoke is sufficient to allow experimental location of a no detectable change level. Subsequently, LSRO can estimate the maximum level to add per cigarette. However, “no detectable change,” or “maximum likely change,” is a difficult concept. It neither implies the absence of any change, nor does it mean that the specified measure lacks any implications for relative risk.

In making its decision about feasibility, LSRO considered the joint implications of analyzing a model smoke and of using indicator substances. Neither approach completely eliminates the possibility that an added ingredient might change the concentration of some substance in cigarette smoke and that this smoke substance might induce an adverse human health effect. Thus, both approaches might be employed, monitoring both the ingredient, and its pyrolysis products, and indicator substances usually found in cigarette smoke.

At a level of an ingredient where exposure-response relationships change, current scientific data do not permit LSRO to predict whether known adverse human health effects might increase or decrease. Therefore, LSRO will regard any change as an indication of a potential adverse effect or as detection of the added ingredient. This approach means that added ingredients also will not necessarily decrease adverse health effects, particularly adverse health effects of smoking cigarettes; it does not address them. Chemical components of the smoke can serve as indicators of changes within the matrix. LSRO will describe some substances, which might make the best indicators, in its Phase Two report, Scientific Criteria. Thus, LSRO will regard any detected change in an indicator substance as potentially adverse development and evaluate it as such, unless the manufacturer can provide convincing evidence to the contrary.

8.2.4 Human Smoking Behavior

Humans have a limited ability to articulate their personal perception of smoke exposure. The evidence favors human smoking behaviors intended to achieve an approximately constant amount of delivered and absorbed nicotine. Smoking behavior determines the delivery of nicotine to the respiratory tract absorptive surfaces. CNS-mediated feedback mechanisms exist which might create the
phenomenon of compensation, such that increasing smoke intake compensates for lower nicotine yield in the smoke.

However, the taste and aroma of cigarette smoke also influence smoking behavior. Ingredients added to cigarettes and designed to affect taste and aroma might contribute to the maintenance of smoking behaviors. Little definitive published data shows that added ingredients might or might not alter the sensory experience of smoking. Sensory cues, such as cigarette flavor and aroma, potentially support the smoking behaviors. Smoking pairs a distinctive aroma and taste ambiance with a pharmacological effect.

The behavior of individual smokers is highly variable. The study of the toxic material to which smokers are exposed is a relatively complex and difficult scientific subject. Because of high inter-individual variation, human studies that compare different smokers will likely encounter difficulties. However, cigarette consumption and smoke exposure are amenable to scientific investigation. Thus, testing of smoking behavior is feasible.

8.2.5 Case-By-Case Evaluation

The wide range of chemical and biological properties of existing ingredients will make the application of over-riding rules impractical and unlikely. The evaluation of an ingredient will require case-by-case expert judgment. The application of written review criteria will help to avoid an inconsistent treatment of similar ingredients, maximize transparency of the process, and support the establishment of an evidence-based consensus.

Evaluation of each ingredient should begin with a scientific review of the available, relevant information by qualified experts. Scientists often will need to proceed with the evaluation of an ingredient on a case-by-case basis. General scientific principles, as described in Chapter 6, should inform and assist the review of a substance. LSRO will further detail the application of these principles in the Phase Two report, Scientific Criteria.

The use of every conceivable test is neither necessary nor desirable. LSRO cannot locate definitive tests that accurately predict specific health endpoints related to cigarette smoking. Beyond a small number of key tests, to establish identity of smoke between a pair of experimental cigarettes, additional tests will yield little additional predictive information and may generate disinformation. A balanced program of qualitatively different tests of ingredients added to cigarettes will be desirable (physical, chemical, subcellular, cellular, intact animal, and human tests). Signal-to-noise ratio and practicality will be important factors in selecting appropriate
tests. LSRO will describe these factors and tests in more detail in its Phase Two report, *Scientific Criteria*.

8.3 DETAILS & EXCEPTIONS

8.3.1 Biological Testing

Testing an added ingredient with traditional animal toxicity tests may be insensitive, because animal bioassays do not predict human health effects associated with cigarette smoking. Any potential animal model would have to predict at least one human health effect associated with cigarette smoking, to be useful in feasible testing. To date, few, if any, short-term biomarkers accurately detect any of the human diseases associated with cigarette smoking. Direct observational studies of chronic adverse human health effects in epidemiological studies take too long and are too cumbersome to help evaluate most added ingredients.

To evaluate the relative risks associated with an ingredient, LSRO will need data about biological changes in smoke chemistry as a function of the amount added. In effect, these tests use a biological measurement to compare smoke from a cigarette containing an ingredient to the cigarettes smoked in existing epidemiological studies. They are measures of biological similarity. Traditional product safety testing in laboratory animals would make little sense in the absence of demonstrable transfer, changes in smoke chemistry, or changes in behavior relevant to smoke exposure.

8.3.1.1 Prediction of human health effects

Over the last two decades, cigarette manufacturers produced extensive test data using traditional animal models and exposures to either (1) diluted, cooled smoke or (2) particulate matter filtered from whole smoke. Both exposure pathways miss potentially crucial aspects of exposure. The first approach may miss pyrolytic or pyrosynthetic changes in the smoke that humans inhale. The second may miss changes in the gas phase of cigarette smoke.

Animal toxicology tests are relatively insensitive methods to detect changes induced in cigarette smoke by an added ingredient, relative to the known toxicity of cigarette smoke. Animal toxicity tests also do not predict the adverse health effects of cigarette smoking observed in epidemiological studies of humans. Testing an added ingredient in isolation almost certainly will miss the more meaningful and relevant effects associated with exposure to a cigarette smoke matrix modified by the additive. Pulmonary absorption of a pyrolysis product in an inhaled plume of hot smoke containing many thousands of chemical substances, but changed amounts or ratios by the presence of the additive, would not be detected in experiments where only the effects of the additive were studied.
Predictive tests of morbidity associated with human smoking also are not yet available, although proximal markers of risk of diseases associated with smoking, such as early biomarkers of effect, have been suggested and are actively being explored. Identification of relevant surrogates of disease will improve the overall review process.

8.3.1.2 Biomarkers of human disease

Biomarkers of effects for the human diseases associated with cigarette smoking are not yet readily available. Biomarkers of exposure, such as cotinine levels, will prove useful to establish whether human smoking behavior changes in response to an added ingredient. Should biomarkers of human adverse health effects arise, toxicologists can study these biomarkers in animal models. Should an animal model prove useful as a predictor of adverse human health effects, toxicologists can employ the model in the paradigm of paired, similar cigarettes, with and without an added ingredient, to detect exposure-response relationships and levels of no detectable change. Toxicologists can compare substances that induce mutagenic reversion in smoke produced by human smokers to substances that induce reversion in their urine and seek animal models that either replicate or allow predictions of these relationships.

8.3.1.3 Duration and other elements of human epidemiology

Observational studies of human epidemiological effects will take too long and prove too cumbersome to evaluate most added ingredients. At best, LSRO can hope for inferential studies comparing populations smoking cigarettes with added ingredient to populations smoking cigarettes lacking added ingredients. Such studies likely will omit identification of specific ingredients in the cigarettes smoked. When possible, results obtained or inferred from testing should undergo validation by post marketing surveillance, epidemiological studies, and/or clinical trials. However, using pack-years, as a measure of exposure is potentially inaccurate, while better than no indicator. Epidemiologists usually obtain exposure information about smoking from questionnaires or interviews. Retrospective, self-reported data may inaccurately characterize the intermittent exposures of individual smokers. As smokers change brands, they will also change exposure to the effects of added ingredients. Measures of cigarette consumption cannot easily account for variable smoking.

A biological model that does predict human disease outcomes of different durations of cigarette smoking would greatly simplify testing of ingredients added to cigarettes.

8.3.2 Mixtures

Besides the presence of mixtures of substances of natural origin in cigarettes and mixtures of many chemical substances in smoke, manufacturers seldom add a
single ingredient in producing cigarettes. Mixtures of ingredients are added to cigarettes. For testing purposes, an added ingredient can be a single added chemical compound or substance, or a specific recipe of compounds or substances, such as a proprietary flavor formula, or a complex mixture, for example, cocoa. The testing of ingredients added to cigarettes, one-by-one, is subject to exceptions:

Exception one: If predictive tests of significant cigarette-associated health effects become available, manufacturers also should apply such tests to all added ingredients in combination.

Exception two: Manufacturers will have to retest the specific mixture of added ingredients, in the proportions used in their brand(s) of cigarettes, to obtain an assurance that the mixture lacks the same effect absent when individual added ingredients are tested, which might arise from an interaction between two or more ingredients.

8.4 PRIORITY SETTING

Because manufacturers already use many ingredients, setting priorities on the evaluation of ingredients makes good sense. Dividing added ingredients into categories of existing and new also makes good sense, because the kinds of tests and the urgency of testing clearly differ for substances already in use. Manufacturers can set priorities for evaluation of ingredients based on the relative maximum mass (or average mass) added to a brand of cigarette, with the highest mass per cigarette evaluated first. Priorities for evaluation which factor the mass of ingredients by measures of toxicity, such as a lethal dose, also make sense, because doses which affect different measures of toxicity in different test systems usually covary. A division of added ingredients into categories of pyrolyzed and unpyrolyzed also makes sense, with the unpyrolyzed receiving more attention.

The need to set priorities explains why an incremental approach to testing is prudent. A checklist of specific tests to apply to each added ingredient is neither feasible nor desirable. Given these exceptions, a balanced program of testing ingredients added to cigarettes will greatly reduce the possibility of any adverse human health effect, beyond the premature mortality and morbidity already seen with cigarette smoking.

Epidemiological studies of the premature mortality and morbidity of cigarettes containing menthol demonstrate that scientists can obtain definitive evidence about ingredients added to cigarettes. Epidemiological studies of the effects of changes in the design of cigarette structure additionally show that premature mortality and morbidity can change. (See Chapter 6, Section 6.4.)
Without some test data, managing changes in the exposures from ingredients in cigarettes will prove difficult. Dose is the critical factor in the possibility of a change in the relative mortality and morbidity of cigarette smoking. However, dose is difficult to measure. At present, no tests appear to predict the major human health effects of chronic cigarette smoking (premature mortality, cancer, cardiovascular effects, and chronic obstructive pulmonary disease). Without predictive tests for specific endpoints, exposure-response relationships from traditional bioassays will prove useful in understanding relative risks and setting limits on the amounts of ingredients in cigarettes that are unlikely to change the risk of smoking, relative to smoking identical cigarettes without the same added ingredients.

A program of testing will put the ingredients added to cigarettes into perspective with each other in a biologically oriented framework and need not prove the complete absence of relative risk. However, such a program raises questions about action limits, which LSRO will address in its report on the second phase of this project, *Scientific Criteria*. Additional information, particularly information aimed at understanding which categories of added ingredients pose the highest potential risks, will serve to target strategies for added ingredients posing the greatest potential health risk.

Test data should include quality assurance and peer review, both within and outside the manufacturing industry. A scientific peer-reviewed report will be accessible to the public and scientific community. Such reports of added ingredient reviews will be especially useful as criterion-based references to build on, when new testing technologies become available and additional relevant data accumulate.
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Added Ingredients Review Ad Hoc Expert Panel:

Carroll E. Cross, M.D. is a pulmonary critical care physician at the University of California at Davis School of Medicine, Division of Pulmonary and Critical Care Medicine. He graduated from Columbia College of Physicians and Surgeons in 1961, completed his internship at the University of Wisconsin Hospital in 1962, his residency at Stanford Hospital Center in 1964 and his clinical and research fellowship training at the University of Pittsburgh Medical Center in 1968. He was certified in internal medicine in 1969 and in pulmonary disease in 1971. He has been at the University of California, Davis since 1968, where he is currently a professor of medicine and physiology. Dr. Cross has published over 200 papers in such fields as air pollutants, antioxidant micronutrients, inflammatory-immune system oxidants, ozone, oxides of nitrogen, cigarette smoke, and related aspects of inhalation toxicology as it relates to respiratory tract diseases. He is a member of several professional organizations including the American Physiological Society, the UK Biochemical Society, the Oxygen Society, the Mount Desert Island Biological Laboratory, the Western Society of Physicians, and the American Society for Clinical Nutrition. He serves on the editorial boards of the American Journal of Clinical Nutrition and Free Radicals in Biology and Medicine, and has served on research review panels for the Veterans Administration, for the NIH and for the Heart, Lung and Cancer Society Associations.

Shayne Cox Gad, Ph.D., D.A.B.T., F.A.T.S. is the Principal at Gad Consulting Services. After majoring in chemistry and biology at Whittier College, he obtained his doctorate in pharmacology/toxicology from the University of Texas. As a fellow at Bushy Run Research Center, Union Carbide Toxicology Laboratory he developed a system for assessment of toxicity of polymer thermal decomposition products. Later, he worked at the Shell Development Laboratory establishing a new inhalation toxicology research facility. As Manager of Mammalian Toxicology at Allied Corporation, Dr. Gad was responsible for all mammalian toxicity testing including the operation of the entire Department of Toxicology laboratory and all external contract testing. Dr. Gad has also worked at G.D. Searle & Co. as Director of Toxicology and Senior Director of Product Safety and Metabolism where he used an interdisciplinary approach to oversee safety/toxicity research programs, assisted with the prioritization of research and development efforts, interacted with foreign firms, regulatory agencies, and developed world wide occupational Airborne Control Objectives. He has also served as the Director of Medical Affairs.
Donald E. Gardner, Ph.D., F.A.T.S. is the President of Inhalation Toxicology Associates. He received his M.S. in medical microbiology from Creighton University and his Ph.D. in environmental health and toxicology from the University of Cincinnati. He has worked for the U.S. Environmental Protection Agency (EPA) and the Public Health Service. Following retirement from EPA, he joined the staff of Northrop/ManTech Corporation as Vice President and Chief Scientist. He held academic appointments at Duke University, North Carolina State University, and the University of Massachusetts. He served on various panels of the National Research Council since 1989, for part of this time as Vice-Chairman of the Committee on Toxicology. He is presently on the Editorial Board of *Toxic Substance Journal* and *Environmental and Nutritional Interactions*. At Inhalation Toxicology Associates he provides consulting services to several Federal Agencies, the World Health Organization, private industry, and law firms. He has published more than 225 peer-reviewed manuscripts and book chapters and is the founding Editor of *The Journal of Inhalation Toxicology*. He is also a co-editor of the *Target Organ Toxicology Series* and *New Perspectives: Toxicology and the Environment*. He is a Fellow of the Academy of Toxicological Science. Among his many accolades, he has received lifetime outstanding achievement awards from the Society of Toxicology in the areas of both inhalation toxicology and immunotoxicology.

Louis D. Homer, M.D., Ph.D., was the Medical Director of Clinical Investigation and Biomedical Research at Legacy Research, Holladay Park Medical Center. He acquired a Ph.D. in physiology and a M.D. from the Medical College of Virginia. He has served as Assistant and Associate Professor at Emory University concentrating on physiological processes and mathematical models. Afterward he served as Associate Professor at Brown University and then moved on to become a Research Medical Officer at the Naval Medical Research Institute concentrating on biometrics, physiology, environmental medicine, metabolic research, and kidney transplant histocompatibility. He served as a consultant to scientists regularly on topics ranging from physiology, mathematics, statistics, and computer applications. He also has reviewed proposals and has served on site visit teams for NIAID, NHLI, NSF, and the Naval Medical Research and Development Command. He has reviewed prospective articles for the *Journal of Theoretical Biology*, *Microvascular Research*, *American Journal of Physiology* and the *Journal of Applied Physiology*. His interest in using mathematical models of physiology in his research has led him to become familiar with a number of computer languages, numerical algorithms, iterative least-squares estimation, and iterative maximum likelihood estimation.
Rudolph Jaeger, Ph.D., D.A.B.T., B.C.F.M., is Research Professor of Environmental Medicine at the New York University School of Medicine. In addition to his academic teaching and research activities, he is Principal Scientist at Environmental Medicine, Inc., a consulting firm specializing in consumer product evaluation, environmental health risk assessment and industrial toxicology. Dr. Jaeger also serves CH Technologies (USA) Inc. as President and Chief Scientific Officer. CH Technologies, a scientific instrument manufacturing company, specializes in low volume inhalation exposure systems for pharmaceutical and infectious disease research. CH Technologies and affiliates manufacture specialized cigarette smoking machines. Dr. Jaeger earned status as a Diplomate of the American Board of Toxicology in 1980. The U.S. Environmental Protection Agency accredited him as an Asbestos Inspector, and he is a Registered Environmental Assessor in the State of California. He is a Certified Lead Inspector and Risk Assessor in the State of New Jersey. He is a Diplomate of the American Board of Forensic Medicine and a Board Certified Forensic Examiner. Dr. Jaeger served on expert panels of the Toxicology Information Program Committee of the Board of Environmental Toxicology of the National Research Council. His research interests include inhalation toxicology, plastics and their monomers, combustion products, pulmonary pathophysiology, liver toxicity and pathophysiology, and the effects of lead and heavy metals on the developing nervous system.

Robert Orth, Ph.D. is a physical chemist at Apis Discoveries, L.L.C. He is also a consultant to Monsanto Company and adjunct Associate Professor of Physical Chemistry at the University of Missouri, where he teaches undergraduate courses in physical chemistry, instrumental analysis and general chemistry. After obtaining his Ph.D. from Case Western Reserve University he completed a post doctoral fellowship at the University of Utah researching mass spectrometry and developed High Pressure Liquid Chromatography/Mass Spectrometry interface using ultrasound. He conducted research in secondary ion mass spectrometry and taught at both the University of Utah and Montana State University. He held positions of increasing responsibility, including Research Fellow, during a 16-year career with the Monsanto Company. He worked on complex problems in environmental chemistry and remediation and in food and agricultural science. His current work at Apis Discoveries, L.L.C. includes setting up business units for ultratrace analysis, consulting for submission to the U.S. Food and Drug Administration of direct and indirect food additives, analysis and remediation of organic pollutants. He has nearly 100 publications and presentations in analytical and physical chemistry, and he is the author of many internal Monsanto publications and presentations. He currently holds two patents.

Resha M. Putzrath, Ph.D., D.A.B.T., was a principal at Georgetown Risk Group during the preparation of this report. After earning an M.S. and a Ph.D. in biophysics from the School of Medicine and Dentistry at the University of Rochester, she was a Research Fellow in Physiology at Harvard Medical School and then a
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Fellow at the Interdisciplinary Programs in Health at Harvard School of Public Health. Dr. Putzrath previously worked for the U.S. Environmental Protection Agency; National Academy of Sciences; Environ Corporation; Organization Resources Counselors, Inc.; and Step 5 Corporation. She currently holds two teaching appointments at Johns Hopkins University, in the Whiting School of Engineering and Applied Science and in the Department of Environmental Heath Sciences at the School of Hygiene and Public Health. She develops methods for improving the accuracy of toxicological evaluations and risk assessments, focusing on models for combining data, primarily for complex mixtures of chemicals including dioxins, pesticides, and particulate matter. Her analyses have included: proposing alternative procedures that reduce uncertainty within regulatory assumptions; delineating the limitations of toxicity equivalency factors; and evaluating numerous hazardous waste sites, occupational exposures, and consumer products. Near the conclusion of this report, Dr. Putzrath, went to work for the Risk Assessment Forum at the U.S. Environmental Protection Agency, and as a full-time employee, she resigned from the Added Ingredients Review Ad Hoc Export Panel.

Emanuel Rubin M.D. is the Attending Physician-in-Chief of Pathology and Chairman of the Department of Pathology, Anatomy and Cell Biology at Thomas Jefferson University Hospital and Medical College, respectively. He obtained a medical degree from Harvard Medical School. After completing a residency at the Children’s Hospital of Philadelphia, he continued as a Dazian Research Fellow in Pathology and as Advanced Clinical Fellow of the American Cancer Society, both at Mount Sinai Hospital. After the fellowship Dr. Rubin spent the next fourteen years at Mount Sinai Hospital’s Pathology Service with increasing responsibility, culminating as the Pathologist-in-Chief. Dr. Rubin then became the Director of Laboratories at the Hahnemann University Hospital. His many academic appointments include the Irene Heinz and John LaPorte Given Professor and Chairman of the Department at Mount Sinai School of Medicine, Professor and Chairman of the Department of Pathology and Laboratory Medicine at the Hahnemann University School of Medicine, Adjunct Professor of Biochemistry and Biophysics at the University of Pennsylvania School of Medicine, and several appointments at Jefferson Medical College, culminating in his current position. Among honors received, the University of Barcelona named him as Doctor Honoris Causa. He was also given the F.K. Mostofi Distinguished Service Award from U.S.-Canadian Academy of Pathology and the NIH MERIT Award, which lasts until 2006. He has held many editorial positions and has served as a consultant to many organizations. He has published over 300 articles, including 13 textbooks and one CD-ROM.

