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EXPOSURE ASSESSMENT IN THE EVALUATION OF POTENTIAL REDUCED-RISK TOBACCO PRODUCTS

LSRO

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In Memoriam: Catherine L. St. Hilaire

The Life Sciences Research Office dedicates this report to the memory of Catherine L. St. Hilaire, Ph.D. Her leadership, vision, and resolve were critical to the successful completion of this project. She will be greatly missed by her many friends and colleagues.
FOREWORD

The Life Sciences Research Office, Inc., (LSRO) provides scientific assessments of topics in the biomedical sciences. Reports published by LSRO are based on comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in relevant areas of science and medicine.

This report, one of three from the LSRO Reduced-Risk Review Project (RRRP), was developed under a contract between Philip Morris USA, Inc., (Philip Morris) and LSRO. The findings, conclusions, and recommendations contained in this report were developed independently of Philip Morris and are not intended to represent the views of Philip Morris or any of its employees.

Several Expert Advisory Committees provided scientific oversight and direction for the RRRP. LSRO independently appointed members of the Expert Advisory Committees on the basis of their qualifications, experience, judgment, and freedom from conflict of interest, with due considerations for balance and breadth in the appropriate professional disciplines. Committee members were selected with the concurrence of the LSRO Board of Directors.

This report provides the findings and recommendations of LSRO on one aspect of the RRRP—methods to assess exposures associated with potential reduced-risk tobacco products. The Exposure Assessment (EA) Committee provided primary oversight of this report’s development; this oversight was supplemented by input from the RRRP Core Committee. Appendix A provides biographical and professional information on each EA and Core Committee member.

The EA Committee held four meetings and one conference call between June 2005 and November 2006 to assess the available data. LSRO invited and accepted written, electronic, and oral submission of data, information, and views bearing on the topic under study. Information about the process, including critical literature and presentations on which the EA Committee based its deliberations, was made publicly available on the LSRO web site www.lsro.org/rrrvw.

LSRO staff drafted this report on the basis of available information and the deliberations and recommendations of the RRRP EA and Core Committees. The draft LSRO EA report was submitted to experts in relevant disciplines...
for independent peer review, and their comments were considered for incorporation by LSRO staff, RRRP EA and Core Committees, and LSRO Board of Directors. Philip Morris reviewed the final report for technical accuracy with respect to its products or other possible factual errors. The EA and Core Committees and LSRO Board of Directors reviewed and approved the final report. Upon approval, LSRO published the report with no additional input or review.

Participation in the preparation of this report or membership on an Expert Advisory Committee, or the LSRO Board of Directors, does not imply endorsement of all statements in the report. LSRO accepts full responsibility for the study conclusions and accuracy of the report.

Michael Falk, Ph.D.
Executive Director
Life Sciences Research Office, Inc.
December 11, 2007
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EXECUTIVE SUMMARY

Cigarette smoking causes cardiovascular disease, chronic obstructive pulmonary disease, and lung cancer (Centers for Disease Control and Prevention, 2005a). Although cigarette smoke contains thousands of constituents that are of biological concern, some of which have been linked to disease development, the components that are most responsible for specific cigarette smoking-related diseases and the exposure thresholds for disease development are unknown.

Tobacco product manufacturers have developed products with modifications intended to reduce exposure to tobacco smoke and/or selected tobacco smoke constituents and, ultimately, disease risk. In 2004, Philip Morris USA, Inc., requested that the Life Sciences Research Office (LSRO) identify the types of scientific information necessary for studying the risk reduction potential of tobacco products; establish criteria to evaluate the scientific information, including identification of comparison products; and define a review process for the scientific information.

LSRO’s overall findings and recommendations were published in the report Scientific Methods to Evaluate Potential Reduced-Risk Tobacco Products (2007b). LSRO also published the report Biological Effects Assessment in the Evaluation of Potential Reduced-Risk Tobacco Products (2007a) which provided an in-depth review of assays, models, and biomarkers of human disease that could be used during premarket evaluation to arrive at scientific conclusions regarding the comparative risks of potential reduced-risk tobacco products (PRRTPs) and conventional cigarettes for smokers who cannot or will not quit. This report, Exposure Assessment in the Evaluation of Potential Reduced-Risk Tobacco Products, provides findings, conclusions and recommendations of the Exposure Assessment (EA) State-of-the-Science Review Committee, which included scientists with the appropriate expertise.