James L. Schardein, M.S., F.A.T.S., consults in reproductive and developmental toxicology. Early in his career, he established one of the first pharmaceutical industry laboratories that investigated the effect of drugs on the developing embryonic animal model. After 23 years of experience in the pharmaceutical sector, Mr. Schardein joined a contract laboratory, International Research and Development
Corporation, as Director of the Reproductive and Development Toxicology group, where he later was named Division Vice President. In 1992, he additionally acquired responsibilities as Associate Director of Research for managing Study Directors and aiding the direction of all research programs at the laboratory. In 1995, he joined WIL Research Laboratories as Director of Research and Senior Vice President, from which he retired March 3, 2000. He has served as consultant to many organizations, including the National Institute of Environmental Health Sciences, the U.S. Environmental Protection Agency, Interagency Regulatory Liaison Group, and the International Life Sciences Institute. He is the author of two books, *Drugs as Teratogens* (1976) and *Chemically Induced Birth Defects* (1985), the latter now in its 3rd (2000) edition.

Thomas Joseph Slaga, Ph.D. is the president and CEO of the Center for Cancer Causation and Prevention at the AMC Cancer Research Center. He completed his doctorate in physiology/biophysics at the University of Arkansas Medical Center and a postdoctoral fellowship at the McArdle Laboratory for Cancer Research at the University of Wisconsin. He held positions of increasing responsibility as an Assistant Member of the Fred Hutchinson Cancer Research Center, Staff Member at the East Tennessee Cancer Research Center, and Senior Staff Member of the Skin Carcinogenesis and Tumor Promotion and Biology Division at the Oak Ridge National Laboratory (part of the Department of Energy). He simultaneously held faculty positions at the University of Washington Medical School, the Oak Ridge Graduate School of Biomedical Sciences, Texas A&M University, and the University of Texas. Several of Dr. Slaga’s former students now work for cigarette manufacturers. Currently, his joint/adjunct positions include Interim Deputy Director and Member of the Comprehensive Cancer Research Center and Member of the Department of Biochemistry and Molecular Biology at the University of Colorado Health Sciences Center. He has served on many scientific advisory committees and editorial boards. He also peer reviews submissions to several scientific journals. His research interests include mechanisms of chemical carcinogenesis, tumor promotion, and mechanisms of action of dermal antitumor agents. Dr. Slaga has published more than 500 papers.

**Added Ingredients Review Meeting Speakers:**

Christopher R.E. Coggins, Ph.D., D.A.B.T., is the Senior Vice President of Science and Technology for Lorillard Tobacco Company. Dr. Coggins has been a board-certified toxicologist since 1986 and has previously been employed by the following companies and organizations: R.J. Reynolds, Research and Development; Battelle-Geneva Research Center; and Animal Diseases Research Association, University of Edinburgh. Dr. Coggins is a member of several professional societies, such as the American Board of Toxicology, the Society of Toxicology, and the Society of Toxicologic Pathologists. He continues to serve as a manuscript reviewer for several scientific journals and is currently a member of the editorial
board of the journal *Inhalation Toxicology*, and is on the Board of Directors of the Tobacco Industry Testing Laboratory. Dr. Coggins has published over 40 articles in the peer-reviewed scientific literature.

Richard N. Dalby, Ph.D. is an Associate Professor and Vice Chair in the Department of Pharmaceutical Sciences at the University of Maryland School of Pharmacy. He was previously a Research Assistant Professor in the Department of Pharmacy and Pharmacuetics at Medical College of Virginia / VCU (1989-1992). Dr. Dalby holds a bachelor of pharmacy degree (1983) from Nottingham University in England, and a Ph.D. in pharmaceutical sciences from the University of Kentucky (1988). Richard’s aerosol research, which encompasses novel lung and nasal formulation development, devices design and product testing, is founded on both his Ph.D. work on sustained release metered dose inhalers, and industrial experience as an Inhalation Formulation Scientist with Fisons Pharmaceuticals (1988-1989). He has more than 30 published papers and 70 abstracts related to aerosol technology, has authored several book chapters, and has spoken at many national and international meetings. He is a co-inventor on three patents concerned with novel MDI formulations, a reviewer for several international journals, and acts as an industrial and FDA consultant. He is a member of the Royal Pharmaceutical Society of Great Britain and the American Association of Pharmaceutical Scientists. He is the director of an annual Inhalation Aerosol Technology Workshop, and co-organizer / publication coordinator of a major international symposium - Respiratory Drug Delivery, now in its 16th year.

Mark W. Frampton, M.D., is an Associate Professor of Medicine and Environmental Medicine in addition to being the Director of the Pulmonary Research Programs at the University of Rochester Medical Center. Dr. Frampton expertise and research interests include the health effects of air pollution. His goals are to determine indicators of individual susceptibility to the adverse effects of pollutants and to develop markers of exposure. He is a member of the American College of Physicians, American Lung Associations of New York State, and the American Thoracic Society.

Wolf-Dieter Heller, Ph.D., received his doctorate in mathematics at the Faculty for Economic Sciences of the University of Karlsruhe in Germany. Along with his teaching responsibilities at the Faculty for Economic Sciences, he is the Editor of *Bieträge zur Tabakforschung International/Contributions to Tobacco Research* and is the Managing Director of the Research Association Smoking and Health in Berlin, Germany.

Lester B. Lave, Ph.D., is University Professor and Higgins Professor of Economics at Carnegie Mellon University, with appointments in the Business School, Engineering School, and the Public Policy School. He has a B.A. from Reed College and a Ph.D from Harvard University. His research has focused on health, safety,
and environmental issues, from the effect of air pollution on health to estimating the benefits and costs of automobile safety standards, risk analysis of carcinogenic chemicals, testing the carcinogenicity of chemicals, valuing natural resources and global climate change. Dr. Lave has served as a consultant to a large number of federal and state agencies and companies. He was elected to the Institute of Medicine of the National Academy of Sciences, is a past president of the Society for Risk Analysis, and has served on many committees of the National Academy of Sciences, AAAS, American Medical Association, and Office of Technology Assessment. Dr. Lave is the director of the CMU university-wide Green Design Initiative. This program is focused on using pollution prevention and sustainable development to boost economic development. He is engaged in a project on studying the toxicity testing of high volume chemicals and is a member of the Alliance for Chemical Awareness Advisory Committee.

Peter N. Lee, M.A., C.Stat., received his degrees at the Wimbledon College and Exeter College, at Oxford. Mr. Lee has served as a Statistician and Research Coordinator at the Tobacco Research Council (now Tobacco Manufacturers Association) in London, England. Presently he is an Independent Consultant in Statistics and Adviser in Epidemiology and Toxicology to a number of tobacco, pharmaceutical and chemical companies. Mr. Lee has published over 170 papers and three books. A number of papers describe statistical methods of analysis of long-term animal studies and their application to numerous experiments. Others concern epidemiological studies and clinical trials, with papers relating to lung cancer, chronic bronchitis, heart disease, stroke, Alzheimer’s disease, Parkinson’s disease, inflammatory bowel diseases, urinary incontinence and other diseases. Agents investigated include aluminium, PCBs, asbestos, vitamin A and other aspects of diet, personality, drugs, and even pet birds. Smoking and environmental tobacco smoke (ETS) exposure are particular interests, with papers relating to lung cancer and a variety of other smoking-related diseases. Two of his books concern ETS, the second being a comprehensive review of the epidemiological evidence relating to mortality in adults. A third is a reference book on international smoking statistics.

Luqi Pei, Ph.D. is Pharmacologist and Toxicologist at the U.S. Food and Drug Administration. He specializes in the nonclinical safety evaluation of inhaled drug products indicated for respiratory diseases. Dr. Pei received his degree in veterinary medicine from Gansu University of Agriculture, China, degree of Master of Science from Colorado State University and degree of Doctor of Philosophy from Texas A&M University College of Medicine. Dr. Pei was a faculty member at the Gansu Agriculture University for four years and a visiting scientist at the Colorado State University for a year prior to his graduate training. He completed his postdoctoral training at the Lovelace Inhalation Toxicology Institute studying toxicity of vapors. Dr. Pei spent about a year in a pharmaceutical company developing inhaled drug products.
Stephen I. Rennard, M.D., is a physician and a professor of Pulmonary and Critical Care Medicine at the University of Nebraska Medical Center. He graduated with honors from Baylor College of Medicine and completed his residency in internal medicine at Washington University’s Barnes Hospital. He then completed a research associateship in developmental biology and anomaly at the National Lung and Blood Institute, National Institutes of Health. Dr. Rennard became the Chief of the Pulmonary and Critical Care Medicine Section at University of Nebraska Medical Center. His research interests have focused on the inflammatory and repair processes of lung tissues. This has included investigations in airway epithelial cell biology, mechanisms of epithelial repair, and peribronchial airways disease. He has more than 200 peer reviewed publications and has served on the committees of many organizations including the Pulmonary Subspecialty Section of the American Board of Internal Medicine, the Graduate Education Committee, the Council of Governors for the American College of Chest Physicians, the Society for Research on Nicotine and Tobacco, the Research Advocacy Committee, the Long-Term Planning committee for the American Thoracic Society and the Bronchoalveolar Task Group of the European Respiratory Society.

Thomas B. Starr, Ph.D., is the Principal of TBS Associates in Raleigh, North Carolina. Dr. Starr is also an Adjunct Associate Professor, Department of Environmental Sciences and Engineering at the University of North Carolina at Chapel Hill School of Public Health. He also serves on the Secretary’s Scientific Advisory Board on Toxic Air Pollutants for the North Carolina Department of Environment, Health, and Natural Resources. In addition, he serves on the Environmental Medicine Residency Advisory Committee of the Duke University Division of Occupational and Environmental Medicine Training Program. He has had over 30 years of professional experience in quantitative assessment of health and environmental risks from exposure to toxic substances, exposure assessment, experimental design and data analysis, comparative risk analysis, toxic torts, and regulatory policy. He is a member of the Society of Toxicology, American Statistical Association, and the Society for Risk Analysis. He has more than 80 publications on human and environmental health effects of exposure to pollutants and other toxic substances, environmental regulation, and mechanisms of toxicity.

C. Joseph Sun, Ph.D., Capt. USPHS received his degree in pharmacology from the University of Mississippi Medical Center. Dr. Sun is Pharmacologist at the U.S. Food and Drug Administration since 1978, where he has served in various divisions within the Center for Drug Evaluation and Research (CDER). Currently, Dr. Sun is the Supervisory Pharmacologist/Team Leader in the Division of Pulmonary Drug Products.

Ashok Teredesai, D.V.M., is the Head of the Department of Pathology at the Institut für Biologische Forschung (INBIFO) in Cologne, Germany. Before his current position he had been a staff scientist at the Department of Veterinary Pa-
thology in Giessen, Germany. Dr. Teredesai holds a National Specialist Title as Fachtierarzt für Pathologie. He is a member of many professional societies including the American, British, and German Societies of Toxicologic Pathology, German Veterinary Medical Association, and the European Society of Veterinary Pathology. He has been published 16 times on subjects including, but not limited to the histopathology of the rodent respiratory tract.

Piter M. Terpstra, D.V.M., M.B.A., is the general manager of CRC (Contract Research Center, a Philip Morris Research Laboratory) in Brussels, Belgium. He received his DVM from the State University of Ghent, Belgium and his MBA from the University of Brussels. He wrote a thesis on the use of novel anesthetics and experimental surgery in the horse. After shortly serving for the European Commission as a trainee, he was employed at Eli Lilly in a research and technical support group for several years. He joined CRC in 1992 and became general manager in 1995.

Patrick Vanscheeuwijck, Ph.D., is the manager of the Bioresearch Department at CRC (Contract Research Center, a Philip Morris Research Laboratory) in Brussels, Belgium. He received his MS in plant biochemistry and physiology and Ph.D. in biochemical pharmacology from the State University of Ghent, Belgium. Postdoctoral training was performed at the University of Arizona, Tucson, Arizona, Department of Pharmacology and Toxicology in molecular pharmacology and at the Catholic University of Leuven, Belgium, in molecular biology. He has published more than 20 peer-reviewed manuscripts as author or co-author.

Hanspeter Witschi, M.D., D.A.B.T., F.A.T.S. is a Professor Emeritus of Veterinary Medicine: Molecular Biosciences at the University of California, Davis where his core research has centered on respiratory toxicology. Dr. Witschi has been a board certified toxicologist since 1980, and received his medical training at the University of Berne and University of Geneva in Switzerland. Dr. Witschi has served as a research associate at the Toxicology Research Unit, British MRC in Carshalton, England, the Department of Environmental Health, of the University of Cincinnati, Ohio and at the Department of Pathology, University of Pittsburgh, Pennsylvania. Other professorships include Associate Professor, Department of Pharmacology, and Faculty of Medicine University of Montreal, Canada. He has also conducted research at the Oak Ridge National Laboratory, Oak Ridge, Tennessee. Dr. Witschi’s principal research interests include the study of cell turnover in the lungs of animals exposed to common air pollutants such as ozone or nitrogen dioxide, and defining the effects of low level exposure and the study events leading to adaptation. Additionally his work has focused on the effects of environmental tobacco smoke on intrauterine growth and lung tumor development.
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LSRO STAFF:

Amin Akhlaghi was an Associate Staff Scientist at the Life Sciences Research Office. He received a B.S. degree in biology from the University of Kentucky and is currently studying at Georgetown University School of Medicine. During his second year of medical school he was an Adjunct Graduate Fellow at the Center for Food and Nutrition Policy formerly at Georgetown University.

Pippa Antonio, B.S. worked at the Life Sciences Research Office in Bethesda, MD, as an Associate Staff Scientist. Ms. Antonio graduated from the University of Stirling, Scotland with distinction. Her honors thesis was on The Effects of Increased Weight Bearing on the Ultra Structure of Collagen Fibers. She was previously employed as a Research Writer for The Myelin Project, an international organization, whose mission is to accelerate research on myelin repair. She recently joined her husband in Virginia Beach, VA.

Daniel M. Byrd III, Ph.D., D.A.B.T. is the Deputy Director of the Life Sciences Research Office and the recent coauthor of the textbook Introduction to Risk Analysis: A Systematic Approach to Science-Based Decision Making (2000). He received both B.A. and Ph.D. degrees from Yale University. He first received certification from the American Board of Toxicology in 1982. Previously, Dr. Byrd taught pharmacology and conducted independent research into the mechanisms and dosimetry of chemotherapeutic drugs at Roswell Park Memorial Institute and at the University of Oklahoma. At the U.S. Environmental Protection Agency he subsequently held positions in the Office of Chemical Control, the Office of Pesticide Programs, the Carcinogen Assessment Group, and the Science Advisory Board (SAB) for which he was awarded with the Silver Medal for Management and Leadership. He also managed committees for three trade associations and was the president of Consultants in Toxicology, Risk Assessment and Product Safety (CTRAPS) a scientific support firm that helps clients acquire, interpret, and use biomedical information. He is the author of more than 100 regulatory documents and 40 scientific articles.

Bonita R. Condon, B.S. worked at the Life Sciences Research Office as the Scientific Literature Specialist/Librarian through March 2002. She received her B.S. in Information Resources Management from the University of Maryland. Prior to joining LSRO, Ms. Condon was Chief of the Information Resources Management Branch, at the Health Resources and Services Institute (HRSA), where she conducted studies of existing procedures and oversaw a team of specialists who provided solutions and services. Prior to HRSA, Ms. Condon served as a Senior Auditor at NIH and conducted studies of internal processes and procedures. In the middle of the Phase One project she left LSRO to join Aspen Associates as the Project Manager for the National Center for Complementary and Alternative Medicine Clearinghouse, NIH, where she managed teams of science writers, editors,
health information specialists, librarians, and technical specialists. Presently, Ms. Condon is a Project Manager for BETAH Associates, Inc. BETAH provides professional and technical services to government and private sector professional services markets.

Dr. Michael Falk, Ph.D is the Director of the Life Sciences Research Office. He received his Ph.D. in biochemistry from Cornell University and completed postdoctoral training at Harvard Medical School. He was employed in various capacities at the Naval Medical Research Institute, Bethesda, MD supervising as many as 80 senior level scientists. As Principal Investigator he was a key member of the Scientific Advisory Board and the Acting Director for the Institute. He was also the Director of the Wound Repair Program and pioneered a new position as the Director of Biochemistry and Cell Biology. Also, as the Director, he rescued the Septic Shock Research Program by cutting inefficiencies and increasing productivity in terms of grant funding and publication production. He managed peer reviews and subject review panels in infectious diseases, environmental sciences, military medicine, and other health-related fields. He reviewed the research proposals for National Science Foundation, Medical Research Council of Canada, and Office of Naval Research. As the Director of the Life Sciences Research Office (LSRO), Dr. Falk manages the evaluation of biomedical information and scientific opinion for regulatory and policy makers in both the public and private sectors. He has written white papers on infant nutrition, food labeling, food safety, and military dental research and has organized two international conferences. He concurrently works at MCF Science Consultants providing analysis and consultation on emerging technologies. Dr. Falk has published over 60 research articles, abstracts, technical reports, and presentations.

Robin S. Feldman, B.S., M.B.A. is the Literature Specialist in the Life Sciences Research Office. She is a seasoned information specialist with experience in the electronic acquisition, analysis, and management of scientific, business, and regulatory information. Ms. Feldman obtained her B.S. from the George Washington University in Washington, D.C. with a major in zoology and her M.B.A. degree from the University of Maryland at College Park with a concentration in science and technology. Previously, she worked as a Biomedical Research Assistant at Consultants in Toxicology, Risk Assessment and Product Safety, where she obtained and researched scientific literature for private and governmental clients. At the National Alliance for the Mentally Ill, she designed and implemented a document management and retrieval system for the Biological Psychiatry Branch of the National Institute of Mental Health and served as Managing Editor of Bipolar Network News, a newsletter for the Stanley Foundation Bipolar Network. At Howard Hughes Medical Institute, she oversaw the implementation of the HHMI Predoctoral Fellowship in Biological Sciences program. While serving as Science Information Specialist at the Distilled Spirits Council of the United States, she managed the installation of a local area network and participated in the development
and maintenance of an electronic research database for the beverage alcohol industry. As a Report Coordinator at Microbiological Associates, Inc. she conducted statistical analyses and prepared technical reports about toxicology studies using animal models. She served as Data Management Administrator for the National Toxicology Program’s sponsored studies. Currently, Ms. Feldman maintains LSRO’s library, responds to requests for reports, and assists LSRO’s scientists in discovering, obtaining, compiling, and documenting the scientific literature required to prepare reports for sponsors.

Catherine J. Klein, Ph.D., R.D., C.N.S.D., is a Staff Scientist at the Life Sciences Research Office. She graduated magna cum laude from the Department of Human Nutrition and Food Science at the University of Maryland at College Park where she also obtained her M.S. and Ph.D. in the Graduate Program in Nutrition. She completed an internship in the Pre-Professional Practice Program in Dietetics at the University of Maryland Medical System (UMMS), and most recently the V.A. Kleinfeld Summer Internship Program at The Food and Drug Law Institute. At the UMMS she developed system-wide guidelines for nutrition assessment, documentation, and continuity of care. As Clinical Coordinator of Research in the Division of Critical Care Medicine at the R. Adams Cowley Shock Trauma Center, she developed, initiated, and administered research projects focused on nutrition issues in critical care. She established a multidisciplinary nutrition task force, which resulted in improvements in clinical practice standards. As Program Coordinator of the Department of Nutrition and Food Science she produced online modules for continuing education in nutrition for health care professionals. She is the primary author on 9 peer-reviewed publications including a book chapter and has lectured or presented at over 25 professional meetings. Dr. Klein received the Pelczar Award for Excellence in Graduate Study from the University of Maryland Graduate School and Sigma Xi and the Dr. E.V. McCollum Award from the Maryland Dietetic Association. Her professional contributions include serving on the Advisory Board of the University of Maryland Dietetics Program and as editor for the Maryland Dietetics Association.

Kara D. Lewis, Ph.D. joined the Life Sciences Research Office in Bethesda, MD, as a Staff Scientist. Dr. Lewis completed postdoctoral research at Yale University. She obtained her Ph.D. in biology with a concentration in neuroscience from Clark University and graduated summa cum laude with a B.S. degree in biology from Spelman College. Dr. Lewis has conducted research on taste and smell of the fruitfly, *Drosophila melanogaster*, and on the molecular mechanisms of sweet taste transduction in the blowfly, *Phormia regina*. She has collegiate teaching experience and three peer-reviewed publications. She is a member of the Association for Chemoreception Sciences.