SPECIFIC OBJECTIVES OF THE EA COMMITTEE

The specific objectives of the EA Committee were to:

- Identify and evaluate methods for assessing exposure of smokers (and other individuals in the smoking environment);
- Identify product characteristics and use behaviors that influence tobacco-product-related exposure; and
- Recommend methods to assess exposure.
The EA Committee was also asked to identify benchmark cigarettes for PRRTP studies and to consider the strengths, limitations, quality, and quantity of the evidence related to exposure assessment methods when evaluating them as tools for assessing PRRTPs. The EA Committee’s conclusions and recommendations are provided below. In the rest of this report, the EA Committee and other Expert Advisory Committees of the RRRP and LSRO staff will be referred to as LSRO.

CONCLUSIONS AND RECOMMENDATIONS

LSRO concluded that the state-of-the-science is adequate for assessing whether a PRRTP is likely to reduce exposure to tobacco product and smoke toxins compared with exposure received from using conventional cigarettes. LSRO recommended consideration of product characteristics, studies of tobacco product or smoke chemistry, and studies of biomarkers of exposure as critical for assessment of PRRTPs. LSRO took a weight of evidence approach to evaluate the relative usefulness of each assessment method. Biomarker studies were identified as the approach in which LSRO has the most confidence. Other types of studies, such as smoking topography, particle deposition and retention, filter analysis, and microarray studies, can supplement biomarker studies.

PRECLINICAL STUDIES

Product characterization

LSRO recommends that a PRRTP evaluation begin with an analysis of the differences in composition, design, and function between the PRRTP and conventional cigarettes. The possible effects of these differences should be described and used to guide later studies of PRRTPs. A consideration of the differences between cigarettes is an initial step in determining whether the user of the PRRTP or individuals in the use environment will be exposed to lower levels of tobacco smoke toxins compared to smokers of conventional cigarettes. Smoke chemistry studies should follow the analyses of differences in product characteristics.

Chemistry of tobacco products and tobacco smoke

Differences in product emissions indicate potential differences in exposure between PRRTPs and conventional cigarettes. LSRO recommends conducting broad analytical screens on smoke from cigarette-like PRRTPs and conventional cigarettes to measure as many constituents as possible. LSRO does not recommend using one smoke constituent as representative of a class of compounds, because substances in the same chemical class...
do not necessarily change in the same direction. Product characteristics should guide prioritization of analytes, and investigators should provide a rationale for selection or exclusion of tobacco smoke constituents for analysis.

LSRO recommends using Kentucky reference cigarettes as analytical controls and at least two conventional cigarettes, each from a separate tar category, as consumer product sample controls. Although no regimen using a cigarette smoking machine can predict actual exposure of smokers to tobacco smoke constituents, LSRO recommends using the smoking machine regimen of the International Organization for Standardization and the US Federal Trade Commission (see Section II.2.2.1) and at least one other method (e.g., the Massachusetts method or Canadian “intense” smoking method) to assess smoke chemistry profiles of PRRTPs and conventional cigarettes. Utilizing more than one method of smoke generation should provide information about the potential range of smoke constituent exposure from PRRTPs.

LSRO recommends using validated non-standard approaches to assess levels of substances in smoke when standardized approaches are unavailable. These new or non-standard methods should be described in adequate detail to allow independent replication and should be published in a peer-reviewed journal. Tobacco product and smoke chemistry studies should guide the clinical studies of tobacco products.

CLINICAL STUDIES

Clinical studies will play a critical role in comparisons of exposure from PRRTPs and conventional cigarettes. The LSRO report Biological Effects Assessment in the Evaluation of Potential Reduced-Risk Tobacco Products (2007a) details guidelines for investigators who conduct such clinical studies. LSRO considers biomarker studies to be the most useful type of clinical studies for PRRTP assessment. LSRO identified biomarkers of exposure\(^1\) used to study tobacco products and ranked them in terms of their usefulness, which was determined by how well they met the following desirable characteristics:

- Tobacco specificity or a substantial difference between smokers and nonsmokers;
- Intra-individual variation that mirrors variation in smoking behavior;
- Existing database on its pharmacokinetics;
- Low analytical method variation;
- Sensitivity and chemical specificity of analytical method(s); and
- Existence of other biomarkers that can confirm the exposure.

\(^1\) A biomarker of exposure is a constituent or metabolite that is measured in a biological fluid or tissue and/or is measured after it has interacted with critical subcellular, cellular, or target tissues.
LSRO also considered the invasiveness of the sample acquisition technique in ranking biomarkers. Detailed discussions of biomarkers of exposure are found in Chapter III of this report.

LSRO recommends using a battery of biomarkers to assess PRRTPs. Because of nicotine’s role in smoking maintenance, all PRRTP evaluations should include measurement of nicotine and at least five of its major metabolites, including cotinine. Investigators should select other biomarkers on the basis of information from product characteristics, smoke chemistry, and other studies. The battery of biomarkers chosen should reflect exposure to both particulate and vapor phase smoke components. LSRO identified biomarkers in which it has the most confidence as “Category A” biomarkers. These biomarkers have been sufficiently studied and provide reliable exposure measurements. LSRO also identified “Category B” biomarkers as those in which it has less confidence but for which there are sufficient data to support their use in exposure studies. LSRO would still recommend inclusion of Category B biomarkers in PRRTP evaluations. Other than nicotine and five of its major metabolites, including cotinine, LSRO does not specify a defined set of biomarkers that should be measured for all PRRTP assessments. Categorization of a biomarker as A or B is not meant to be prescriptive (see Section III.4).

LSRO determined that it has the lowest level of confidence in a third group of biomarkers “Category C”, as measures of tobacco product or smoke exposure (see Section III.4). Category C biomarkers are those that are not considered sufficiently reliable for routine use in exposure studies. However, if smoke chemistry or other studies indicate a significant difference in levels of a specific smoke constituent, biomarkers that measure exposure to the constituent of interest should be used in evaluation of the PRRTP regardless of category. Other types of clinical studies, such as smoking topography, filter analysis, and microarray studies, can supplement biomarker studies.

LSRO determined that the state-of-the-science is adequate for studying exposure assessment but acknowledges considerable limitations in the state-of-the-science. For example, additional biomarkers should be identified, validated, and used to compare exposure from PRRTPs with exposure from conventional cigarettes.

LSRO’s recommendations about scientific methods for assessing exposure are limited to what is possible at present. Advancements in the state-of-the-science for exposure assessment will influence recommendations about exposure assessment of PRRTPs. Exposure assessment is an important
step in the evaluation of PRRTPs, but ultimately, the biological effects arising from these exposures play the crucial role in determining risk.
INTRODUCTION

I.1 TOBACCO PRODUCT HARM REDUCTION

Tobacco products intended to decrease morbidity and mortality from diseases associated with cigarette smoking by reducing exposure to tobacco product and smoke toxicants are currently on the market as well as under development. A critical question concerning these products is whether they do in fact reduce risks of such diseases for individuals who use them and for other individuals in the environment.

In 1999, the US Food and Drug Administration commissioned the Institute of Medicine (IOM) to assess the state of the science for determining whether tobacco-based and non-tobacco-based potential reduced-exposure products (PREPs) are likely to reduce tobacco-product-related harm. The IOM concluded, in part, that “for many diseases attributable to tobacco use, reducing risk of disease by reducing exposure to tobacco toxicants is feasible,” and that “currently available PREPS have been or could be demonstrated to reduce exposure to some of the toxicants in most conventional tobacco products” (Institute of Medicine, 2001). The report further noted that PREPs “have not yet been evaluated comprehensively enough (including for a sufficient time) to provide a scientific basis for concluding that they are associated with a reduced risk of disease compared to conventional tobacco product use.” Since the publication of the IOM report, significant efforts have been made to develop methods and frameworks for assessing tobacco products that may pose lower health risks than conventional cigarettes (Hatsukami et al., 2005a, 2006a).