Negin P-M. Royaee, B.S. worked as an Associate Staff Scientist during the preparation of the Phase One report. She was previously employed at Biophysical Soci-
ety, FASEB, where she assisted in the production of the *Biophysical Journal*. Ms. Royaee obtained her bachelor’s degree from Lake Forest College in Chicago, IL with a major in biology and minor in art history. Ms. Royace has volunteered for sick and under-privileged children and was a Weekend Resident Manager at The Children’s Inn at the National Institutes of Health. Currently Ms. Royaee resides in Boston, MA.

Paula M. Nixon, Ph.D. joined the Life Sciences Research Office in Bethesda, MD, as a Staff Scientist. Dr. Nixon completed her postdoctoral research at The Babraham Institute in Cambridge. She obtained her Ph.D. in molecular biology from Imperial College London as part of Cancer Research UK. She graduated with an honors degree in molecular biology from the University of Manchester, UK. Dr. Nixon has conducted research on the MEK5/ERK5 MAP kinase pathway and the role of the AP-2 transcription factors in the control of gene expression in breast cancer. In addition she was involved in research to identify the Currarino syndrome gene and the development of mitochondrial DNA profiling techniques for forensic science. She has three peer-reviewed publications and is a member of the Biochemistry Society.

Kristine K. Sasala, B.S. recently joined the Life Sciences Research Office in Bethesda, MD as an Associate Staff Scientist. Ms. Sasala obtained her bachelor’s degree in biology from the University of Cincinnati, OH with a focus on cell biology. Prior to joining the LSRO, Ms. Sasala was previously employed at Nerac, Inc. as an Information Scientist. She has been involved in researching the molecular biology of osteoporosis, anti-Brucella vaccine development, and pharmacology of xenobiotics, in addition to graduate work in protistology. Ms. Sasala has two peer-reviewed publications and several conference publications. At the LSRO, she assists with the Added Ingredients Review and various other projects.

James Cecil Smith Jr., Ph.D., a Senior Scientific Consultant at Life Sciences Research Office obtained his doctorate in nutritional sciences/biochemistry at the University of Maryland and post-doctorate in the Department of Biological Chemistry, UCLA. He served two years as Health Service Officer at the National Institutes of Health. Dr Smith completed 36 years in federal research laboratories with increasing responsibilities in the Veterans Administration Medical system and the U.S. Department of Agriculture (USDA). Best known for contributions to trace element nutrition, he is also an authority in the area of vitamin and mineral interactions. Original research he directed identified a link between zinc and vitamin A. His other research accomplishments include studies that revealed an interaction between copper and dietary carbohydrates. He directed two large human investigations, funded in part by the National Cancer Institute that established the USDA Laboratory and Research Center at Beltsville, MD as original leaders in research to elucidate the role of dietary carotenoids. Dr. Smith has published more than 375 articles. He served as an editorial board member, assistant editor, and ad hoc
reviewer for several journals and is a member of several professional societies. His recognitions include the Klaus Schwarz Award for Excellence and Leadership in Trace Element Research sponsored by the International Association, Bioinorganic Scientists and was recently named a Fellow of the American Society for Nutritional Sciences.

LSRO STAFF WRITERS:

Ms. Tina Adler, M.S., obtained her M.S. degree from the School of Journalism at Columbia University, concentrating on health and science issues. She has worked as a writer for many organizations including the Washington Post, the U.S. National Institutes of Health, Burlington Free Press, the American Psychological Association, and Science News, covering a wide array of scientific subjects.

Ms. Wendy Meltzer, M.P.H., earned her M.P.H. degree from the School of Public Health and Health Services at George Washington University. She has worked at Georgetown University Medical Center’s Women’s Interagency HIV Study and at the Center for Science in the Public Interest. She also has been the Managing Editor and Executive Producer at FoodFit.com, a nutrition and healthy living web site. She is a member of the Delta Omega Public Health Honor Society.

Dr. Susan A. Stern PhD., earned a doctorate in biochemistry at the East Tennessee State University College of Medicine and completed a postdoctoral fellowship in clinical immunology at the University of Minnesota School of Medicine. She currently is employed as senior manager at Constella Health Sciences. At the time of her work on this project, she was the Principal at Stern Consulting where she provided medical and scientific writing, advice on strategic planning, and various analysis and consulting services to professional service firms and companies. An immunologist with nearly 20 years of experience in biotechnology, consulting, and technology management she has held management positions at several biotechnology companies in the Washington metropolitan area. Dr. Stern is the author of seven original publications and is a member of several professional organizations including the American Association for the Advancement of Science, Drug Information Association, and the American Writers Association.
APPENDIX B

GLOSSARY

Absorption
The transfer of smoke constituents from the lung air space and/or surface into lung tissue (Byrd & Cothern, 2000).

Accuracy
The ability of a measurement or method to determine the true value of the quantity being quantified.

Active transport
The process of molecules or ions crossing a membrane against its concentration gradient. This requires energy, saturation, and could be affected by competitive inhibition.

Added Ingredient
A substance added to an already defined mixture.

Additive
A substance added in small amounts to something else to improve, strengthen or otherwise alter it.

Aerodynamic diameter
For ideal smoke particles (spheres with densities of 1 gram/cubic cm) diameter and aerodynamic diameter are equivalent. If the particle’s shape or density differs from ideal, then the diameter and aerodynamic diameter will differ. Particles smaller than 0.5 µm diameter behave like gases aerodynamically, and diffusion dominates their motion. So, a physical diameter is used.

Aerosol
A two-phase system consisting of a collection of solid and/or liquid particles (particulate matter) and the gas in which they are suspended (Hinds, 1999).

After cut
Alcoholic solution containing aromatic and/or flavoring agents (e.g., menthol) and stabilizing agent (e.g., glycol) sprayed onto tobacco after it is cut into shred.

Air-cured
The drying (curing) process of Maryland and Burley tobaccos in open-air barns or scaffolding, using ambient air conditions without external heat or humidity. The rate of curing depends on prevailing temperature, humidity, airflow and sunlight and may extend over a period of 8 weeks or longer.
Alveolus
   Pl. Alveoli. Vascularized air sac extending from the respiratory bronchioles, at the interface between the airway and the pulmonary tissue where gas is exchanged.

Animal model
   Animals used for *in vivo* studies as surrogates for humans. Rodents are most frequently used for studies involving cigarette smoke.

Ash
   The powdery residue remaining after combustion of tobacco.

Bias
   A statistical sampling or testing error systematically favoring one outcome over others.

Biological activity
   Processes, usually biochemical, that are required to carry out metabolic functions associated with supporting life.

Biologically effective dose
   The amount of tobacco constituent or metabolite that binds to or alters a macromolecule; estimates of BED might be performed in surrogate tissues. (Institute of Medicine, 2001).

Biomarker
   A biological response variable that is indicative of exposure, and/or effect (*i.e.*, disease initiation; disease progression; remission; death).

Blended cigarette
   Cigarettes made from tobacco that has been mixed in proportions from other tobaccos.

Bright tobacco
   Flue-cured tobacco.

Bronchiole
   Extension of the bronchus; terminal bronchioles are ciliated, respiratory bronchioles extending from terminal bronchioles are not ciliated and contain alveoli.

Bronchus
   One of two main branches (bronchi) of airway from the trachea to the lungs.

Burley tobacco
   The main light air-cured tobacco that is grown in Kentucky and surrounding states, setting the standard for that grown in other parts of the world.

Cambridge filter pad
   A glass fiber filter stabilized by an organic binder, originally made by the Cambridge Filter Corporation, Syracuse, New York. It is used in the FTC standard method to separate the vapor and particulate phases of cigarette smoke.
Casing
Solution applied to tobacco leaf by dipping or spraying to retain moisture in the tobacco and improve sensory characteristics of the smoke. Casing solutions may include sugar, glycerin or glycol, licorice and cocoa.

Char
The remaining carbonized residue following combustion of cigarette tobacco. Char reacts with oxygen in the air producing carbon monoxide, carbon dioxide and water leaving ash.

Chronic obstructive pulmonary disease (COPD)
COPD “is a disease characterized by a progressive airflow limitation caused by an abnormal inflammatory reaction to chronic inhalation of particles.” (Pauwels, 2000)

Cigarette smoke condensate
See ‘Tobacco smoke condensate’

Compensation
Behavioral responses of smokers after changing to lower yielding cigarettes to preserve the nicotine intake prior to switching; these responses include larger, more rapid or frequent puffs, deeper inhalation, blocking of ventilation holes by the lips/fingers or an increase in cigarettes smoked.

Complex mixture
A heterogeneous group of many chemical substances found together, for example, as in tobacco or in cigarette smoke.

Concordance
Agreement between two sets of data; a confirmation of results.

Condensate
See ‘Tobacco smoke condensate’

Confounder
A factor that can compromise the validity of experimental data and may be difficult to identify.

Correlation
The simultaneous numerical increase or decrease of two random variables.

Cotinine
A metabolite of nicotine found in the plasma, saliva and urine of smokers and used as a biomarker of exposure to cigarette smoke.

Deposition
Process resulting in a fraction of the inspired smoke that is not expired (Brain & Valberg, 1979).

Dilution
See ‘Ventilation’
Direct additive
A chemical substance added to a cigarette during manufacturing.

Distillation
A process that separates a chemical mixture into component fractions by the application and removal of heat.

DNA adducts
Covalent chemical products formed when an electrophile, typically a metabolite of a mutagen or carcinogen, binds to DNA.

Dose
The amount of a substance absorbed by an organism.

Dosimetry
The accurate measurement of doses. (Pickett, 2000)

Dynamics
The effect of a substance on the body, specifically its mechanism of action, pathway and biological effect.

Endpoint
Measure of outcome (e.g., cancerous tumor; heart attack; death)

Environmental tobacco smoke (ETS)
The cigarette smoke that is present in the ambient air, consisting of exhaled mainstream smoke plus sidestream smoke. The highest concentrations are in near proximity of smokers in confined areas with poor ventilation.

Error
A numerical value that deviates from the true value.

Existing additive
A chemical substance added to a cigarette during manufacturing and already in use, as a chemical substance listed in Appendix D.

Exposure
The potential dose of a substance, available for absorption (Byrd & Cothern, 2000).

Exposure Biomarker
A chemical substance (for example, a tobacco constituent or its metabolite) in a biological fluid or tissue that measures internal dose (Institute of Medicine, 2001).

External validity
Confirmation of data/test results from other authoritative sources.

Facilitated diffusion
Movement across a membrane down a concentration gradient involving a transporter and subject to saturation and competitive inhibition.

False Negative
A negative result from a test, when the true outcome was positive.
False Positive
A positive result from a test, when the true outcome was negative.

Filter (cigarette)
A device positioned at the end of cigarette, which serves as a mouthpiece, usually composed of cellulose acetate fibers in the U.S. and encased by a wrapper.

Filter wrapper
Paper wrapping the filter.

599 list
In 1994 U.S. manufacturers published a list of 599 substances that might be added to some cigarettes. (See Appendix D)

Flue-cured
Tobaccos (Bright or Virginia) that are dried (cured) in tightly constructed barns with artificial heat beginning at 35 °C and ending at about 75 °C over a 5 to 7 day period.

Gas phase
The nonliquid (vapor) phase of cigarette smoke.

Gold standard
A technique, method, substance or measure considered the most accurate, precise, or accepted by authoritative experts.

Initiation
“Instantaneous event during exposure, leading to manifest mutation that is irreversible at least until the mutated cell or its offspring is eliminated or escapes further development by differentiation.” (Tornqvist & Ehrenberg, 2001)

Humectant
Substances or compounds added to cigarette tobacco to retain moisture and plasticity. The principal humectants in cigarette manufacturing are glycerol and propylene glycol.

Indirect additive
A chemical substance, detectable in a cigarette, and distinct from its tobacco paper, introduced into cigarettes through treatment during the growing and handling of the tobacco or manufacture and handling of paper.

Ingredient
Substances except for tobacco and paper used in the manufacture or preparation of cigarettes. Also see (European Parliament, 2001).

Internal Validity
Confirmation of test results based on reference standards or materials employed concurrently with the procedure.

Kinetics
The rates of processes, e.g., pharmacokinetics or toxicokinetics. How rapidly the body processes a substance, specifically the rates of absorption, distribution, metabolism and excretion of chemical substances.
Light cigarette
A cigarette designed to lower the delivery of tar when smoked under standard conditions.

Mainstream smoke
Smoke drawn through the butt end of the cigarette into the mouth as a smoker puffs on a cigarette.

Maryland tobacco
Light bodied, mild, air-cured tobacco grown on the sandy soils in southern Maryland.

Maximum tolerated dose (MTD)
In theory, an MTD is an estimate of the maximum daily dose or exposure which a species can experience over a lifetime and experience no adverse effects. In practice, MTDs for animal species usually are estimated from experimental data about lifespan or inhibition of body weight gain.

Microexplosions
Small components of a cigarette (e.g., sugar, protein) that break off before they burn during pyrolysis and are transported in the smoke intact.

Molecular epidemiology
The study of biomarkers in an epidemiological context.

Mouth spill
Smoke lost from the mouth before inhalation (Bentrovato et al., 1995).

Multiplicity
Several approaches or tests designed to establish/validate an effect.

New additive
A chemical substance added to a cigarette during manufacturing not previously used.

N-Nitrosamine
A chemical substance formed by nitrosation of secondary and tertiary amines. Tobacco-specific nitrosamines (TSNA) are formed by the nitrosation of the major tobacco alkaloid, nicotine. (Brunnemann et al., 1996)

Nicotine
A cyclic tertiary amine composed of a pyridine and a pyrrolidine ring. It is a colorless to pale yellow, water soluble, oily, volatile, and hygroscopic liquid derived from plants of the Nicotiana genus.

Nicotinic receptors
A subclass of cholinergic receptors, which are ligand-gated and control the influx of calcium ions into the cell.

Noise
Background interference due to internal vibrations or electronics of instruments.
Oriental tobaccos
The varieties of tobaccos derived from the main species *Nicotiana tabacum*; specific oils and resins from these tobaccos provide distinctive taste and aroma.

Pack-year
A unit measure of smoking exposure. One pack-year represents the consumption of 20 cigarettes per day (one pack) for one year by one person.

Paper
Cigarette paper (wrapper) is composed of inorganic filler such as CaCO₃ and cellulose fibers from wood pulp and/or flax. The filler holds the fibers apart, creating pores that allow air permeability.

Parenchyma
Cells of an organ, essential to its function, distinct from the membrane that encloses it and the connective tissue that supports it.

Particulate matter
In smoke, the solid particles and liquid droplets (typically < 1 µm in diameter) in vapor, altogether forming an aerosol (Hinds, 1999).

Particulate phase
In smoke, the solid and/or liquid phase of an aerosol, which is suspended in gas. Liquid particles are referred to as droplets (Hinds, 1999).

Passive diffusion
Molecules or ions that cross permeable membranes to equilibrate concentrations on both sides. Movement occurs predominantly down the concentration gradient.

Peak puff flow
Maximum rate (in mL per second) of mainstream smoke flow from cigarette to smoker

Pinocytosis
Transport of small molecules or liquids into cells by invagination of the cell wall and engulfment of the substance.

Plasticizer
A bonding agent, such as triacetin, used to modify and accelerate the reaction between chemical subunits that compose a plastic.

Plug wrap
Paper wrapping the cigarette filter.

Polycyclic aromatic hydrocarbons (PAHs)
Organic chemical substances containing fused aromatic ring systems, formed during incomplete combustion. Some PAHs form DNA adducts.

Potential reduced-exposure products (PREPs)
Pharmaceutical substances or products, such as nicotine replacement therapy or bupropion, and modified tobacco products with the potential to reduce morbidity and mortality.
Precision
Consistent reproduction of a measurement.

Pressure drop
The difference between atmospheric pressure and the pressure inside the cigarette during a standard flow rate. A large pressure drop indicates more difficulty (greater suction) in drawing smoke. Pressure drop indicates dilution. A reduction in the holes-open pressure drop indicates greater ventilation (dilution).

Processing aids
Ingredients that facilitate the manufacturing procedures and enhance the efficiency of production of cigarettes.

Promoter/Promotion
“...promotion is a reversible effect that, according to experiments, requires the chronic presence of the promoter. The term promoter is an operational definition for a variety of agents that have cell proliferation and clonal expansion as a common effect.” (Tornqvist & Ehrenberg, 2001)

Puff duplicator
An automated smoking machine that can be programmed with data, such as puff profile, from a puff recorder.

Puff interval
Length of time (in seconds) between puffs.

Puff profile
A graphic representation of the rate of the flow of mainstream smoke plotted as a function of time during the puff. The puff profile can be used to calculate puff volume. (International Organization for Standardization, 2000).

Puff recorder
A device to measures parameters of smoking behavior while a subject smokes, such as puff flow and puff duration.

Puff volume
The volume (in mL) of smoke inhaled from one puff.

Pyrolysis
Thermal degradation of a chemical substance, generally resulting in smaller chemical fragments.

Pyrosynthesis
Formation of additional compounds by the recombination of fragments arising from incomplete combustion of a parent chemical/compound during pyrolysis.

Reconstituted sheet tobacco (RST)
A processed tobacco widely used in tobacco blends made by a paper making-like process or from slurry of small scraps not suitable for cut filler. In a typical American blended cigarette it constitutes 10-25 % of content.
Reference cigarette
Cigarettes prepared under controlled conditions with uniform, documented source tobaccos and producing standardized yields of tar, nicotine and carbon monoxide. The Tobacco & Health Research Institute at the University of Kentucky manufactures the 1R2 and 2R4F reference cigarettes.

Relative risk
Expression of a risk in relation to another risk, e.g., the risk of death at work is approximately twice the risk of death from drowning.

Residence time
The time between inhalation and exhalation when a smoker holds smoke temporarily in the respiratory tract; estimated to vary from 4 sec to 24 sec.

Resistance-to-draw (Draw resistance)
The amount of negative pressure (suction) applied to pull a puff of mainstream smoke through the butt end of the cigarette in Pascal units (Pa).

Retention
The amount of smoke (and its constituents) found in the lungs at any time, including deposited and absorbed smoke (Brain & Valberg, 1979).

Returns
A list of all additives and ingredients included in tobacco products of major manufacturers and the maximum amount (as percentage) used.

Reverse smoking
A method of generating smoke by machine in which air is forced through the lighted cigarette by applying an elevated pressure to the burning end. This method has been used in animal exposure systems to minimize the time to deliver the smoke to the animal (Schultz & Wagner, 1975).

Risk
The probability of a future loss. In this report the probability of a mortality or morbidity within a stated time (Byrd & Cothern, 2000).

Sales-weighted
Statistical adjustment of data about cigarette brands, such as tar contents, by their sales volumes.

Sensitivity
The capacity to detect a signal (response) above background interference (noise).

Sensory impact
The effect of a stimulus which changes behavior dependent on sensate pathways, e.g., an attractant odor or the localized sensation in a smoker’s throat after inhaling (Junker et al., 2001).

Sidestream smoke
The smoke emitted directly into the air from the burning end of the cigarette, largely during the smolder interval between puffs.
Signal-to-noise ratio
The ratio of the quality of the signal (data) obtained to the unwanted interference.

Smoke
Usually a suspension of fine particles in air that scatters light and is physically visible. A cigarette smoke contains many chemical substances in both gas and liquid state, suspended in a dynamic aerosol created by incomplete combustion and changing both physically and chemically with time.

Smolder phase
The part of combustion that is slow burning.

Specificity
Discrimination between the true signal (response) and competing (interfering) signals.

Surrogate endpoint
A measurement, such as a biomarker, that can be substituted for an endpoint, such as a disease. Surrogate endpoints are used to compare interventions (Fleming et al., 1994).

Tar
The Federal Trade Commission refers to tar as the weight in grams of the total particulate matter collected on a Cambridge filter minus the weight of alkaloids, as nicotine, and water (Federal Trade Commission, 1967a).

Teratogen
A substance causing abnormal fetal development resulting in congenital malformation.

Thorax
Region between neck and abdomen delineated by 12 thoracic vertebrae and by the diaphragm; contains lungs and heart.

Tip
The portion of the cigarette that separates the body of the cigarette (tobacco column) from the smoker’s lips. The tip retains the tobacco in the cigarette so that tobacco particles cannot touch and stick to smoker’s lips (Allseits et al., 1968).

Tipping paper
The paper (wrapper) that joins the filter plug to the tobacco column covering the plug and overlapping the tobacco column.

Tobacco smoke condensate
Residual substance trapped on a filter as the smoke passes through a filter; usually obtained from mainstream smoke.