I.2 REDUCED-RISK REVIEW PROJECT: OBJECTIVES AND APPROACH

In 2004, Philip Morris USA, Inc., requested that Life Sciences Research Office (LSRO):

• Identify the types of scientific information needed to assess risk reduction;
• Establish criteria to evaluate the scientific information, including identification of comparison products; and
• Define a review process for the scientific information.
Because LSRO’s charge was to assess the risk-reduction potential of tobacco products, LSRO used the term “potential reduced-risk tobacco products” (PRRTPs) and named the project the Reduced-Risk Review Project.

LSRO’s overall findings and recommendations were published in the report Scientific Methods to Evaluate Potential Reduced-Risk Tobacco Products (2007b). LSRO also published the Biological Effects Assessment State-of-the-Science Review Committee’s report Biological Effects Assessment in the Evaluation of Potential Reduced-Risk Tobacco Products (2007a) which provides a detailed review of various assays, models, and biomarkers of human disease that could be used during premarket evaluation to draw scientific conclusions regarding the comparative risks of PRRTPs and conventional cigarettes for smokers who cannot or will not quit. This report provides detailed findings and recommendations of the Exposure Assessment (EA) State-of-the-Science Review Committee.

The reason for studying exposure assessment is that epidemiological studies have related biological effects of smoking to the duration and dose of cigarette smoke exposure, usually measured as pack-years\(^2\) (Doll & Hill, 1954; Doll & Peto, 1976; Doll et al., 2004; Stampfer et al., 2000; Wynder & Graham, 1950). People who quit smoking reduce their number of pack-years and significantly lower their risk of lung cancer compared with continuing smokers. Their risk declines further with additional years of abstinence (Peto et al., 2000).

Reducing the number of cigarettes smoked \textit{per} day by heavy smokers is an approach used in an attempt to decrease the dose of cigarette smoke toxins and reduce risks associated with cigarette smoking (Bolliger \textit{et al.}, 2002; Hatsukami \textit{et al.}, 2005b). However, such “cigarette reducers” (former heavy smokers who switch to smoking fewer than 15 cigarettes/day) have significantly higher levels of exposure to a tobacco smoke carcinogen than do consistent light smokers when exposure is measured at the point when cigarettes smoked \textit{per} day is equal (Hatsukami \textit{et al.}, 2006b). Light smokers are individuals who have smoked fewer than 15 cigarettes/day for an extended period of time. Compared with continuous heavy smokers, cigarette reducers who decrease cigarette consumption by at least 50\% do not lower their risk of hospitalization for chronic obstructive pulmonary disease (Godtfredsen \textit{et al.}, 2002b), myocardial infarction (Godtfredsen \textit{et al.}, 2003, 2005), or all-cause mortality (Godtfredsen \textit{et al.}, 2002a). Although these reducers decrease their risk of lung cancer compared with continuing heavy smokers, the risk reduction is substantially lower than the related smoking reduction, and the risk is higher

\(^2\) Pack-year is a unit of measure of smoking exposure. One pack-year represents the consumption of 20 cigarettes (one pack) \textit{per} day for one year by one person.
than the risk to light smokers (Godtfredsen et al., 2005). Other approaches to lowering the dose of cigarette smoke constituents involve altering the cigarette by means of design changes, such as inclusion of filters, puffed tobacco, reconstituted tobacco sheets, and different blends of tobacco (Burns & Benowitz, 2001).

Cigarette smoke contains a particulate phase and a vapor phase. The particulate phase is that part of the smoke retained on a Cambridge filter pad when smoke is pulled through the pad (Baker, 1999). Cambridge filter pads trap smoke particles larger than 0.1 μm with 99.9% efficiency (Baker, 1999). The total particulate matter (TPM), which includes alkaloids and water, is also known as wet particulate matter or cigarette smoke condensate. Tar is defined as the particulate matter from which alkaloids (such as nicotine) and water have been subtracted (Federal Trade Commission, 1967b). TPM produces tumors when applied to the skin of mice (Curtin et al., 2006; Meckley et al., 2004; Wynder et al., 1953, 1957). The vapor phase of cigarette smoke includes volatile organic compounds and gases of toxicological concern (Fowles & Dybing, 2003) and is associated with lung tumors in mice (Leuchtenberger & Leuchtenberger, 1971; Witschi et al., 1997; Witschi, 2005, 2006).

Filters were added to cigarettes to decrease smoke delivery to smokers. Filter cigarettes were widely introduced in the 1950s and by the 1990s accounted for 97% of cigarettes sold in the US (Hoffmann et al., 2001). Although some epidemiological studies reported a reduced risk for lung cancer for smokers of low-tar or low-yield cigarettes compared with smokers of high-tar cigarettes, overall mortality rates from lung cancer did not decline for low-tar cigarette smokers to the extent expected (Burns et al., 2001). Furthermore, the influence of filter cigarettes on mortality differed in the UK and the US. The reduction in mortality from lung cancer for men after filter cigarettes were introduced occurred earlier and the decline was greater in the UK than in the US (Burns et al., 2001; Peto et al., 2000).

One factor that likely contributed to this lower than expected decline in mortality rate from lung cancer was an altered puffing behavior when smokers switched from smoking high-yield cigarettes to smoking low-yield cigarettes (Peach et al., 1986; Robinson et al., 1983). In support of this idea, uptake of cigarette smoke constituents for smokers of low-tar cigarettes was determined to be equal to uptake for smokers of high-tar cigarettes (Hecht et al., 2005). Some smokers also blocked filter ventilation holes while

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3 A Cambridge filter pad is “a glass fiber filter stabilized by an organic binder” originally made by the Cambridge Filter Corporation in Syracuse, NY. It is used in the FTC method to separate the vapor and particulate phases of cigarette smoke.
smoking. Filter ventilation was intended to dilute MS, reducing the amount of smoke in the burning cone of the cigarette (Kozlowski & O’Connor, 2002). Filter ventilation holes are not blocked when low-yield cigarettes are machine-smoked according to a standard protocol for testing cigarettes, the US Federal Trade Commission (FTC) protocol (see Section II.2.2.1).

Lee and Sanders (2004) reviewed whether any reduced risk associated with switching to lower tar products is decreased when relative risks are adjusted for the number of cigarettes smoked per day. Based on an examination of epidemiological data they reported that the risk reductions for low tar cigarette smokers were maintained irrespective of gender, race, study location period, study design, and for adjusted and unadjusted estimates.

The natural experiment on the effect of switching to lower tar cigarettes on mortality underscores the need for an integrated approach to assessing exposure to tobacco product and smoke constituents, one that involves chemical analyses, clinical studies, and other types of studies.

Elimination of all toxicants from cigarette smoke is not possible. PRRTPs are intended to reduce disease risk by decreasing the dose of smoke constituents for individuals who cannot or will not quit smoking cigarettes or for those who are in the smoking environment.

The specific objectives of the EA Committee were to:

- Identify and evaluate methods for assessing exposure of smokers (and other individuals in the smoking environment);
- Identify product characteristics and use behaviors that influence tobacco-product-related exposure; and
- Recommend methods to assess exposure.

The EA Committee considered the strengths, limitations, quality, and quantity of the evidence related to these methods when evaluating them as tools for assessing PRRTPs.

1.3 SMOKING-RELATED EXPOSURES

Some tobacco products generate smoke when used as intended and some do not. PRRTPs include modified cigarettes, such as those incorporating novel filters, genetically modified tobacco, or tobacco grown or processed under altered conditions; cigarette-like products that mostly or only heat tobacco and therefore yield significantly lower levels of combustion or pyrolysis products in aerosols they produce compared to conventional
cigarettes and smokeless tobacco products. Therefore, exposures differ depending on the type of PRRTP. This report focuses on exposures from smoked tobacco products.

Cigarette smoking generates mainstream smoke (MS), sidestream smoke (SS), and environmental tobacco smoke (ETS). MS is drawn by smokers from the butt end of a cigarette into the mouth. SS is emitted directly into the air from the burning end of a cigarette largely during the smolder interval between puffs. Although SS and MS contain essentially the same constituents, SS has higher absolute quantities of most smoke constituents compared with MS (Baker & Proctor, 1990; Guerin et al., 1992). One reason for this is the higher burning temperature during smoke inhalation (U.S. Environmental Protection Agency, 1992). Some smoke constituents occur predominantly in the particulate phase of smoke, some partition mostly in the vapor phase of smoke, and others occur at similar levels in both phases. The smoke constituent’s primary phase may differ for different types of smoke. For example, nicotine is found primarily in the particulate phase of MS but mostly in the vapor phase of ETS (Baker, 1999). The relative yield per cigarette of compounds in MS and SS varies for smoke constituents.