Total particulate matter
Particles in smoke or smoke condensate larger than 1 μm in diameter.
**Tow**

The filling of cigarette filters. Most U.S. brands use cellulose diacetate filling (Norman, 1999).

**Tow item**

The number of filaments in a tow band calculated by dividing the total weight of the tow band (total denier) by the weight in grams of a single filament (denier per filament), TD/dpf (Norman, 1999).

**Toxicokinetics**

The time course of the absorption, distribution, metabolism, and excretion of chemical substances by the body.

**Toxicological**

Related to toxic circumstances that alter biological function, such as a change in morbidity or mortality.

**Toxicology**

The study of the interactions between chemical, physical, or biological agents and biological systems.

**Transition**

A purine-to-purine or pyrimidine-to-pyrimidine mutation.

**Transversion**

A purine-to-pyrimidine or pyrimidine-to-purine mutation.

**True Negative**

A negative result from a test when the true outcome was negative.

**True Positive**

A positive result from a test when the true outcome was positive.

**Vapor phase**

Material in the gas state, usually material that passes through a filter.

**Ventilation or Dilution**

Perforations in the filter tipping paper or paper wrapping the tobacco rod that dilute total air flow inside the rod and reduce the holes-open pressure drop measurement; expressed as a percentage with higher numbers indicating greater ventilation.

**Virginia tobacco**

A dark, flue-cured tobacco.

**Yield or Level**

Cigarettes classified based on the amount of a substance, such as tar, nicotine, or carbon monoxide, as produced in smoke under standard smoking conditions.
LITERATURE CITATIONS


APPENDIX C

PUBLIC AND INVITED COMMENTS

Organizations Submitting Information on the Review of Ingredients Added to Cigarettes

Open Meeting Participants¹

The Open Meeting on the Review of Ingredients Added to Cigarettes was held on August 26, 2002. One oral presentation was made.

Wolf-Deiter Heller, Ph.D.
Faculty for Economic Sciences
University of Karlsruhe
Berlin, Germany

The following individuals submitted written materials for consideration by the Ad Hoc Expert Panel:

James E. Trosko, Ph.D.
Department of Pediatrics/Human Development
College of Human Medicine
Michigan State University
East Lansing, Michigan

Randall J. Ruch, Ph.D.
Department of Pathology
Medical College of Ohio
Toledo, Ohio

Dr. Thilo Paschke
Verbend der Cigarettenindustrie
Berlin, Germany

¹ Copies of the Open Meeting transcript are available from Ace-Federal Reporters, 1120 G Street, NW, Washington, DC 20005
Abstract of written comments by Trosko and Ruch about gap junctions, as edited by George J. Christ, Ph.D., of Albert Einstein College of Medicine, in response to LSRO’s call for comments from the scientific community to guide our evaluation of the relative risks of non-tobacco ingredients added to cigarettes. May 20, 2003

The development of the most efficacious strategy for the prevention and treatment of cancers is based on understanding the underlying mechanisms of carcinogenesis. This includes the knowledge that the carcinogenic process is a multi-step, multi-mechanism process and that no two cancers are alike, in spite of some apparent universal characteristics, such as their inability to have growth control, to terminally differentiate, to apoptose abnormally and to have an apparent extended or immortalized life span. The multi-step process, involving the “initiation” of a single cell via some irreversible process, with the clonal expansion of this initiated cell by suppressing growth control and inhibiting apoptosis (promotion step), leads to a situation whereby additional genetic and epigenetic events can take place (progression step) to confer the necessary phenotypes of invasiveness, and metastasis (neoplastic stage). While it is clear that, in principle, prevention of each of these three steps is possible, in practical terms, while it would make sense to minimize the initiation step, one can never reduce this step to zero. On the other hand, since the promotion step is the rate-limiting step of carcinogenesis, intervening to block this step makes the most sense. Also, by understanding the ultimate biological function that confers growth control, terminal differentiation or apoptosis for cells, there is even some hope of treating some, but not all, malignant cells such that they can regain some ability to perform these vital cellular functions.

Gap junctional intercellular communication (GJIC) has been speculated to be a necessary, if not sufficient, biological function of metazoan cells for the regulation of growth control, differentiation and apoptosis of normal progenitor cells. Normal, contact-inhibited fibroblast and epithelial cells have functional GJIC, while most, if not all, tumor cells have dysfunctional homologous or heterologous GJIC. Cancer cells are characterized by the lack of growth control, inability to terminally differentiate or apoptose under normal conditions and have extended or immortalized life spans. Chemical tumor promoters, growth factors and hormones have been shown to inhibit GJIC. Several oncogenes and anti-sense connexin genes have been shown to down-regulate GJIC function. Anti-oncogene drugs, anti-tumor promoting natural and synthetic chemicals, as well as GJIC-deficient neoplastic cells, transfected with various connexin genes, have been shown to re-gain GJIC and growth control with the loss of tumorigenicity. Therefore, the hypothesis for a rational approach to identify anti-tumor promoting chemopreventive drugs and anti-carcinogenic treatments is to use the prevention of the down regulation of GJIC by the tumor promoters and the restoration of GJIC in neoplastic cells.
While previous and many current strategies for chemoprevention and therapy have been based on treating specific oncogene products or cell signalling mechanisms, as well as advance molecular modifications of older strategies, none have specifically used the prevention of GJIC by agents during the rate limiting step of carcinogenesis or the restoration of GJIC in neoplastic cells which are deficient in GJIC. Since there are multiple mechanisms by which GJIC is down regulated during the tumor promotion phase and since stable GJIC deficiencies in neoplastic cells can be the result of transcriptional, translational or posttranslational mechanisms, it should be clear there would not be one “golden bullet” approach to resolve either the chemoprevention or therapeutic approach. Even with the hypothesis that GJIC, which depends on the transcription of normal connexin genes, their normal translation, trafficking, assembly and function, it should be clear that cells with normal connexin genes and potentially normal GJIC might not have functional GJIC because of dysfunction of other defects in cancer cells, namely cell-adhesion or cell-matrix problems (both of which are necessary for GJIC to occur). In essence, if dietary or chemopreventive/therapy is to be effective, the strategy must either ameliorate the growth stimulatory effects of exogenous chemicals, growth factors or hormones, that trigger various mitogenic /anti-apoptotic signal transducing systems.

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Abstract relevant to oral presentation by Wolf-Dieter Heller, Ph.D. at the open meeting.

This paper presents a literature review of published scientific studies of the effects of tobacco product ingredients and various experimental additives on cigarette smoke composition and its biological activity. The format of this work is that of an uncommented reference paper rather than a critical scientific review. Therefore, the mention of an ingredient in this survey does not imply that it is used by the tobacco industry or that it is covered by existing national regulations. A broad range of scientific papers and patents on tobacco ingredients is included as well as studies on experimental ingredients. This review may provide public health officials as well as scientists in government agencies and in the tobacco industry with a helpful overview of published information on tobacco product ingredients, their transfer into mainstream cigarette smoke, pyrolysis products, and influence on the biological activity of mainstream cigarette smoke.

APPENDIX D

LIST OF INGREDIENTS

BACKGROUND

In 1994 six major cigarette manufacturers reported their most current and complete list of ingredients (Doull et al., 1994). This list, also known as the “599 Ingredients List,” is annotated with references to materials approved for use as food additives by the U.S. Food and Drug Administration (FDA) as “Generally Recognized as Safe” (GRAS). The Flavor and Extracts Manufacturers Association of the United States (FEMA) also recognizes many of these ingredients as safe for ingestion. This list is a subset of approximately 700 ingredients that tobacco companies annually report to the Centers for Disease Control and Preventions (CDC) Office on Smoking and Health (OSH) which is a division of the National Center for Chronic Disease Prevention and Health Promotion. The Department of Health and Human Services (DHHS) list (R.J.Reynolds Tobacco Company, 2000a) is treated as a trade secret or confidential information in accordance with the Comprehensive Smoking Education Act (U.S. Congress, 2002).

INFORMATION SOURCES

Several versions of the 599 ingredients list can be found via the Internet.


➤ The Tobacco Institute (1994), the Indiana Prevention Resource Center at Indiana University (1998) and the Alternative Cigarettes websites (2002) provide lists of the 599 ingredients. Of these only the Tobacco Institute numerically lists the ingredients and indicates whether FEMA and/or GRAS approved the ingredients.

➤ R.J. Reynolds Tobacco Company, Philip Morris USA, Brown & Williamson Tobacco Corporation, and the Liggett Group Inc. (2000a; 2000; 2002; 2002) also provide lists of ingredients on their company websites. Of these lists, R.J. Reynolds, Philip Morris and Brown & Williamson list the “Maximum Use Level” (MUL), which is the maximum amount of each ingredient that these companies add to cigarette tobacco. Due to the differences in cigarette brand characteristics, the actual levels of ingredients will vary. Brown & Williamson’s list is annotated with the Chemical Abstract Services Registry Numbers (CAS Nos.).
The CAS number is a unique code to identify a chemical, which may have more than one common or scientific name. Liggett Group’s list indicates tar and nicotine level, for each of the company cigarette brands but does not annotate the list with MULs or references to materials that are approved by GRAS or FEMA. Lorillard Tobacco Company does not provide an ingredients list on its website.

OBSERVATIONS

Our review of the original 1994 report found spelling errors in the lists predominantly in the amino acids, e.g., \textit{dl}-alanine, \textit{l}-leucine listed as \textit{d1}-alanine and \textit{l}-leucine, respectively. Since the Indiana Prevention Resource Center and Alternative Cigarettes websites copied the original list to their websites, these errors carried through. Our comparison of the DHHS list with the original 1994 report list found that the DHHS list contained only 483 ingredients. However, the spelling errors found in the 599 list are not repeated here. LSRO developed a spreadsheet to determine which ingredients are on both the 599 list (Doull \textit{et al.}, 1994) and the DHHS (R.J.Reynolds Tobacco Company, 2000b) lists and which ingredients are on one list but not the other.

LITERATURE CITATIONS


**LIST OF INGREDIENTS**

**Tobacco Companies’ Combined Ingredients List For the U.S. Department of Health and Human Services (DHHS) (R.J. Reynolds Tobacco Company, 2000b)**

Most of these ingredients are commonly used in foods and beverages, or permitted for use in foods by the U.S. Food and Drug Administration (FDA), or have been given the status “Generally Recognized as Safe in Foods” (GRAS) by FDA, the Flavor and Extract Manufacturers Association (FEMA) or other expert committees.

**Ingredient**

1. Acetanisole  
   FDA-approved food additive; FEMA GRAS; found in beef, cranberry, guava, grape, mango, peppermint; used in frozen dairy products, hard candies.

2. Acetic acid  
   FDA GRAS; FEMA GRAS; found in banana, beer, beef, apple juice, apricot, blue cheese, blueberries; used in condiment relishes.

3. Acetoin  
   FDA GRAS; FEMA GRAS; found in apples, butter, yogurt, asparagus, black currants, blackberry, wheat, broccoli, brussel sprouts, cantaloupe; used in baked goods.

4. Acetophenone  
   FDA-approved food additive; FEMA GRAS; found in apple, cheese, apricot, banana, beef, cauliflower; used in chewing gum.
5. 2-Acetylpyrazine
FEMA GRAS; found in beef, coffee, popcorn, sesame seed, almond, wheat bread, cocoa, peanut, pork, potato chips; used in frozen dairy products.

6. 2-Acetylpyridine
FEMA GRAS; found in cocoa, coffee, roasted peanut, potato chips, tea, beer, wheat bread, hazelnut, lamb/mutton, potato; used in breakfast cereals, ice cream, candy.

7. 3-Acetylpyridine
FEMA GRAS; found in roasted filbert, cocoa; used in nonalcoholic beverages, ice cream, candy, gelatins & puddings, baked goods.

8. 2-Acetylthiazole
FEMA GRAS; found in bean, potatoes, artichoke, asparagus, beef, beer, brazil nuts, rice, boiled shrimp; used in snack foods.

9. Aconitic acid
FDA GRAS; FEMA GRAS; found in beet root, sugarcane; used in alcoholic beverages, baked goods, chewing gum.

10. dl-Alanine, l-Alanine
FDA-approved food additive; natural constituent of protein in plants and animals; found in apple, beef, carob, pea, soybean, wine, zucchini.

11. Alfalfa extract
FDA GRAS; FEMA GRAS; found in alfalfa; used in baked goods.

12. Allspice oil (pimento berry)
FDA GRAS; FEMA GRAS; used in soups, candies, chewing gum, meats.

13. Allyl Hexanoate
FDA-approved food additive; FEMA GRAS; found in baked potato; used in gelatins & puddings.

14. alpha-Methylbenzyl acetate
FDA-approved food additive; FEMA GRAS; found in grape brandy, rice, tea, tomato, tomato paste; used in chewing gum.

15. Ambergris tincture
FDA GRAS; FEMA GRAS; used in nonalcoholic beverages, ice cream, candy.
16. Ammonium alginate  
FDA GRAS; FEMA GRAS; used in confections, frostings, gelatins & puddings, sauces. Ammonium alginate is used as a binder in the carbon heat source of cigarettes that primarily heat tobacco and also in some tobacco sheets.

17. Ammonium hydroxide  
FDA GRAS; found in cured pork.

18. Ammonium phosphate dibasic  
FDA GRAS; used in dough, ice cream, gelatins & puddings.

19. Amyl alcohol  
FDA-approved food additive; FEMA GRAS; found in apple, banana, cheese, chicken, coffee, potato, raspberry, strawberry, tomato; used in baked goods, candy, gelatins & puddings, chewing gum.

20. Amyl butyrate  
FDA-approved food additive; FEMA GRAS; found in bananas, beer, apple juice, apricots, strawberries, wine; used in syrup, candy, chewing gum.

21. Amyl formate  
FDA-approved food additive; FEMA GRAS; found in apples, strawberry, brandy, honey, tomatoes, whiskey; used in nonalcoholic beverages, candy, chewing gum.

22. Amyl octanoate  
FDA-approved food additive; FEMA GRAS; found in strawberry, apple, cognac; used in baked goods, candy, gelatins & puddings.

23. alpha-Amylcinnamaldehyde  
FDA-approved food additive; FEMA GRAS; found in black tea, olibanum; used in candy, baked goods, chewing gum.

24. Amyris oil  
FDA-approved food additive; found in brandies, liqueurs, amyris balsamifera; used in brandies, liqueurs, oriental specialties.

25. trans-Anethole  
FDA GRAS; FEMA GRAS; found in cheese, tea, apple, licorice; used in alcoholic beverages.

26. Angelica root extract and oil  
FDA GRAS; FEMA GRAS; used in nonalcoholic beverages, alcoholic beverages, baked goods, chewing gum.
27. Anise oil  
FDA GRAS; FEMA GRAS; found in star anise; used in ice cream, ices, baked goods, candy, chewing gum, meats, condiments.

28. Anisyl acetate  
FDA-approved food additive; FEMA GRAS; found in currant; used in baked goods, candy, gelatins & puddings, chewing gum.

29. Anisyl alcohol  
FDA-approved food additive; FEMA GRAS; found in honey, tomato; used in gelatins & puddings.

30. Anisyl formate  
FDA-approved food additive; FEMA GRAS; found in vanilla; used in candy, baked goods.

31. Apple juice concentrate and extract  
Common food item found in apple; used in juices, baked goods.

32. Apricot extract  
Common food item found in apricot; used in condiments.

33. l-Arginine  
FDA-approved food additive; natural constituent of proteins in plants and animals.

34. Ascorbic acid  
FDA GRAS; FEMA GRAS; found in citrus fruit, tea leaves; used in baked goods, sweet sauce, soups, candy, gelatins & puddings, dairy products.

35. l-Aspartic acid  
FDA-approved food additive; FEMA GRAS; found in proteins, licorice; used in seasonings.

36. Balsam peru and oil  
FDA GRAS; FEMA GRAS; found in Peru balsam; used in baked goods, syrups, candy, chewing gum.

37. Bay oil  
FDA GRAS; FEMA GRAS; found in bay leaves; used in condiments, meats.

38. Beeswax resinoid and absolute  
FDA GRAS; FEMA GRAS; used in baked goods, candy, honey.
39. Beet juice concentrate
Beets are included among “Miscellaneous Vegetables” in FDA Standards of Identity and are also covered by a USDA Standards for Grades.

40. Benzaldehyde
FDA GRAS; FEMA GRAS; found in apple juice, almond, apricot, artichoke, asparagus, beans, beef, beer; used in baked goods, chewing gum.

41. Benzaldehyde glycercyl acetal
FDA-approved food additive; FEMA GRAS; used in baked foods, candy, gelatins & puddings, chewing gum.

42. Benzoic acid
FDA GRAS; FEMA GRAS; found in cinnamon, strawberry, tea, apple, beer, bread, cocoa, honey; used in baked goods, cheese, candy, chewing gum, condiment relish.

43. Benzoin, resin, resinoid, gum, and absolute
FDA-approved food additive; FEMA GRAS; used in baked goods, gelatins & puddings, chewing gum.

44. Benzophenone
FDA-approved food additive; FEMA GRAS; found in grape, apples, papaya; used in frozen dairy products.

45. Benzyl alcohol
FDA-approved food additive; FEMA GRAS; found in apricot, beef, beer, almonds, apple, apple juice, asparagus, bananas, black currants, blackberries; used in chewing gum, candy, baked goods.

46. Benzyl benzoate
FDA-approved food additive; FEMA GRAS; found in celery, parsley, black currants, butter, guava, pineapple, papaya; used in ice cream, baked goods, candy.

47. Benzyl butyrate
FDA-approved food additive; FEMA GRAS; found in apple, apple juice, apricot, banana, parmesan cheese, grape, honey, mango, melon, muskmelon, orange juice, papaya; used in candy.

48. Benzyl cinnamate
FDA-approved food additive; FEMA GRAS; used in nonalcoholic beverages, baked goods, candy.
49. Benzyl salicylate
FDA-approved food additive; FEMA GRAS; found in cranberry, apple flowers; used in baked goods.

50. Bergamot oil
FDA GRAS; FEMA GRAS; found in oranges; used in icings, gelatin, alcoholic beverages.

51. Bois de Rose (Peruvian) oil
FDA GRAS; FEMA GRAS; used in baked goods, candy, chewing gum.

52. Bornyl acetate
FDA-approved food additive; FEMA GRAS; found in carrot, black currants, gin, ginger, kiwi fruit, pistacia, plum, sweet potato, soy sauce, black & green tea; used in gelatins & puddings, candy, ice cream.

53. Brown sugar
Found in food sugar sources; used in baked goods, candy, breakfast cereals.

54. Buchu leaf oil
FDA-approved food additive; FEMA GRAS; used in ice cream, ices, candy, condiments.

55. 1,3-Butanediol
FDA-approved food additive; used as a solvent for natural and synthetic flavors.

56. 4-(2-Butenylidene)-3,5,5-trimethyl-2-cyclohexen-1-one
Found in white-flesh nectarine, starfruit, grapefruit juice.

57. Butter, butter esters, and butter oil
FDA-approved food additive; FEMA GRAS; found in butter; used in frozen dairy products.

58. Butyl Acetate
FDA-approved food additive; FEMA GRAS; found in apple, banana, beer, black currant, cashew nuts, cheese, raspberry, apricot, blackberry brandy, cantaloupe; used in cheeses, baked goods, candy.

59. Butyl Alcohol
FDA-approved food additive; FEMA GRAS; found in apple juice, banana, celery, cheese (cheddar and Swiss), peach, potato; used in nonalcoholic beverages, alcoholic beverages, ice cream ices, candy, cream, baked goods.
60. Butyl Butyrate  
FDA-approved food additive; FEMA GRAS; found in banana, apricot, blackberry, brandy, parmesan cheese, honey, mango, melon, muskmelon, orange juice, papaya; used in candy.

61. Butyl butyryl lactate  
FDA-approved food additive; FEMA GRAS; used in nonalcoholic beverages, baked goods, candy, sweet sauces.

62. n-Butyl isovalerate  
FDA-approved food additive; FEMA GRAS; found in apple, apricot, banana, parmesan cheese, olives, pear, plum, strawberry, wine; used in baked goods.

63. Butyl phenylacetate  
FDA-approved food additive; FEMA GRAS; found in papaya, alfalfa; used in baked goods, ice cream, candy.

64. 3-Butylidenephthalide  
FEMA GRAS; found in celery, celery stalk; used in soups, condiments, meats.

65. Butyric acid  
FDA GRAS; FEMA GRAS; found in apple, beef, beer, black currants, blueberries, wheat bread, butter, blue cheese; used in snack foods, candy, margarine.

66. Camphene  
FDA-approved food additive; FEMA GRAS; found in carrot, cheddar cheese, ginger, apricot, black currants, blackberry, celery, gin, kiwi fruit; used in candy, baked goods, ice cream.

67. Cananga oil  
FDA GRAS; FEMA GRAS; used in nonalcoholic beverages, candy, baked goods.

68. Caramel and caramel color  
FDA GRAS; FEMA GRAS; found in sugars; used in gravies, meats, condiments.

69. Caraway oil  
FDA GRAS; FEMA GRAS; found in caraway seeds; used in baked goods, condiments.

70. Carbon  
Carbons are used in air and water filtration systems. Carbons are also used as clarifiers and decolorizers in the food and beverage industry. Carbon is the main heat source constituent in cigarettes that primarily heat tobacco.
71. Carbon dioxide  
FDA GRAS; used in beverages, meat products, processed fruits, dairy products.

72. Cardamom oleoresin, oil, extract, seed oil, and powder  
FDA GRAS; FEMA GRAS; found in cardamom; used in baked goods, pickles, meats.

73. Carob bean and extract  
FDA GRAS; FEMA GRAS; found in carob beans; used in baked goods, candy, gelatins puddings, icings & toppings.

74. beta-Carotene  
FDA GRAS; found in carrot, pumpkin, spinach, broccoli; used in processed fruit and fruit juices, dairy products.

75. Carrot oil, seed  
FDA GRAS; FEMA GRAS; found in carrots; used in baked goods.

76. Carvacrol  
FDA-approved food additive; FEMA GRAS; found in pepper, spearmint, tea; used in baked goods, condiments, candy, gelatins & puddings, chewing gum.

77. 4-Carvomenthenol  
FDA-approved food additive; FEMA GRAS; found in carrot, celery seed, cocoa powder, grape, grapefruit, orange, tea, wine; used in nonalcoholic beverages, candy, baked goods, gelatins & puddings, chewing gum.

78. l-Carvone  
FDA GRAS; found in grapefruit juice, honey, hops oil, orange juice, beer, cherries, endive, guava, hazelnuts; used in candy, condiments, baked goods.

79. beta-Caryophyllene oxide  
FDA-approved food additive; found in rosemary; used in beverages, ice cream, candy, condiments.

80. beta-Caryophyllene  
FDA-approved food additive; FEMA GRAS; found in carrot, artichoke, banana, cashews, apples, celery, chervil, chicken, cocoa; used in chewing gum, ice cream, beverages.

81. Cascarilla oil and bark extract  
FDA GRAS; FEMA GRAS; used in baked goods, candy, condiments.
82. Cassia bark, buds, oil and extract
FDA GRAS; FEMA GRAS; found in cassia; used in meat products.

83. Cassie absolute
FDA-approved food additive; FEMA GRAS; found in cassie; used in candy, baked goods, ice cream.

84. Castoreum extract, tincture, liquid, and absolute
FDA GRAS; FEMA GRAS; found in castor; used in baked goods, condiments, candy, chewing gum.

85. Cedar leaf oil
FDA-approved food additive; FEMA GRAS; found in cedar tree; used in alcoholic beverages, meats, candy.

86. Cedarwood oil terpenes
FDA-approved food additive; found in cedarwood, clary sage oil.

87. Celery seed extract, solid, oil, and oleoresin
FDA GRAS; FEMA GRAS; found in celery seeds, celery; used in baked goods, meats, soups, condiments, pickles.

88. Cellulose and Cellulose fiber
Natural polysaccharide, which is the most abundant carbohydrate in nature; found in all plant material; used in grated cheese, fruit preserves/jams, fruit jellies. Cellulose fiber is used as a sheet strengthener in tobacco sheets.

89. Chamomile flower oil and extract
FDA GRAS; FEMA GRAS; found in chamomile flowers; used in nonalcoholic beverages, ice cream, baked goods, gelatins & puddings.

90. Chicory extract
FDA GRAS; FEMA GRAS; found in chicory; used in baked goods, nonalcoholic beverages, ice cream.

91. Chocolate
Common food item.

92. 1,8-Cineole
FDA-approved food additive; FEMA GRAS; found in black currants, blueberries, brandy, cantaloupe, cheese, cocoa, grapes, thyme, babaco fruit, bilberry, corn; used in chewing gum, ice cream, baked goods.
93. Cinnamaldehyde
FDA GRAS; FEMA GRAS; found in beer, brandy, blueberries, cantaloupe, capers, cranberries, gin, guava, melon; used in gravies, candy, ice cream, meat.

94. Cinnamic acid
FDA-approved food additive; FEMA GRAS; found in beer, blackberry, capers, cherry, grape, guava, malt, mango, mushroom, passion fruit, strawberry; used in soft candy.

95. Cinnamon leaf oil, bark oil, and extract
FDA GRAS; FEMA GRAS; found in cinnamon tree; used in baked goods, chewing gum, candy, meats, condiments, pickles.

96. Cinnamyl acetate
FDA-approved food additive; FEMA GRAS; found in guava; used in candy, ice cream condiments.

97. Cinnamyl alcohol
FDA-approved food additive; FEMA GRAS; found in blackberry, blueberry, cantaloupe, cranberry, guava, melon, raspberry, strawberry, watermelon; used in nonalcoholic beverages.

98. Cinnamyl cinnamate
FDA-approved food additive; FEMA GRAS; found in storax; used in baked goods, ice cream, candy.

99. Cinnamyl isovalerate
FDA-approved food additive; FEMA GRAS; found in chestnut flowers; used in candy, gelatins & puddings, chewing gum.

100. Citral
FDA GRAS; FEMA GRAS; found in grapefruit juice, orange, orange juice, celery, apricot, black currants, grape, hops, kiwi fruit, mango, mango ginger, melon, plum, raspberry, rum; used in baked goods, candy, ice cream.

101. Citric acid
FDA GRAS; FEMA GRAS; widely found in fruits and vegetables; used in fruit juices, meats, poultry, beverages.

102. Citronella oil
FDA GRAS; FEMA GRAS; found in citronella; used in alcoholic beverages, ice cream, baked goods.
103. *dl*-Citronellol FDA-approved food additive; FEMA GRAS; found in apple, apricot, beer, black currants, blackberry, blueberry, orange juice, passion fruit, peach; used in soft candy.

104. Citronellyl isobutyrate
FDA-approved additive; FEMA GRAS; used in candy, gelatins & puddings, nonalcoholic beverages, baked goods.

105. Civet absolute
FDA GRAS; FEMA GRAS; found in civet; used in ice cream, candy, baked goods, chewing gum.

106. Clary sage oil and extract
FDA GRAS; FEMA GRAS; found in clary sage; used in alcoholic beverages, baked goods, condiments.

107. Cocoa, cocoa shells, extract, distillate, powder, alkalized, absolute and tincture
FDA GRAS; found in cocoa, cocoa shells; used in baked goods.

108. Coffee and coffee solid extract
FDA GRAS; found in coffee; used in baked goods, candy, syrups.

109. Cognac white and green oil
FDA GRAS; FEMA GRAS; found in cognac brandy; used in alcoholic beverages, ice cream, baked goods.

110. Copaiba oil
FDA-approved food additive; found in copaiba.

111. Coriander extract, oil, and seed
FDA GRAS; FEMA GRAS; found in coriander; used in baked goods, meats, condiments.

112. Corn silk
FDA GRAS; FEMA GRAS; found in corn; used in baked goods, beverages, candy, desserts.

113. Corn syrup
FDA GRAS; found in food sugar sources; used in baked goods, candy, breakfast cereals.

114. Costus root oil
FDA-approved food additive; FEMA GRAS; found in costus root; used in candy, baked goods.
115. para-Cymene
FDA-approved food additive; FEMA GRAS; found in apricot, banana, beans, beli, black currants, brandy apple, carrots, celery; used in chewing gum.

116. l-Cysteine
FDA GRAS; FEMA GRAS; natural constituent of protein in plants and animals; found in pippali fruit (India); used in condiments relish, beverages, meats, baked goods, dairy products.

117. Dandelion root solid extract
FDA GRAS; FEMA GRAS; found in dandelions; used in baked goods.

118. Davana oil
FDA-approved food additive; FEMA GRAS; found in Artemisia plant; used in alcoholic beverages.

119. 2,4-Decadienal
FEMA GRAS; found in chicken, cranberry, peanut, tomato; used in vegetables, baked goods, meat, candy, chewing gum, cereals.

120. delta-Decalactone
FDA-approved food additive; FEMA GRAS; found in apricot, beef, butter, beer, blue cheese, grape brandy, plum brandy, wheat bread, cantaloupe, cheese; used in baked goods, frozen dairy products.

121. gamma-Decalactone
FDA-approved food additive; FEMA GRAS; found in apricot, beef fat, butter, black currants, blackberry, blue cheese, cheddar cheese, chicken, coconut, cranberry, cream; used in baked goods, margarine, candy.

122. Decanal
FDA GRAS; FEMA GRAS; found in almond, apple, apple flowers, apricot, artichoke, avocado, beef, wheat bread; used in baked goods, beverages, ice cream, candy.

123. Decanoic acid
FDA-approved food additive; FEMA GRAS; found in apple, apple flowers, banana, beef, beer, blackberries, blue cheese, brandies, wheat bread, butter, heated butter; used in imitation dairy goods.

124. Decanoic Acid, Ester with 1,2,3 - Propanetriol Octanoate (Coconut oil)
FDA GRAS; found in coconut; used in shortening, candies, chocolate.
125. Dextrin
FDA-approved food additive; FEMA GRAS; found in apple, apple juice, apricot, asparagus, banana, beer, brandy apple, butter; used in frozen dairy goods, ice cream, beverages, candy.

126. Diacetyl
FDA GRAS; FEMA GRAS; found in apple, bean, beef, butter, artichoke, avocado, black currants, blueberry, blue cheese, grape brandy, wheat, brussel sprouts; used in meat products.

127. Diethyl malonate
FDA-approved food additive; FEMA GRAS; found in whiskey, wine, blackberry, grape brandy, strawberry wine.

128. 2,3-Diethylpyrazine
FEMA GRAS; found in wheat bread, wheat, hazelnut, baked potato, soy sauce; used in candy, gelatins & puddings.

129. 5,7-Dihydro-2-methylthieno (3,4-d) pyrimidine
FEMA GRAS; used in breakfast cereals, beverages, ice cream, candy, dairy products.

130. Dill oil
FDA GRAS; FEMA GRAS; found in dill; used in cheese, meats, sauces, dips, baked goods.

131. meta-Dimethoxybenzene
FDA-approved food additive; FEMA GRAS; found in brandy grape, salami, filberts; used in meat products, beverages, ice cream, candy, baked goods.

132. para-Dimethoxybenzene
FDA-approved food additive; FEMA GRAS; found in tea, hyacinth oil, peppermint oil; used in gelatins & puddings, beverages, ice cream, candy, baked goods.

133. 2,6-Dimethoxyphenol
FEMA GRAS; found in maple syrup, rum, smoked sausage, wine; used in seafood, meat, baked goods, candy, soups.

134. 3,4-Dimethyl-1,2-cyclopentadione
FEMA GRAS; found in roasted coffee; used in nut products, beverages, ice cream, candy.
135. 3,7-Dimethyl-1,3,6-octatriene
FDA-approved food additive; FEMA GRAS; found in apricot, guava, pineapple, tomato; used in frozen dairy products.

136. 4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one
FEMA GRAS; found in almond, asparagus, wheat bread, butter, chicken, steamed clams, cocoa, coconut, coffee, corn; used in baked goods, sweet sauce.

137. 6,10-Dimethyl-5,9-undecadien-2-one
FDA-approved food additive; FEMA GRAS; found in almond, asparagus, beans, beef, beer, cashew nuts, parmesan cheese, chicken; used in baked goods, candy, dairy products.

138. 3,7-Dimethyl-6-octenoic acid
FEMA GRAS; found in peppermint oil; used in baked goods.

139. alpha,para-Dimethylbenzyl alcohol
FEMA GRAS; used in nonalcoholic beverages, ice cream, ices, candy.

140. alpha,alpha-Dimethylphenethyl butyrate
FDA-approved food additive; FEMA GRAS; found in sake; used in frozen dairy goods, beverages, candy, baked goods.

141. 2,3-Dimethylpyrazine
FEMA GRAS; found in asparagus, peanut, coffee, potato; used in gravies, beverages, candy, baked goods.

142. 2,5-Dimethylpyrazine
FEMA GRAS; found in beef, blackberry, grape brandy, cantaloupe, corn, endive, grapefruit juice; used in breakfast cereal.

143. 2,6-Dimethylpyrazine
FEMA GRAS; found in citronella, camphor oil; used in milk products, meat, candy.

144. delta-Dodecalactone
FDA-approved food additive; FEMA GRAS; found in beef, butter, milk, blue cheese, cheddar cheese, chicken, coconut, lamb/mutton, peach, plum, pork; used in meat products, baked goods, candy.

145. gamma-Dodecalactone
FDA-approved food additive; FEMA GRAS; found in apricot, beef, beer, blackberry, blue cheese, butter, carambola (starfruit), cheddar cheese, chervil, chicken; used in baked goods, beverages, ice cream, candy.
146. Ethyl 2-methylbutyrate
FDA-approved food additive; FEMA GRAS; found in apple, apple juice, beer, blackberry, blackberry, brandy apple, brandy grape, cantaloupe, fig, grape, honeydew melon; used in hard candy, beverages, ice cream.

147. Ethyl acetate
FDA GRAS; FEMA GRAS; found in apple, apple juice, banana, beans, beef, beer, blue cheese, blueberry; used in chewing gum, beverages, ice cream, candy.

148. Ethyl acetoacetate
FDA-approved food additive; FEMA GRAS; found in passion fruit, sherry, strawberry, wine; used in soft candy.

149. Ethyl alcohol, including Specially Denatured Alcohol (SDA) No. 4
FDA GRAS; FEMA GRAS; found in apple, banana, bread, coffee, cucumber, potato. As required by the U.S. Bureau of Alcohol, Tobacco and Firearms regulations, nicotine sulfate is used to denature the alcohol, which is used as a solvent to apply flavors during processing. There is no measurable effect on the nicotine level of the finished cigarette as a result of this process.

150. Ethyl benzoate
FDA-approved food additive; FEMA GRAS; found in apple, apricot, arctic bramble, babaco fruit, banana, beer, beli, bilberry, bilberry wine, black currants, blackberry, brandy apple; used in gelatins & puddings, beverages, ice cream, candy.

151. Ethyl butyrate
FDA GRAS; FEMA GRAS; found in apple, apple juice, banana, beer, apricot, beef, blue cheese, brandy; used in chewing gum.

152. Ethyl cinnamate
FDA-approved food additive; FEMA GRAS; found in beer, blackberry, brandy apple; used in baked goods, beverages, ice cream, candy.

153. Ethyl decanoate
FDA-approved food additive; FEMA GRAS; found in apple juice, banana, beef, wheat bread, butter, cheddar cheese; used in frozen dairy foods, ice cream, candy.

154. Ethyl heptanoate
FDA-approved food additive; FEMA GRAS; found in cashew, apple, cocoa, grape, grapefruit juice, roasted hazelnut, hops, milk, olive, papaya mountain, passion fruit, peach; used in chewing gum.
155. Ethyl hexanoate
FDA-approved food additive; FEMA GRAS; found in banana, beer, cheese, beef, black currants, blackberry, brandies, broccoli; used in baked goods, ice cream, candy.

156. Ethyl isovalerate
FDA-approved food additive; FEMA GRAS; found in banana, celery, apple, beer, brandy, cantaloupe, cashew apple, parmesan cheese; used in condiments, ice cream, baked goods.

157. Ethyl lactate
FDA-approved food additive; FEMA GRAS; found in apple, beer, cocoa, pineapple, apricot, bilberry wine, brandy, butter, capers, chicken, meat, elderberry, elderberry juice, grape peas, plum; used in chewing gum, ice cream, baked goods.

158. Ethyl laurate
FDA-approved food additive; FEMA GRAS; found in apple, beer, cheddar cheese, apricot, bilberry wine, blackberry, brandy, wheat bread, butter; used in baked goods, candy, ice cream.

159. Ethyl levulinate
FDA-approved food additive; FEMA GRAS; found in bilberry wine, brandy grape, wheat bread, cherimoya, cocoa, onion roasted, rum, wine; used in frozen dairy goods, beverages, candy, baked goods.

160. Ethyl maltol
FDA-approved food additive; FEMA GRAS; found in apple juice; used in sweet sauce, soups, meat, candy.

161. Ethyl methyl phenylglycidate
FDA GRAS; FEMA GRAS; used in condiment relish, beverages, candy, ice cream.

162. Ethyl myristate
FDA-approved food additive; FEMA GRAS; found in cheddar cheese, grape wine, Bartlett pear; used in nonalcoholic beverages, alcoholic beverages, ice cream, ices, candy, baked goods.

163. Ethyl nonanoate
FDA-approved food additive; FEMA GRAS; found in apple, apricot, banana, beef, beer, bilberry wine, brandy apple, wheat bread, cocoa, elderberry, grape, nectarine, olive, peach; used in baked goods, beverages, ice cream, candy.

164. Ethyl octadecanoate
FEMA GRAS; found in grapes, beer, brandy, maple syrup; used in nonalcoholic beverages, ice cream, ices, candy, alcoholic beverages.
165. Ethyl octanoate
FDA-approved food additive; FEMA GRAS; found in apple, banana, beer, blue cheese, apricot, bilberry wine, blackberry, brandy, wheat bread, broccoli, butter, capers; used in frozen dairy products.

166. Ethyl oleate
FDA-approved food additive; FEMA GRAS; found in melons, grapes, brandy, maple syrup; used in nonalcoholic beverages, candy, baked goods, gelatins & puddings, condiments & relishes.

167. Ethyl palmitate
FEMA GRAS; found in cheddar cheese, maple syrup, grape wine; used in nut products.

168. Ethyl phenylacetate
FDA-approved food additive; FEMA GRAS; found in apple, crisp bread, honey, beer, beli, bilberry wine, brandy, wheat bread, cantaloupe, chempedak fruit, cocoa, grape, grapefruit juice, guava, licorice, melon; used in gelatin & puddings, syrups, baked goods.

169. Ethyl propionate
FDA-approved food additive; FEMA GRAS; found in apples, apricot, banana, beer, bilberry, blackberry, brandy, cantaloupe, cheddar cheese, cocoa, fig, grape, guava; used in baked goods, meat products, ice cream.

170. Ethyl valerate
FDA-approved food additive; FEMA GRAS; found in apple, banana, grape, apricot, bilberry wine, black currants, brandy, cashew apples, parmesan cheese, fig, guava, honey, kiwi fruit, melon, muskmelon; used in chewing gum, baked goods, beverages.

171. Ethyl vanillin
FDA GRAS; FEMA GRAS; found in vanilla beans; used in alcoholic beverages, imitation vanilla extract, breakfast cereals.

172. Ethyl vanillin glucoside
Composed of ethyl vanillin, a common flavoring, and glucose, a sugar. Ethyl Vanillin is FDA/FEMA GRAS; found in vanilla beans and used in alcoholic beverages, imitation vanilla extract, breakfast cereals. Glucose is found in food sugar sources; used in baked goods, candy, breakfast cereals.

173. 2-Ethyl-1-hexanol
FEMA GRAS; used in nonalcoholic beverages, ice cream, ices, candy, chewing gum.
174. 3-Ethyl-2-hydroxy-2-cyclopenten-1-one
FEMA GRAS; found in coffee, maple syrup, peanuts, pork; used in baked goods, soups, cereals, condiments, milk & dairy products.

175. 2-Ethyl-3,(5 or 6)-dimethylpyrazine
FEMA GRAS; found in beef, coffee, bread; used in baked goods, cereals, candy, dairy products.

176. 5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone
FEMA GRAS; nature identical by FEMA; found in beef, beer, wheat bread, cashew nuts, chicken, cocoa, coconut, coffee, crayfish, eggs, hazelnut; used in chewing gum, meat products.

177. 2-Ethyl-3-methylpyrazine
FEMA GRAS; found in beef, whole egg, chicken, heated corn oil, krill, lamb/mutton, boiled shrimp, fermented soy sauce; used in candy, ice cream, beverages.

178. 4-Ethylbenzaldehyde
FEMA GRAS; found in oranges, carrots, broccoli, tomatoes; used in baked goods, meat products, candy, gelatins & puddings, confectionery/frosting, cereals, dairy products.

179. 4-Ethylguaiacol
FDA-approved food additive; FEMA GRAS; found in coffee, cranberry, smoked pork, rum, smoked sausage, tea, wine; used in nonalcoholic beverages, ice cream, ices.