ETS is defined as a mixture of aged diluted SS and exhaled MS. SS released from the tip of the burning cigarette is the major contributor to ETS (Baker & Proctor, 1990), accounting for more than 50% of particulate phase constituents and almost all vapor phase constituents (Baker, 1999; U.S. Environmental Protection Agency, 1992).

Exposures of concern from smoked tobacco products include MS and ETS constituents; therefore, exposure reduction relates to decreased exposure to MS and ETS constituents for the smoker and to ETS for other individuals in the smoking environment.
II.1 PRODUCT CHARACTERIZATION

Manufacturers of potential reduced-risk tobacco products (PRRTPs) have employed diverse strategies intended to reduce exposure to toxicants found in the smoke from conventional cigarettes. These include modifying composition, design, engineering, and manufacturing of the tobacco product (World Health Organization, 2003b). Table II-1 provides examples of smoked PRRTPs, their modifications, and their market status in the US. Assessing structural, chemical, and functional differences between PRRTPs and conventional cigarettes is a useful starting point for PRRTP evaluation. Consideration of the possible effects of identified PRRTP modifications on tobacco-product-related exposure should guide the design of further studies. Examples of PRRTP modifications and some implications for exposure assessment are described below.

PRRTPs that expose tobacco to a lower temperature, such as Accord® and Eclipse®, could heat the tobacco instead of burning it (ECLIPSE Expert Panel, 2000; Stabbert et al., 2003). These PRRTPs may generate lower levels of combustion and pyrolysis products than tobacco-burning conventional cigarettes. Exposure-related concerns about such products include whether it in fact produces significantly lower levels of pyrolysis and combustion products in mainstream smoke (MS) and environmental tobacco smoke (ETS) compared with conventional cigarettes, whether it produces significantly higher levels of substances not found in smoke from conventional cigarettes, and whether levels of biological markers that measure exposure to smoke constituents after PRRTP use differ significantly from levels occurring after conventional cigarette use.

Inclusion of a modified filter in a cigarette may reduce levels of targeted smoke constituents compared with levels found for conventional cigarettes with standard filters. Evaluation of a PRRTP with a modified filter should examine whether amounts of targeted smoke constituents decline significantly, levels of other smoke constituents increase significantly, biomarkers of exposure reflect lower amounts of targeted smoke constituents,
other biomarkers reflect increased levels of other smoke constituents, and users are exposed to significant levels of filter components.

Product design and composition can also affect ETS exposure. A PRRTP that produces substantially lower sidestream smoke (SS) than conventional cigarettes has the potential for reducing ETS levels. In contrast, a PRRTP that produces lower levels of MS constituents may not necessarily produce lower levels of SS constituents. Cigarettes with filters that reduce MS constituent yields may have no effect on SS yields of the same constituents (U.S. Environmental Protection Agency, 1992).

Table II-1. Examples of Potential Reduced-Risk Tobacco Products

<table>
<thead>
<tr>
<th>PRRTP</th>
<th>Modification</th>
<th>US Market Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accord®/Premier®</td>
<td>Heats but does not burn tobacco. Heating occurs only when the cigarette is puffed while inserted into a microchip-controlled, battery-operated, hand-held device (Philip Morris USA, 2001)</td>
<td>In test market: Richmond, VA</td>
</tr>
<tr>
<td>Advance®</td>
<td>Includes a 3-part “Trionic” filter containing cellulose acetate, a carbon compound, and an ion exchange resin. Contains Starcure™ tobacco, which has reduced levels of TSNAs (Brown &amp; Williamson Tobacco, 2002)</td>
<td>Not on the market</td>
</tr>
<tr>
<td>Eclipse®</td>
<td>Primarily heats tobacco. “Eclipse is designed to burn only 3% as much tobacco as other cigarettes” (R.J. Reynolds Tobacco Company, 2006)</td>
<td>On the market</td>
</tr>
<tr>
<td>Omni®</td>
<td>Contains tobacco cured under modified conditions and treated with catalysts to reduce levels of carcinogens; uses a carbon filter (Vector Tobacco Inc., 2001a,b)</td>
<td>Not on the market</td>
</tr>
<tr>
<td>Quest®</td>
<td>Contains genetically modified tobacco lacking an important gene for nicotine synthesis. (Vector Tobacco Inc., 2006)</td>
<td>On the market: NY, NJ, PA, OH, IN, IL, MI and AZ</td>
</tr>
<tr>
<td>Marlboro UltraSmooth®</td>
<td>Cigarette contains a novel carbon filter (Beran, 2005)</td>
<td>In test market: Atlanta, GA; Tampa, FL; and Salt Lake City, UT</td>
</tr>
</tbody>
</table>