180. para-Ethylphenol
FEMA GRAS; found in cocoa, coffee, peanut, tomato, wine; used in baked goods, candy, gelatins & puddings, meat products.

181. 3-Ethylpyridine
FEMA GRAS; found in beef, chicken, coffee, corn oil, almond, barley, beer, wheat bread, cocoa, eggs; used in candy, ice cream, meat, baked goods.

182. Farnesol
FDA-approved food additive; FEMA GRAS; found in oranges, lemongrass; used in nonalcoholic beverages, ice cream, candy, baked goods, gelatins & puddings.

183. Fenchone
FDA-approved food additive; FEMA GRAS; found in anise, basil, fennel, peppermint, saffron, thyme; used in ice cream, candy, baked goods.

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184. Fenugreek, extract, resin, and absolute
FDA GRAS; FEMA GRAS; found in fenugreek; used in nonalcoholic beverages, gelatins & puddings, syrups.

185. Fig juice concentrate and extract
Common food item; found in figs; used in fruit breads, cookies, cakes, sauces, fillings and cereals.

186. Food starch modified
FDA-approved food additive; widespread use; used in cured pork.

187. Fructose
Found in food sugar sources; used in baked goods, candy, breakfast cereals.

188. Furfuryl mercaptan
FEMA GRAS; found in coffee, beef, chicken, meat, popcorn; used in nonalcoholic beverages, ice cream, ices, candy, baked goods, gelatins & puddings, icings.

189. 4-(2-Furyl)-3-buten-2-one
FEMA GRAS; found in coffee; used in nonalcoholic beverages, ice cream, ices, candy, baked goods, gelatins & puddings, alcoholic beverages.

190. Galbanum oil and resinoid
FDA-approved food additive; FEMA GRAS; found in galbanum; used in meat products, baked goods, ice cream.

191. Geraniol
FDA GRAS; FEMA GRAS; found in apples, apricot, beer, bilberry, black currants, blackberries; used in baked goods, candy, ice cream.

192. Geranium rose and bourbon oil
FDA GRAS; FEMA GRAS; found in geranium leaves and stems; used in chewing gum, ice cream, baked goods.

193. Geranyl acetate
FDA GRAS; FEMA GRAS; found in celery, cocoa, black currants, chervil, gin, ginger, grape, grapefruit juice, orange juice, passion fruit, pineapple, plum; used in baked goods, ice cream, syrups.

194. Geranyl butyrate
FDA-approved food additive; FEMA GRAS; found in celery, tomatoes, passion fruit; used in gelatins & puddings, ice cream, candy.
195. Geranyl formate
FDA-approved food additive; FEMA GRAS; found in hops, black and green tea; used in baked goods, ice cream, candy.

196. Ginger oil and oleoresin
FDA GRAS; FEMA GRAS; found in ginger; used in candy, baked goods, meats.

197. Glucose/ Dextrose
FDA GRAS; found in food sugar sources; used in baked goods, candy, breakfast cereals.

198. L-Glutamic acid
FDA GRAS; FEMA GRAS; natural constituent of proteins in plants and animals; used in baked goods, meat, soups, milk & dairy products, condiments, pickles, cereal.

199. Glycerol
FDA GRAS; FEMA GRAS; found in beer, cherry, wine; used in milk products, baked goods, meat products.

200. Graphite
Carbons are used in air and water filtration systems. Carbons are also used as clarifiers and decolorizers in the food and beverage industry. Graphite is a soft, crystalline form of carbon. Graphite is a heat source constituent in cigarettes that primarily heat tobacco.

201. Guaiac wood oil
FDA-approved food additive; FEMA GRAS; found in guaiac wood; used in meat products, ice cream, chewing gum.

202. Guaiacol
FDA-approved food additive; FEMA GRAS; found in celery, cocoa, coffee, rum, soybean, tea, tomato, whiskey, wine; used in ice cream, ices, baked goods, meat, chewing gum, dairy products.

203. Guar gum
FDA GRAS; FEMA GRAS; found in the seed of the guar plant, which is similar to the soybean plant; used in breakfast cereal, dairy products, gravies, processed vegetables, baked goods.

204. 2,4-Heptadienal
FEMA GRAS; found in avocado, beef, black currants, bread, wheat, broccoli, butter, heated butter, cabbage, cauliflower; used in meat products, baked goods, soups, candy.
205. gamma-Heptalactone
FDA-approved food additive; FEMA GRAS; found in mango, passion fruit, peach, black tea, asparagus, butter, hazelnut, lamb/mutton, leek, licorice, nectarine, papaya, pineapple; used in candy, baked goods, ice cream.

206. Heptanoic acid
FEMA GRAS; found in apple, beer, banana, beef, dried blue cheese, brandy, bread, wheat, butter, cheddar cheese; used in baked goods, margarine, ice cream.

207. 2-Heptanone
FDA-approved food additive; FEMA GRAS; found in apples, banana, beer, beef, apricot, asparagus, beans, blue cheese, wheat butter; used in gravies, ice cream, condiments, baked goods.

208. 3-Hepten-2-one
FEMA GRAS; found in capsicum peppers, hazelnut, hops, muruci; used in gelatins & puddings, ice cream, baked goods.

209. 4-Heptenal
FDA-approved food additive; FEMA GRAS; found in butterfat, soybean oil; used in nonalcoholic beverages, baked goods, meat, candy, cereal, dairy products.

210. Heptyl acetate
FEMA GRAS; found in apples, grapes, bananas, pears, plums, whiskey; used in baked goods, gelatins & puddings, chewing gum.

211. omega-6-Hexadecenlactone
FDA-approved food additive; FEMA GRAS; found in ambrette seed; used in baked goods, ice cream, candy.

212. gamma-Hexalactone
FDA-approved food additive; FEMA GRAS; found in apricot, beef, butter, apple, asparagus, beer, blackberry, brandy, wheat bread; used in candy, baked goods, ice cream.

213. Hexanal
FDA-approved food additive; FEMA GRAS; found in apples, bananas, beef, berries, apricot, artichoke, asparagus, avocado, barley, beer, blackberry, blueberry, others; used in frozen dairy goods, baked goods, meat.

214. Hexanoic acid
FDA-approved food additive; FEMA GRAS; found in apple, beef, beer, apricot, banana, barley, blackberry, blue cheese, blueberry, bread, wheat; used in condiment relish, ice cream, candy.

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215. 2-Hexen-1-ol
FDA-approved food additive; FEMA GRAS; found in apples, apricots, bananas, beer, Swiss cheese, peaches; used in nonalcoholic beverages, candy, baked goods, gelatins & puddings.

216. 3-Hexen-1-ol
FDA-approved food additive; FEMA GRAS; found in apple, banana, bean, celery, grape, apricot, cantaloupe, pineapple, honeydew melon; used in chewing gum, ice cream, candy, baked goods.

217. cis-3-Hexen-1-yl acetate
FEMA GRAS; found in apple juice, apricot, artichoke, asparagus, avocado, banana, beans, beef, beer, blackberry, blueberry, dried bonito, grape brandy, wheat bread; used in ices, candy, baked goods.

218. Hexen-2-al
FDA-approved food additive; FEMA GRAS; found in apple, banana, raspberry, strawberry, beer, chicken, fat, grape, guava, hops, peach, pork, black and green tea; used in frozen dairy goods, baked goods, candy.

219. 3-Hexenoic acid
FEMA GRAS; found in apples, bananas, beer, apple juice, artichoke, beans, beed, blackberry, blueberry; used in beverages, candy, baked goods, gelatins & puddings, frozen desserts.

220. trans-2-Hexenoic acid
FEMA GRAS; found in banana, pork fat, raspberry, black tea; used in nonalcoholic beverages, ice cream, ices, dairy products, candy, gelatins & puddings, chewing gum.

221. cis-3-Hexenyl formate
FEMA GRAS; found in tea, cognac; used in baked goods, chewing gum, preserves and spreads.

222. Hexyl acetate
FDA-approved food additive; FEMA GRAS; found in apples, bananas, beer, apricot, beef, black currants, blackberry, blueberry, brandy, cantaloupe, capers, coffee; used in candy, baked goods, meat products.

223. Hexyl alcohol
FDA-approved food additive; FEMA GRAS; found in apple, banana, beef, chicken, coffee, pineapple, potato; used in baked goods, gelatins & puddings, dairy products.
224. Hexyl phenylacetate
FDA-approved food additive; FEMA GRAS; found in grape, tea, scotch, spearmint, black and green tea; used in frozen dairy products, candy, baked goods.

225. High Fructose Corn Syrup
FDA GRAS; found in food sugar sources; used in baked goods, candy, breakfast cereals.

226. Honey
Common food item; used in condiments, sauces, deli meats and salad dressings.

227. Hydrolyzed soy protein
Found in plants; used in baked goods, milk and dairy products, meat products, baby formulas, soups, condiments and relishes.

228. 4-Hydroxy-2,5-dimethyl-3(2H)-furanone
FEMA GRAS; found in beef, maple syrup, cassia oil; used in frozen dairy products, baked goods, candy.

229. 2-Hydroxy-3,5,5-trimethyl-2-cyclohexenone
FEMA GRAS; found in rose oil, gardenia; used in frozen dairy goods, candy.

230. 4-Hydroxy-3-pentenoic acid lactone
FEMA GRAS; found in bread, grapes, soy beans; used in ice cream, ices, baked goods, gelatins & puddings, meat, meat sauces, soups, milk & dairy products, cereals.

231. 4-Hydroxybutanoic acid lactone
FEMA GRAS; found in apricot, bread, coffee, cocoa, mushroom, onion, wine; used in dairy products, breakfast cereals, meat products.

232. Hydroxycitronellal
FDA-approved food additive; FEMA GRAS; found in beef, mushroom, nectarine, peach; used in candy, ice cream, baked goods.

233. 6-Hydroxydihydrotheaspirane
FEMA GRAS; found in black tea; used in nonalcoholic beverages, ice cream, candy, gelatins & puddings.

234. 4-(para-Hydroxyphenyl)-2-butanone
FDA-approved food additive; FEMA GRAS; found in almond, beef, coffee, grape, guava, hazelnut, pineapple, popcorn, raspberry, soy sauce, strawberry; used in chewing gum, ice cream, baked goods.

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235. Hydroxypropyl cellulose
FDA-approved food additive; used in food as an emulsifier, film former, protective colloid, stabilizer, suspending agent or thickener.

236. Immortelle absolute and extract
FDA GRAS; FEMA GRAS; used in baked goods, candy, gelatins & puddings, chewing gum, frozen dairy products.

237. Invert sugar
FDA GRAS; found in food sugar sources; used in baked goods, candy, breakfast cereals.

238. alpha-Ionone
FDA-approved food additive; FEMA GRAS; found in raspberry, almond, banana, blackberry, grape brandy, raspberry brandy, capers, carrots, celery, cherry, grapefruit juice, kumazasa, mango ginger, peach, peas, plum; used in chewing gum, ice cream, baked goods.

239. beta-Ionone
FDA-approved food additive; FEMA GRAS; found in carrot, almonds, apricot, beer, blackberry, brandy, broccoli, capers, cherry, endive; used in candy, baked goods, ice cream.

240. Isoamyl acetate
FDA-approved food additive; FEMA GRAS; found in apple, banana, beer, apricot, blackberry, blackberry brandy, wheat bread, butter; used in chewing gum, ice cream, baked goods.

241. Isoamyl benzoate
FDA-approved food additive; FEMA GRAS; found in beer, cherries, cocoa, papaya; used in baked goods, candy, gelatins & puddings.

242. Isoamyl butyrate
FDA-approved food additive; FEMA GRAS; found in banana, blue cheese, grape, apple, apricot, beer, apple brandy, grape brandy, guava, honey, mango; used in non-alcoholic beverages, ice cream, baked goods, chewing gum.

243. Isoamyl cinnamate
FDA-approved food additive; FEMA GRAS; found in wine, cinnamon, styrax; used in baked goods, candy, gelatins & puddings.
244. Isoamyl formate
FDA-approved food additive; FEMA GRAS; found in apple, beer, chicken, honey, eggs, rum, strawberries, tea, vinegar, grape brandy, whiskey, wine; used in baked goods, candy.

245. Isoamyl hexanoate
FDA-approved food additive; FEMA GRAS; found in apple, apricot, banana, grape-fruit juice, plums, strawberries, beer, grape brandy, plum brandy, rum, sherry; used in candy and gum.

246. Isoamyl isovalerate
FDA-approved food additive; FEMA GRAS; found in banana, tomato, beer, sherry, spearmint, scotch; used in frozen dairy goods, candy.

247. Isoamyl phenylacetate
FDA-approved food additive; FEMA GRAS; found in peppermint oil; used in baked goods, candy, chewing gum.

248. Isobutyl acetate
FDA-approved food additive; FEMA GRAS; found in apples, bananas, cantaloupe, cocoa, figs, honeydew melon, beer, grape, guava, mango, melon; used in chewing gum, gelatins, and puddings.

249. Isobutyl alcohol
FDA-approved food additive; FEMA GRAS; found in beef, blackberry, apple, apricot, banana, barley, brandy; used in gelatin and puddings, candy, baked goods.

250. Isobutyl cinnamate
FDA-approved food additive; FEMA GRAS; found in coffee, tomato, mullein leaves; used in candy, ice cream, baked goods.

251. Isobutyl phenylacetate
FDA-approved food additive; FEMA GRAS; found in cocoa; used in baked goods, ice cream, candy.

252. 2-Isobutyl-3-methoxypyrazine
FEMA GRAS; found in bean, coffee, pea, pepper, potato, spinach, grape; used in nonalcoholic beverages, ice cream, dairy products.

253. alpha-Isobutylphenethyl alcohol
FDA-approved food additive; FEMA GRAS; used in nonalcoholic beverages, alcoholic beverages, ice cream, ices, candy, gelatins, and puddings, baked goods.
254. Isobutyraldehyde  
FDA-approved food additive; FEMA GRAS; found in apple, banana, barley, beans, beef, beer, blue cheese, brandy, bread, wheat, butter; used in gelatins & puddings, candy, frozen dairy products.

255. Isobutyric acid  
FDA-approved food additive; FEMA GRAS; found in apple, beef, beer, celery, banana, blue cheese, grape brandy, wheat bread, cashews, apples, cheddar cheese; used in baked goods, candy, gelatins & puddings.

256. 2-Isopropylphenol  
FEMA GRAS; found in Japanese whiskey; used in meat, soups, condiments.

257. Isovaleric acid  
FDA-approved food additive; FEMA GRAS; found in apple, beer, banana, blue cheese, grape brandy, wheat bread, heated butter, capers, cashews, apples, cheddar cheese; used in frozen dairy goods, candy, cheese.

258. Jasmine absolute  
FDA GRAS; FEMA GRAS; found in jasmine flowers; used in baked goods, chewing gum, candy.

259. Kola nut extract  
FDA GRAS; FEMA GRAS; found in kola nut; used in gelatins & puddings, ice cream, candy.

260. Labdanum oils, absolute  
FDA-approved food additive; FEMA GRAS; used in baked goods, frozen dairy products, gelatins & puddings.

261. Lactic acid  
FDA GRAS; FEMA GRAS; found in apple juice, beef, beer, bread, cocoa, coffee, wheat bread, cherry, grape, guava, mango milk, papaya, dry salami, sherry, tomato; used in cheese, candy, chewing gum, baked goods.

262. Lauric acid  
FDA-approved food additive; FEMA GRAS; found in apple, beer, blue cheese, banana, beef, blackberry, brandy, wheat bread, butter, heated butter, cantaloupe, cashew nuts; used in baked goods, candy, ice cream.

263. Lavandin oil  
FDA GRAS; FEMA GRAS; found in lavender plant; used in baked goods, candy, chewing gum.
264. Lemon oil
FDA GRAS; FEMA GRAS; found in lemons; used in candy, breakfast cereals, frozen dairy products.

265. Lemongrass oil
FDA GRAS; FEMA GRAS; found in lemongrass; used in chewing gum, ice cream, baked goods.

266. Levulinic acid
FDA-approved food additive; FEMA GRAS; found in wheat bread, papaya; used in reconstituted vegetables, ice cream, baked goods.

267. Licorice root, fluid extract and powder
FDA GRAS; FEMA GRAS; found in glycyrrhizia; used in candy, baked goods, meat products.

268. Lime oil and lime oil terpeneless
FDA GRAS; FEMA GRAS; found in lime; used in frozen dairy goods, candy.

269. Linalool
FDA GRAS; FEMA GRAS; found in banana, beer, blackberry, beans, blueberry, apple, apricot, arctice bramble, artichoke, grape brandy, plum brandy; used in meat products.

270. Linalool oxide
FDA-approved food additive; FEMA GRAS; found in oranges, apricot, coffee; used in ice cream, baked goods, candy.

271. Linalyl acetate
FDA GRAS; FEMA GRAS; found in bergamot, clary sage, lemon oil, pepper, tomato, lavender; used in baked goods, dairy products, candy.

272. L-Lysine
FDA-approved food additive; amino acid, natural constituent of plant and animal proteins; used in meat products, breakfast cereals.

273. Lovage oil and extract
FDA-approved food additive; FEMA GRAS; found in levisticum; used in sweet sauce, alcoholic beverages, ice cream.

274. Mace powder and oil
FDA GRAS; FEMA GRAS; found in mace; used in alcoholic beverages, candy, frozen dairy products.
275. l-Malic acid
FDA GRAS; FEMA GRAS; found in celery, cocoa, orange juice, grape brandy, sour cherry, gin, grapefruit juice, honey, hops, kiwi fruit, mango, mushroom; used in frozen dairy goods, candy, baked goods.

276. Malt and malt extract
FDA GRAS; found in barley; used in beer, frozen dairy products, baked goods.

277. Maltodextrin
FDA GRAS; used in baked goods, candy, frozen dairy products.

278. Maltol
FDA-approved food additive; FEMA GRAS; found in barley, cocoa, coffee, beef, wheat bread, butter, hazelnut, licorice, malt, milk, peanut; used in frozen dairy goods, jellies, baked goods.

279. Mandarin and tangerine oil
FDA GRAS; FEMA GRAS; found in tangerines, mandarin oranges; used in candy, frozen dairy products.

280. Maple syrup
Common food item; used in sauces, dressings, frostings, icings, candies and desserts.

281. Mate leaf, absolute, extract and oil
FDA GRAS; found in mate leaves; used in flour, meat, poultry.

282. para-Mentha-8-thiol-3-one
FEMA GRAS; used in frozen dairy, soft candy, gelatins & puddings, baked goods.

283. Menthol and L-Menthol
FDA-approved food additive; FEMA GRAS; found in peppermint plant, honey, mint, rum, cocoa, eggs, guava, raspberry, rice, spearmint; used in candy, mouthwash.

284. Menthone
FDA-approved food additive; FEMA GRAS; found in celery, clams, cocoa, peppermint, raspberries, rice, spearmint; used in baked goods, candy.

285. Menthol Acetate
FEMA GRAS; found in peppermint oil, orange juice, raspberries; used in baked goods, frozen dairy, soft candy, gelatins & pudding.
286. Menthyl Isovalerate
FDA-approved food additive; FEMA GRAS; found in nutmeg, peppermint oil; used in alcoholic beverages, baked goods, chewing gum, frozen dairy goods, gelatins & puddings, soft candy.

287. 2-, 5-, or 6-Methoxy-3-methylpyrazine (mixture of isomers)
FEMA GRAS; found in beef, coffee, bread; used in baked goods, cereals, candy, dairy products.

288. 2-Methoxy-4-methylphenol
FDA-approved food additive; FEMA GRAS; found in cocoa, sausage, banana, beer, coffee; used in baked goods, meat products.

289. 2-Methoxy-4-vinylphenol
FDA-approved food additive; FEMA GRAS; found in bean, coffee, sherry, whiskey, banana, beer, cocoa, cured ham, malt; used in meat products, ice cream, baked goods.

290. para-Methoxybenzaldehyde
FDA-approved food additive; FEMA GRAS; found in coffee, tea, tomato, beer, krill; used in baked goods, candy, dairy products.

291. 1-(para-Methoxyphenyl)-1-penten-3-one
FDA-approved food additive; FEMA GRAS; found in jasmine, ylang ylang; used in soft candy, sweet sauce, baked goods.

292. 1-(para-Methoxyphenyl)-2-propanone
FDA-approved food additive; FEMA GRAS; found in chervil; used in baked goods, frozen dairy products, soft candy, gelatins & puddings.

293. Methoxypyrazine
FEMA GRAS; found in beef, cocoa; used in meat products, soups, gravies, baked goods.

294. Methyl 2-furoate
FEMA GRAS; found in almond, cocoa, coffee, filbert, peanut, wine; used in nonalcoholic beverages, ice cream, candy, baked goods.

295. Methyl 2-octynoate
FDA-approved food additive; FEMA GRAS; used in baked goods, frozen dairy products, gelatins & puddings.
296. Methyl 2-pyrrolyl ketone  
FEMA GRAS; found in apple juice, cocoa, coffee, onion, peanut; used in baked goods, candy, gelatins & puddings, meat products.

297. para-Methyl anisate  
FDA-approved food additive; FEMA GRAS; used in nonalcoholic beverages, frozen dairy products, baked goods, soft candy, gelatins & puddings.

298. Methyl anthranilate  
FDA GRAS; FEMA GRAS; found in cocoa, grape, tea, wine, coffee, strawberry; used in baked goods, candy, frozen dairy products.

299. Methyl benzoate  
FDA-approved food additive; FEMA GRAS; found in banana, cherry, coffee, brandy, butter, cashew, apple; used in chewing gum, ice cream, candy.

300. Methyl cinnamate  
FDA-approved food additive; FEMA GRAS; found in guava, strawberry, cranberry, pineapple, plum; used in baked goods, candy, gelatins & puddings.

301. Methyl dihydrojasmonate  
FEMA GRAS; used in nonalcoholic beverages, ice cream, ices, baked goods, candy.

302. Methyl ester of rosin, partially hydrogenated  
FDA-approved food additive; used in baked goods, candy.

303. Methyl isovalerate  
FDA-approved food additive; FEMA GRAS; found in apple, peach, pineapple, banana, blackberry, parmesan cheese, coffee, honey, nectarine, olives, peas, strawberries; used in candy, baked goods.

304. Methyl linoleate (48%) methyl linolenate (52%) mixture  
FEMA GRAS; found in banana, grape, grapefruit juice, melon, strawberries; used in ice cream, baked goods, candy.

305. Methyl phenylacetate  
FDA-approved food additive; FEMA GRAS; found in coffee, cocoa; used in candy, syrups, baked goods.

306. Methyl salicylate  
FEMA GRAS; found in blackberry, broccoli, butter, cherry cake, coffee; used in candy, ice cream, syrups.
307. Methyl sulfide
FDA-approved food additive; FEMA GRAS; found in asparagus, bean, beef, beer, white bread, brussel sprouts, butter, cabbage, carrot, cauliflower, broccoli; used in meat products, candy, ice cream.

308. 5-Methyl-2-phenyl-2-hexenal
FEMA GRAS; found in Romano cheese, potato chips; used in soft candy.

309. 6-Methyl-3,5-heptadien-2-one
FDA-approved food additive; FEMA GRAS; found in almonds, asparagus, beef, beer, wheat bread, cashew nuts, chicken; used in snack foods.

310. 1-Methyl-3-methoxy-4-isopropylbenzene
FEMA GRAS; found in tangerine peel, thyme; used in nonalcoholic beverages, baked goods, meat, candy, condiments.

311. Methyl-3-methylthiopropionate
FDA-approved food additive; FEMA GRAS; found in cantaloupe, pineapple; used in candy, baked goods, beverages, syrups.

312. 2-Methyl-4-phenylbutyraldehyde
FEMA GRAS; used in nonalcoholic beverages, ice cream, candy, gelatins & puddings.

313. 6-Methyl-5-hepten-2-one
FDA-approved food additive; FEMA GRAS; found in guava, mango, potato, rum; used in gravies, candy, baked goods.

314. 4-Methyl-5-thiazoleethanol
FEMA GRAS; found in cocoa, malt, roasted peanuts; used in meat products.

315. 4’-Methylacetophenone
FDA-approved food additive; FEMA GRAS; found in hop oil, cocoa powder, black currant; used in chewing gum.

316. Methyl-alpha-ionone
FDA-approved food additive; FEMA GRAS; used in baked goods.

317. para-Methyl-anisole
FDA-approved food additive; FEMA GRAS; found in cocoa, malt, peanut, pork, potato chips, sesame seeds; used in baked goods.
318. 2-Methylbutyraldehyde  
FDA-approved food additive; FEMA GRAS; found in beef, apple, cheddar cheese, coffee, cranberry, eggs, fish, lettuce, olive, onion, peas, tomato; used in gelatins & puddings.

319. 3-Methylbutyraldehyde  
FDA-approved food additive; FEMA GRAS; found in apple, banana, bread, tomato, rice, blackberry; used in baked goods.

320. 2-Methylbutyric acid  
FDA-approved food additive; FEMA GRAS; found in apple, apricot, avocado, beef, beer, blackberry, brandy, butter, cantaloupe, carrots; used in cheese, ice cream, candy.

321. alpha-Methylcinnamaldehyde  
FDA-approved food additive; FEMA GRAS; found in blackberry, cauliflower, cherry, cocoa, endive, guava, honey, peach; used in candy.

322. Methylcyclopentenolone  
FDA-approved food additive; FEMA GRAS; found in carambola (starfruit), cheese, tomato; used in breakfast cereals, baked goods, candy.

323. 2-Methylheptanoic acid  
FDA-approved food additive; FEMA GRAS; found in gardenia flower oil, almonds, cocoa, coffee, soy sauce, onions; used in baked goods, ice cream, candy.

324. 2-Methylhexanoic acid  
FEMA GRAS; found in apple, avocado, banana, barley, beans, beef, beer, blackberry, blue cheese, wheat bread, butter; used in baked goods, candy, gelatins & puddings.

325. 3-Methylpentanoic acid  
FEMA GRAS; found in apple, apricot, beer, blackberry, blueberry, wheat bread, Romano cheese, chicken; used in baked goods.

326. (Methylthio)methylpyrazine (mixture of isomers)  
FEMA GRAS; used in baked goods, candy.

327. 2-Methylpyrazine  
FEMA GRAS; found in peppermint oil, tomato, popcorn; used in milk products, baked goods, candy.
328. 5-Methylquinoxaline
FEMA GRAS; found in roasted almonds, coffee; used in frozen dairy, beverages, candy, gelatin.

329. 3-(Methylthio)propionaldehyde
FEMA GRAS; found in bean, bread, cheese, cocoa bean, roasted nuts, milk, soy sauce, tomato; used in ice cream, ices, baked goods, dairy products, fats/oils.

330. Methyl-trans-2-butenoic acid
FEMA GRAS; found in celery oil, orange juice crystals, coffee, strawberry; used in baked goods, meat products, soups.

331. 2-Methylvaleric acid
FDA-approved food additive; FEMA GRAS; found in almond, barley, wheat bread, cocoa, coffee, hazelnut, licorice, malt, peanut, soy sauce, lamb/mutton; used in frozen dairy goods, candy.

332. Mimosa absolute
FDA-approved food additive; FEMA GRAS; found in mimosa flowers; used in frozen dairy products.

333. Molasses, blackstrap, sugarcane and extract
FDA GRAS; found in refined sugars; common food item.

334. Mountain maple solid extract
FDA-approved food additive; FEMA GRAS; found in mountain-maple-tree sap; used in baked goods.

335. Myristic acid
FDA-approved food additive; FEMA GRAS; found in apple, banana, beef, beer, blackberry, brandy grape, butter, cantaloupe, cashew nuts, cheese (blue, cheddar); used in nonalcoholic beverages, candy, baked goods.

336. Myrrh oil and absolute
FDA-approved food additive; FEMA GRAS; found in myrrh; used in alcoholic beverages.

337. Nerol
FDA-approved food additive; FEMA GRAS; found in apricot, beer, blackberry, blueberry, brandy grape, cranberry, gin, grape, grapefruit juice, honey, hops, wine; used in frozen dairy goods.
338. Neroli bigarade oil
FDA GRAS; FEMA GRAS; found in oranges; used in baked goods, candy.

339. Nerolidol
FDA-approved food additive; FEMA GRAS; found in grapefruit, hops, lime, grapefruit oil; used in nonalcoholic beverages, ice cream, ices, candy, baked goods.

340. Nona-2-trans,6-cis-dienal
FEMA GRAS; found in apple, banana, beef, beer, blue cheese, cheddar cheese, brandy plum; used in frozen dairy goods.

341. 2,6-Nonadien-1-ol
FDA-approved food additive; FEMA GRAS; found in cucumber, frozen peas, whole soybean, tomato; used in baked goods, candy, gelatins & puddings, gravies.

342. gamma-Nonalactone
FDA-approved food additive; FEMA GRAS; found in beer, wheat bread, capers, cherry, chicken, clam; used in candy, baked goods, ice cream.

343. Nonanal
FDA-approved food additive; FEMA GRAS; found in apricot, asparagus, beef, blackberry, wheat bread, cantaloupe, cocoa; used in baked goods.

344. Nonanoic acid
FDA-approved food additive; FEMA GRAS; found in apple, apricot, artichoke, avocado, banana, beef, beer, wheat bread; used in baked goods, candy, meat products.

345. 2-Nonanone
FDA-approved food additive; FEMA GRAS; found in strawberry; used in dairy products, condiments.

346. trans-2-Nonen-1-ol
FEMA GRAS; found in asparagus, brandy grape, cucumber, melon, nectarine, plum, prickly pear, wine; used in baked goods.

347. Nonyl alcohol
FDA-approved food additive; FEMA GRAS; found in apples, bananas, and oranges; used in baked goods, soft candy, frozen dairy, and chewing gum.

348. Nutmeg powder and oil
FDA GRAS; FEMA GRAS; found in nutmeg; used in condiments, baked goods.
349. Oak moss absolute  
FDA-approved food additive; FEMA GRAS; found in essential oil of lichen, oak moss; used in meat products, candy, ice cream.

350. 9,12-Octadecadienoic acid (48%) and 9,12,15-octadecatrienoic acid (52%) (mixture) FEMA GRAS; found in potato, tomato, apple, beer, cheese, country ham; used in preserves, spreads, candy, gelatins & puddings.

351. delta-Octalactone  
FEMA GRAS; found in apricot, beef, blackberry, butter, cheese (blue, cheddar, parmesan), cranberry, cream, coconut; used in candy, margarine, baked goods.

352. gamma-Octalactone  
FDA-approved food additive; FEMA GRAS; found in apricot, asparagus, beef, beer, blackberry, blue cheese, brandy grape, cantaloupe, cherry, chicken, cranberry; used in baked goods, candy, ice cream.

353. Octanal  
FDA-approved food additive; FEMA GRAS; found in apple, apricot, artichoke, avocado, beef, beer, blackberry, brandy, wheat bread, butter; used in frozen dairy products, beverages, baked goods, candy.

354. Octanoic acid  
FDA GRAS; FEMA GRAS; found in apple, banana, beef, blackberry, plum brandy, wheat butter, beer, blue cheese; used in snack foods, baked goods, candy.

355. 2-Octanone  
FDA-approved food additive; FEMA GRAS; found in apple, banana, beef, cheese, coffee, cocoa, milk, peanut, tea, wine; used in baked goods, candy, gelatins & puddings, dairy products.

356. 1-Octen-3-ol  
FDA-approved food additive; FEMA GRAS; found in mushroom, peppermint, spearmint; used in processed vegetables.

357. 2, trans-Octenal  
FEMA GRAS; found in asparagus, beer, banana, beans, blue cheese, butter, cantaloupe, capers, chicken; used in snack foods, baked goods, dairy products.

358. Octyl isobutyrate  
FDA-approved food additive; FEMA GRAS; found in hops, plum; used in baked goods, beverages, ice cream, candy.
359. Oleic acid
FDA-approved food additive; FEMA GRAS; found in apple, banana, grape, ginger, potato, strawberry, tomato; used in condiment relish, citrus fruit, yeast, sugar beets.

360. Olibanum oil
FDA-approved food additive; FEMA GRAS; found in gum-resin exudate; used in nonalcoholic beverages, ice cream, ices, candy, baked goods.

361. Opoponax oil
FDA-approved food additive; found in opoponax, opoponas; natural flavor; used in alcoholic beverages.

362. Orange leaf absolute
FDA GRAS; FEMA GRAS; found in oranges; used in nonalcoholic beverages, ice cream, ices, candy, baked goods.

363. Orange Oil (sweet orange oils terpeneless, sour/ bitter orange oils)
FDA GRAS; FEMA GRAS; found in oranges; used in nonalcoholic beverages, ice cream, ices, baked goods, condiments, candy, gelatins & puddings, chewing gum.

364. Orange oil and extract (Sweet, distilled orange oils and terpeneless)
FDA GRAS; FEMA GRAS; found in oranges; used in nonalcoholic beverages, ice cream, ices, baked goods, condiments, candy, gelatins & puddings, chewing gum.

365. Origanum oil
FDA GRAS; FEMA GRAS; found in origanum flowers; used in soups.

366. Orris concrete oil and root extract
FDA-approved food additive; FEMA GRAS; found in orris roots; used in gelatins & puddings, alcoholic beverages.

367. Palmarosa oil
FDA GRAS; FEMA GRAS; found in geranium; used in baked goods.

368. Palmitic acid
FDA-approved food additive; FEMA GRAS; found in apple, beer, celery, cheddar cheese, milk, potato, tomato; used in meat products, baked goods.

369. Parsley seed oil
FDA GRAS; FEMA GRAS; found in parsley; used in soups.
370. Patchouly oil and absolute
FDA-approved food additive; FEMA GRAS; found in dried leaves of Pogostemon cablin Benth; used in nonalcoholic beverages, ice cream, ices, baked goods, candy, chewing gum.

371. Pectin
FDA GRAS; found in plants; used in frozen desserts, canned fruits, jellies, preserves.

372. omega-Pentadecalactone
FDA-approved food additive; FEMA GRAS; used in baked goods, ice cream, candy.

373. 2,3-Pentanedione
FDA-approved food additive; FEMA GRAS; found in nuts, beef, beer, bread, chicken, cocoa, coffee, tomato, yogurt; used in baked goods, candy, gelatins & puddings.

374. 2-Pentanone
FDA-approved food additive; FEMA GRAS; found in apple juice, banana, beef, butter, oil, cheese, chicken, grape, ham, honey, peanut; used in nonalcoholic beverages, ice cream, ices, candy, baked goods.

375. Pepper oil, black
FDA GRAS; FEMA GRAS; found in pepper corns; used in condiments, ice cream, baked goods.

376. Peppermint oil and peppermint oil terpeneless
FDA GRAS; FEMA GRAS; found in peppermint; used in chewing gum, meat products, ice cream, baked goods.

377. Petitgrain absolute, oil, and terpeneless oil
FDA GRAS; FEMA GRAS; found in bitter orange tree, leaves, twigs, oranges; used in baked goods, condiments, candy.

378. alpha-Phellandrene
FDA-approved food additive; FEMA GRAS; found in apple, gin, hops, mango, nectarine, papaya, paprika, parsley, beans, carrots; used in milk products, baked goods, candy.

379. 2-Phenethyl acetate
FDA-approved food additive; FEMA GRAS; found in apple, banana, beer, brandy, raspberry, wheat bread, butter, cantaloupe; used in candy, ice cream, baked goods.
380. Phenethyl alcohol
FDA-approved food additive; FEMA GRAS; found in apple juice, banana, beef, beer, blackberry, blueberry, apple, apricot, asparagus; used in chewing gum, ice cream, baked goods.

381. Phenethyl butyrate
FDA-approved food additive; FEMA GRAS; found in beer, banana, apple brandy, grape, strawberry, wine; used in baked goods, candy, ice cream.

382. Phenethyl cinnamate
FDA-approved food additive; FEMA GRAS; used in nonalcoholic beverages, alcoholic beverages, ice cream, ices, baked goods, candy, gelatins & puddings.

383. Phenethyl isobutyrate
FDA-approved food additive; FEMA GRAS; found in beer, brandy, grape, olive, rum; used in chewing gum, baked goods, candy.

384. Phenethyl isovalerate
FDA-approved food additive; FEMA GRAS; found in peppermint, spearmint, banana, beer, brandy, grape; used in chewing gum, candy, frozen dairy products.

385. Phenethyl phenylacetate
FDA-approved food additive; FEMA GRAS; used in baked goods, candy, cheese.

386. 3-Phenyl-1-propanol
FDA-approved food additive; FEMA GRAS; found in cinnamon, honey, tea; used in frozen dairy, baked goods, soft candy, gelatins & puddings, chewing gum.

387. 2-Phenyl-2-butenal
FEMA GRAS; found in beer, chicken, tomato, almonds, asparagus, cocoa, tea, hazelnuts; used in gelatin and puddings, candy, ice cream.

388. 4-Phenyl-3-buten-2-one
FDA-approved food additive; FEMA GRAS; used in nonalcoholic beverages, frozen dairy, baked goods, soft candy; gelatins & puddings.

389. Phenylacetaldehyde
FDA-approved food additive; FEMA GRAS; found in chicken, strawberry; used in baked goods, ice cream, candy.

390. Phenylacetic acid
FDA-approved food additive; FEMA GRAS; found in almond, asparagus, cocoa, coffee, mushroom, peanut, pork, potato chips, sesame seed, tea; used in sweet sauce, baked goods, candy.
391. l-Phenylalanine
FDA-approved food additive; FEMA GRAS; found in meats, eggs, breads, cereals, milk, cheese, fish, corn, beans, potatoes, asparagus, peas; used in frozen dairy, baked goods, candy, condiments, meat products.

392. 3-Phenylpropionaldehyde
FDA-approved food additive; FEMA GRAS; found in beer, chicken, tomato; used in baked goods, candy, condiments.

393. 3-Phenylpropionic acid
FDA-approved food additive; FEMA GRAS; found in beef, beer, blue cheese, grape brandy, wheat bread, broccoli, apricot, artichoke, asparagus, banana, beans; used in baked goods, candy.

394. 3-Phenylpropyl acetate
FDA-approved food additive; FEMA GRAS; used in nonalcoholic beverages, ice cream, baked goods, candy, chewing gum, condiments.

395. Phosphoric acid
FDA GRAS; FEMA GRAS; component of living organisms; used in cheese, baked goods, candy, gelatins & puddings, meat products.

396. Pine needle oil
FDA-approved food additive; FEMA GRAS; found in pine tree needles; used in candy, baked goods, ice cream.

397. Pine oil, scotch
FDA-approved food additive; FEMA GRAS; found in pine trees; used in candy, baked goods, nonalcoholic beverages.

398. Pineapple juice concentrate
Found in pineapple; defined as a fruit juice under FDA Standards of Identity.

399. alpha-Pinene
FDA-approved food additive; FEMA GRAS; found in apple, blueberry, plum brandy, carrots, celery, cheddar cheese, chicken; used in condiments, candy, meat products.

400. beta-Pinene
FDA-approved food additive; FEMA GRAS; found in apricot, plum brandy, butter, cantaloupe, carrots, celery, cheddar cheese, cocoa, cranberry; used in baked goods, candy, meat products.

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401. Piperonal
FDA GRAS; FEMA GRAS; found in cantaloupe, capers, melon, sherry; used in candy, baked goods.

402. Pipsissewa leaf extract
FDA GRAS; FEMA GRAS; used in nonalcoholic beverages, candy.

403. Plum juice, concentrate and extract
Found in plums.

403. Potassium carbonate
FDA GRAS; used in wine and juice prior to or during fermentation. Potassium carbonate is a flavor enhancer that facilitates the release of natural tobacco aroma and flavor.

405. Potassium sorbate
FDA GRAS; FEMA GRAS; found in mountain-ash-tree berries; used in cheese.

406. l-Proline
FDA-approved food additive; FEMA GRAS; essential amino acid, found in proteins, plants and animals; used in breakfast cereals, baked goods.

407. Propenylguaethol
FDA-approved food additive; FEMA GRAS; used in sweet sauce, baked goods, candy.

408. Propionic acid
FDA GRAS; FEMA GRAS; found in apple, apple juice, beef, beer, blueberry juice, bread, cheese, coffee, grape juice, maple syrup, orange juice, raspberry, rum; used in fruit, candy, gelatins & puddings, dairy products.

409. Propyl acetate
FDA-approved food additive; FEMA GRAS; found in banana, grape, apple juice, beer, wheat bread, cantaloupe, capers, cocoa, guava, honey, fig, honeydew, melon, heated corn oil; used in beverages, ice cream, baked goods.

410. Propyl para-hydroxybenzoate
FDA-approved food additive; FEMA GRAS; found in licorice; used in processed vegetables.

411. Propylene glycol
FDA GRAS; FEMA GRAS; found in sesame seed, mushroom; used in confection frostings, cheese, candy.
412. 3-Propylidenephthalide
FDA-approved food additive; FEMA GRAS; found in lovage; used in frozen dairy products, baked goods, candy.

413. Prune juice and concentrate
Common food item; found in prunes; used in beverages, bread and other bakery products.

414. Pyridine
FDA-approved food additive; FEMA GRAS; found in bean, bread, cheese, cocoa, coffee, fish, onion, peanut, pecan, popcorn, potato, rum, tea, tomato; used in ice cream, baked goods, condiments, meat products.

415. Pyroligneous acid and extract
FDA-approved food additive; FEMA GRAS; found in birch tree; used in alcoholic beverages, baked goods, meat products.

416. Pyruvic acid
FDA-approved food additive; FEMA GRAS; found in beer, wheat bread, celery, asparagus, milk, onion, sake; used in frozen dairy products, baked goods, candy.

417. Raisin juice concentrate and extract
Common food item; found in raisins; used in baked goods.

418. L-Rhamnose
FEMA GRAS; found in food-sugar sources; used in baked goods, candy, breakfast cereals.

419. Rhodinol
FDA-approved food additive; FEMA GRAS; found in geranium flowers; used in chewing gum, baked goods, ice cream.

420. Rose absolute and oil
FDA GRAS; FEMA GRAS; found in roses; used in chewing gum, ice cream, baked goods.

421. Rosemary oil and extract
FDA GRAS; FEMA GRAS; found in rosemary; used in condiments, meat products, baked goods.

422. Rum and rum extract
Alcoholic beverages; natural flavor.
423. Rum ether
FDA-approved food additive; FEMA GRAS; found in rum; used in nonalcoholic beverages, alcoholic beverages, frozen dairy products, baked goods, candy.

424. Rye Extract
Found in rye plant; used in rye bread.

425. Sage oil, oleoresin, and powder
FDA GRAS; FEMA GRAS; found in sage; used in baked goods, condiments, meat products.

426. Salicylaldehyde
FDA-approved food additive; FEMA GRAS; found in beer, butter, chicken, coffee, cranberry, grape, potato, rum, sherry, tea, tomato, whiskey; used in baked goods, condiments, candy.

427. Sandalwood oil, yellow
FDA-approved food additive; FEMA GRAS; found in sandalwood; used in candy, baked goods, ice cream.

428. Sclareolide
FEMA GRAS; found in clary sage; used in milk products, baked goods, candy, meat products, breakfast cereals.

429. Sodium benzoate
FDA GRAS; FEMA GRAS; used in baked goods, margarine, dietary supplements.

430. Sodium bicarbonate
FDA GRAS; natural mineral; main component of baking powder and baking soda.

431. Sodium carbonate
FDA GRAS; used in baked goods, desserts, margarine, poultry. Sodium carbonate is a heat source constituent in cigarettes that primarily heat tobacco.

432. Sodium citrate
FDA GRAS; FEMA GRAS; used in evaporated milk, general purpose food additive.

433. Sorbic acid
FDA GRAS; found in mountain-ash-tree berries (Merck); used in flavorings.

434. d-Sorbitol
FDA GRAS; FEMA GRAS; found in cherry, plum, apple; used in soft candy, chewing gum, frozen dairy desserts.
435. Spearmint oil
FDA GRAS; FEMA GRAS; found in spearmint; used in chewing gum, ice cream, candy, baked goods.

436. Storax and styrax, extract, gum, and oil
FDA-approved food additive; FEMA GRAS; found in storax; used in baked goods, candy, jellies.

437. Sucrose
FDA GRAS; found in food sugar sources; used in baked foods, candy, breakfast cereals.

438. Sucrose octaacetate
FDA-approved food additive; FEMA GRAS; found in ginger ale; used in candy, gelatins & puddings, baked goods.

439. Tagetes oil
FDA-approved food additive; FEMA GRAS; found in marigold flowers; used in condiment relish.

440. l-Tartaric acid, dl-Tartaric acid
FDA GRAS; FEMA GRAS; found in wine grapes; used in fruit juices, baked goods, ice cream.

441. Tea extract
FDA GRAS; natural food extractive.

442. alpha-Terpineol
FDA-approved food additive; FEMA GRAS; found in apple, apple juice, apricot, artichoke, beans, beef, beli, bilberry, blueberry, plum brandy; used in chewing gum, baked goods, ice cream.

443. Terpinolene
FDA-approved food additive; FEMA GRAS; found in thyme, valencia oranges; used in baked foods, ice cream, candy.

444. alpha-Terpiny1 acetate
FDA-approved food additive; FEMA GRAS; found in apricot, beer, blackberry, carrots, celery, cranberry, gin, ginger; used in meat products, baked goods, candy.

445. 5,6,7,8-Tetrahydroquinoxaline
FEMA GRAS; found in beef, wheat bread, cocoa, peanut, pork; used in candy, baked goods, dairy products.
446. 1,5,5,9-Tetramethyl-13-oxatricyclo (8.3.0.0(4,9)) tridecane
FEMA GRAS; found in clary sage oil; used in nonalcoholic beverages, ice cream, ices, baked goods, candy, gelatins & puddings.

447. 2,3,5,6-Tetramethylpyrazine
FEMA GRAS; found in wheat bread, sake, shrimp, beef, beer, coffee, peanut; used in baked goods, dairy products, candy.

448. Thyme oil
FDA GRAS; FEMA GRAS; found in thyme; used in condiments, meats, soups, baked goods.

449. Thymol
FDA-approved food additive; FEMA GRAS; found in blueberry, Romano cheese, papaya, peppermint, pistacia, fruit tea, wine; used in candy, baked goods, ice cream.

450. Tolu balsam gum, resinoid, and extract
FDA-approved food additive; FEMA GRAS; found in balsam tolu; used in baked goods, candy, syrups.

451. Tolualdehydes (ortho,meta,para)
FDA-approved food additive; FEMA GRAS; found in beef, beer, butter, coffee, endive, rum, tea; used in chewing gum, baked goods, ice cream.

452. para-Tolyl 3-methylbutyrate
FEMA GRAS; found in raspberry, coffee, tea, rum; used in baked goods, ice cream, candy.

453. para-Tolyl acetate
FDA-approved food additive; FEMA GRAS; found in cananga, ylang ylang; used in candy, ice cream, baked goods.

454. para-Tolyl isobutyrate
FDA-approved food additive; FEMA GRAS; used in baked goods, ice cream, candy.

455. para-Tolyl phenylacetate
FDA-approved food additive; FEMA GRAS; used in baked goods, candy, cheese, ice cream.

456. Triacetin
FDA GRAS; FEMA GRAS; found in papaya; used in candy, baked goods, ice cream.
457. 2-Tridecanone
FEMA GRAS; found in cheese, coffee, coconut oil, hops; used in ice cream, ices, baked goods, margarine, candy, gelatins & puddings.

458. Triethyl citrate
FDA GRAS; FEMA GRAS; found in red currant; used in chewing gum, baked goods, ice cream.

459. 3,5,5-Trimethyl-1-hexanol
FEMA GRAS; used in baked goods, condiments, pickles.

460. 4-(2,6,6-Trimethylcyclohex-1-enyl)but-2-en-4-one
FEMA GRAS; found in rose, rum, brandy, tea; used in baked goods, candy, chewing gum.

461. 2,6,6-Trimethylcyclohex-2-ene-1,4-dione
FEMA GRAS; found in apricot, beer, blackberry, grape, hops, kiwi fruit; used in soft candy.

462. 4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-2-en-4-one
FEMA GRAS; found in apples, black tea, Riesling wine; used in beverages, frozen dessert, baked goods, candy, gelatins & puddings, preserves, condiments.

463. 2,2,6-Trimethylcyclohexanone
FEMA GRAS; found in bilberry, passion fruit, tea; used in nonalcoholic beverages, frozen dessert, confectionery, ice cream, ices, candy.

464. 2,3,5-Trimethylpyrazine
FEMA GRAS; found in barley, almond, asparagus, beef, wheat bread, chicken, cocoa, coffee; used in baked goods, candy, dairy products, cereals.

465. delta-Undecalactone
FEMA GRAS; found in beef, butter, coconut, milk; used in baked goods, candy, dairy products, cereals.

466. gamma-Undecalactone
FDA-approved food additive; FEMA GRAS; found in apple, apple juice, apricot, heated butter, peach, plum, pork, rice; used in chewing gum, candy, ice cream, baked goods.

467. 2-Undecanone
FDA-approved food additive; FEMA GRAS; found in coconut oil, banana, beer, beef, cheese, cocoa, coffee, wine, milk, mushroom, peanut, strawberry; used in nonalcoholic beverages, ice cream, baked goods, candy, dairy products.
468. Urea
FDA GRAS; found in mushrooms; used in baked goods.

469. Valeraldehyde
FDA-approved food additive; FEMA GRAS; found in apple, apple juice, apricot, artichoke, asparagus, avocado, banana, beef, blue cheese; used in candy, baked goods, ice cream.

470. Valerian root extract, oil and powder
FDA-approved food additive; FEMA GRAS; found in valerian root; used in baked goods, chewing gum.

471. Valeric acid
FDA-approved food additive; FEMA GRAS; found in banana, beef, beer, blue cheese, blueberry, wheat bread, butter; used in imitation dairy goods.

472. gamma-Valerolactone
FEMA GRAS; found in beef, beer, cocoa, coffee, mushroom, peach, peanut, wheat bread, heated butter, honey; used in candy, meat products, baked goods.

473. l-Valine
FDA-approved food additive; FEMA GRAS; found in plants, lemons, oranges, grapefruits; used in ice cream, candy, baked goods.

474. Vanilla extract and oleresin
FDA GRAS; FEMA GRAS; found in vanilla bean; used in baked goods, gelatins & puddings, condiments.

475. Vanillin
FDA GRAS; FEMA GRAS; found in asparagus, barley, beer, brandy, blackberry, blueberry, coffee, cranberry; used in confection frosting, baked goods, candy.

476. Veratraldehyde
FDA-approved food additive; FEMA GRAS; found in coffee, raspberry; used in baked goods, ice cream, candy.

477. Vetiver oil
FDA-approved food additive; found in vetiver flowers.

478. Violet leaf absolute
FDA GRAS; FEMA GRAS; found in violets; used in baked goods, ice cream, candy.
479. Walnut hull extract
FDA-approved food additive; FEMA GRAS; found in walnuts; used in breakfast cereals, ice cream, candy.

480. Water
Common food item.

481. Wheat absolute
Common food component.

482. Wine and wine sherry
Common beverages, found in grape fermentation/distillation.

483. Ylang ylang oil
FDA GRAS; FEMA GRAS; used in baked goods, soft candy, frozen diary, and chewing gum.
APPENDIX E

LEGAL DEFINITION OF “CIGARETTE”

The Food and Drug Administration and the Bureau of Alcohol, Tobacco and Firearms define a cigarette as “any product which contains nicotine, is intended to be burned under ordinary conditions of use, and consists of: (1) Any roll of tobacco wrapped in paper or in any substance not containing tobacco; or (2) Any roll of tobacco wrapped in any substance containing tobacco which, because of its appearance, the type of tobacco used in the filler, or its packaging and labeling, is likely to be offered to, or purchased by, consumers as a cigarette” (21CFR897.3, 1999; 27CFR290.11, 1999).

LITERATURE CITATIONS


APPENDIX F

STANDARD METHODS FOR TOBACCO AND SMOKE ANALYSIS

<table>
<thead>
<tr>
<th>Method</th>
<th>AOAC(^2)</th>
<th>ISO(^3)</th>
<th>CORESTA(^4)</th>
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<td>Tobacco</td>
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<td>Chlorides in tobacco</td>
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<td>Determination of silica content</td>
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<td>Determination of chlorophyll residues content (green index)</td>
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<td>Determination of dithiocarbamate pesticide residues</td>
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<td>Determination of maleic hydrazide residues</td>
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<td>4-1976</td>
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<td>Determination of nitrate in tobacco by continuous flow analysis</td>
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<td>Determination of organochlorine pesticide residues</td>
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<td>Determination of reducing substances in tobacco by continuous flow analysis</td>
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<td>Determination of residual stem content</td>
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<td>12195-1995</td>
<td>17-1991</td>
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<td>Determination of residues of the suckercide Flumetralin (Prime Plus, CGA-41065) on tobacco</td>
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<td>30-1991</td>
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\(^1\) Adapted and modified from (Green & Rodgman, 1996)
\(^2\) Association of Official Analytical Chemists (2000)
\(^3\) International Organization of Standardization (Bialous & Yach, 2001; 2002)
\(^4\) Cooperation Centre for Scientific Research Relative to Tobacco (1994)

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<th>ISO⁶</th>
<th>CORESTA⁷</th>
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⁵ Association of Official Analytical Chemists (2000)
⁶ International Organization of Standardization (Bialous & Yach, 2001; 2002)
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\(^{12}\) International Organization of Standardization (Bialous & Yach, 2001; 2002)

\(^{13}\) Cooperation Centre for Scientific Research Relative to Tobacco (1994)
LITERATURE CITATIONS


February 15, 2002

Dr. Edward Carmines
Philip Morris
P.O. Box 26583
Richmond VA 23261

Dear Dr. Carmines:

LSRO’s Added Ingredients Review Committee (AIRC) has now held four meetings and one conference call. The Committee has heard from seven outside speakers in addition to experts from PM. The Committee has reviewed three books and forty-six articles from the peer-reviewed scientific literature as a group, in addition to work by individual members. In the progress of our discussions, we have discussed the feasibility of verifying claims about ingredients added to cigarettes separately from the scientific criteria to apply in validating them.

In general, the consensus of the AIRC is that a review of the effects of the non-tobacco ingredients added to cigarettes, while challenging, is feasible, scientifically achievable, and potentially useful for public health considerations.

Our full report regarding feasibility will sharpen the definitions of some terms in this summary letter, including cigarette, additive, feasibility, and testing. The current draft has more than ten chapters. Of necessity with a lengthy report, we will require time for editing and peer review before we have a version suitable for public review. Therefore, we are providing our summary conclusion to you in advance.

Sincerely yours,

Daniel M. Byrd III, Ph.D., D.A.B.T.
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APPENDIX H

POWER CALCULATIONS

One question for clinical study to address is whether an ingredient in a cigarette induces a change in the number smoked. This change is determined by comparing cigarettes containing a specific ingredient to essentially identical cigarettes that lack that ingredient. The simplest approach to this question is to randomize smokers between two groups in a double-blind study and after an appropriate interval, enumerate and compare cigarette consumption between the two groups. Subsequent crossover studies, based on these study groups, can compare each subject with themselves (the same subject), as well as replicate the original study with the subject groups reversed (Senn, 2002).

Various additional factors in this study design merit attention, particularly assurance of subject compliance. For example, investigators will want to monitor whether subjects continue to smoke cigarettes as fully in both groups, by collecting and measuring butt length. However, a second question becomes important. If the ingredient does not induce a meaningful difference in cigarette consumption, how can a study design investigate smoking behavior to understand whether the smoking exposure changed?

A simple method to determine whether an ingredient has altered a smoker’s exposure to cigarette smoke would be to monitor a biomarker of smoking exposure, for example, cotinine levels in urine. Urine collection is non-invasive. Also, investigators may want to validate twenty-four hour samples by collecting first morning void samples from the same subject for compliance comparisons.

This design brings up the issue of how many smokers we should study. However, establishing a specific number of smokers to study raises two questions: 1) Are there any reasons for using that particular number? 2) How large a difference can we detect? A statistical method to predict these data is a power analysis. Broadly, a power analysis is the prospective examination of the expected properties of proposed studies, even though the quantity to be calculated might be the number of samples needed, or the sensitivity of study (Gad & Rousseaux, 2002). To conduct a power analysis, the investigator must select answers to four of the five questions below, and the answer to the fifth emerges by calculation. The five questions are:

1. How large a difference (or other measure) is important?
2. What is the standard deviation of the thing that matters?
3. How sure do you wish to be that a declared difference is not due to chance?

4. How sure do you wish to be that the difference declared in question 1 would in fact be detected if it were truly present?

5. How many samples must be collected?

Having answered all four of these questions, the fifth may be calculated.

Apart from the five questions above, two other questions arise in the planning. One is, “Does the measure give rise to a one-sample test or a two-sample test?” The other is, “Do we use a one-tail rejection region or a two-tail rejection region?”

1. **How large a difference (or other measure) is important?**

   The larger the difference of interest the easier will be the study. If we are interested only if the ingredient causes a 100% increase in blood cotinine levels this will be an easier study than if we declare that we wish to be sensitive to a 1% increase. If the study fails to find a difference due to the ingredient then we would like to be able to declare that we are pretty sure there was not a 100% increase in cotinine levels. It would be more reassuring to be able to say we are pretty sure there was not a 5% or even a 1% increase. We will not be able to say there was no increase. We will be able to say there was no detectable increase, but the reflective person will naturally wish to know what we might reasonably have been able to detect (the answer to question 4).

2. **What is the standard deviation of the thing that matters?**

   Surrogates for smoke exposure show different degrees of scatter. One of the reasons cotinine is often chosen is that the standard deviation associated with repeated measurements is less than that of other surrogates. Generally, a number for the reproducibility will be known from the literature. Small standard deviations are good, larger ones require larger studies, or the tolerance of greater uncertainty.

   If a new surrogate is to be used, a preliminary study will be required to determine the reproducibility under conditions of the study planned. For example, blood levels of the surrogate are measured at 1, 2 and 3 months following a switch to cigarettes containing the ingredient. A preliminary study using measurements at 0, 1, 2, and 3 months without a change in smoking habits will give us the information needed to calculate the standard deviation of paired differences from 0. This can be used to answer question 2 for a study following a switch to a cigarette containing the ingredient.
3. How sure do you wish to be that a declared difference is not due to chance?

This is usually taken to be 0.05, called alpha, and referred to as a type I error. The type I error is the probability that one would reject the null hypothesis by chance even though it is true. This is the chance of declaring a significant difference even though the true difference is zero. In the present setting where it will be important to assert confidently that we really should have been able to detect a difference if it was truly there, consideration might be given to using an alpha of 0.1 or larger. In accepting a larger risk of declaring a difference by chance we would also be increasing the probability of detecting a true difference if it is present. (Question 4)

4. How sure do you wish to be that the difference declared in question 1 would in fact be detected if it were truly present?

This is less standardized than the choice for type I errors although in many clinical trials a value of 0.8 is used. This is called the power of the test and is also 1 minus the probability of a type II error. The type II error is the probability of not rejecting the null hypothesis though it is false. There really is a difference but we couldn’t find it. Naturally we wish this number to be large. Making the number of measurements larger, reducing the standard deviation, increasing the sought for difference, or increasing the risk of a type I error will all reduce type II errors.

5. How many samples must be collected?

This is often chosen as the question to be answered by calculation, though occasionally, costs, time or just availability of subjects constrains this number. One seeks instead to answer question 1 (how large a difference are we likely to detect), or perhaps 2 (how small must we make the standard deviation to accomplish our aims), or 3 or 4 (what sort of uncertainty are we forced to tolerate).

One-Sample or Two-Sample?

One-sample tests compare the mean or median of a single set of data to some fixed reference, for example zero. Paired differences give rise to one-sample tests. Two-sample tests compare two sets of data with one another. The reason this matters is that computer programs for calculating power will be expecting the standard deviation of a single set of data for the one-sample test, but will be expecting a common standard deviation for the two sets of data for the two-sample, and will calculate the standard deviation of the difference between the two means.

One-tail or Two-tail?

If the interest is in detecting any change whose magnitude exceeds the answer to question one above, then a two-tail rejection region is appropriate. On the other hand, one might argue that if smoke exposure declines with the use of an ingredi-
ent, this is of no consequence since no claim of improvement is intended. It will also suffice to say that no increased exposure was detected and that we would have expected a 90% chance of detecting an increase exceeding 10%. By sacrificing the detection of a decrease, we can increase the sensitivity of detection of an increase for the same sample size.

Calculations

Calculations will be made using commercial software that requires data from the previous questions. If the software does not require the information above, then it is making assumptions about whatever is omitted. Many software programs are based on normal theory, which is usually appropriate if it is anticipated that 10 or more patients will be entered in each of two groups, or 10 or more pairs of observation are expected (Desu & Raghavarao, 1990). As a practical requirement, it is likely that at least 20 subjects in each group will be considered useful for experiments that compare smoke exposure in subjects smoking a cigarette with or without an ingredient. In such a case normal theory should be quite adequate for study planning and analysis.

If for some reason the normal theory is deemed not suitable, then the chief nonparametric approaches would be the Wilcoxon rank sum test for comparison of unpaired samples or the Wilcoxon signed rank test for paired observations. Both approaches assume there will be no ties in the data, and the signed rank test makes the further assumption of asymmetry. If either of these conditions is likely to be violated, then the power calculations available in commercial software should be avoided.

If a sample of residuals is available for data similar to that to be collected, then bootstrap estimates of the power can be obtained (Efron & Tibshirani, 1993).

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