*Premier® was an earlier version of Accord®.

TSNAs: tobacco-specific nitrosamines (suspected human carcinogen)
A significant behavioral component is associated with ETS exposure. Exposure can increase if smoking continues for extended periods, if more people smoke, or if toxicants in SS or exhaled MS increase (World Health Organization, 2003b).

II.2 CHEMISTRY

Assessment of tobacco smoke chemistry is a useful next step in PRRTP evaluation.

II.2.1 Control Tobacco Products

An important element of product chemistry studies is defining appropriate tobacco products for control and comparison purposes.

II.2.1.1 Kentucky reference cigarettes

Kentucky reference cigarettes, developed jointly by the US National Cancer Institute, the Agricultural Research Service of the US Department of Agriculture, and the Tobacco and Health Research Institute of the University of Kentucky, serve as research standards for chemical and biological studies of cigarettes (Davies & Vaught, 1990). These cigarettes are designed to reflect characteristics of cigarettes with various tar levels. The Kentucky reference 3R4F cigarette (which replaced 1R4F and 2R4F cigarettes when supplies were exhausted) approximates the tar delivery of the sales-weighted average of US low-tar cigarettes. The Kentucky reference 1R5F cigarette approximate ultra-low-tar cigarettes (Chepiga et al., 2000).

II.2.1.2 Conventional cigarettes

Conventional cigarettes are commercial cigarettes that incorporate materials and designs typical of those that have been used in cigarette manufacturing for a number of years (Counts et al., 2006), but they do not constitute a static group. Hoffmann and Hoffmann (2001) described design changes in cigarettes now available, such as inclusion of cellulose acetate filters (with or without perforations), charcoal filter tips, additives, or porous cigarette paper; and changes in physical parameters of cigarettes (e.g., length, circumference, and width of cut of the tobacco filler). Manufacturers have also included different types of tobacco (e.g., flue-cured, air-cured, light air-cured, dark air-cured, or sun-cured) and have incorporated reconstituted or expanded tobacco in cigarettes (Hoffmann & Hoffmann, 2001).
Conventional cigarettes fall into three categories based on nicotine and tar yields when cigarettes are machine-smoked according to the US Federal Trade Commission (FTC) method: full flavor (“regular”); full flavor, low tar (“light”); and ultra-low tar (“ultra-light”). The amount of tar per cigarette produced by each cigarette type is higher than 14.5 mg for regular, 6.5–14.5 mg for light, and less than 6.5 mg for ultra-light (Chepiga et al., 2000).

A recent toxicological assessment in a comparative subchronic inhalation study of 1R4F and 2R4F reference cigarettes indicated that there was no biologically significant difference when rats were exposed to smoke from each type of cigarette (Higuchi et al., 2004). MS yields of total particulate matter (TPM) and nicotine were 11.7 mg TPM and 0.83 mg nicotine per cigarette for the 1R4F cigarette and 9.2 mg TPM and 0.75 mg nicotine per cigarette for the 2R4F cigarette.

II.2.1.3 Recommendations for control cigarettes

For a PRRTP evaluation, the Life Sciences Research Office (LSRO) recommends comparing smoke constituent yields for a PRRTP with at least one Kentucky reference cigarette and at least two conventional cigarettes. Kentucky reference cigarettes serve as analytical controls. LSRO does not recommend specific control conventional cigarettes for PRRTP studies but does recommend that investigators provide their rationale for selecting the control cigarettes. The selection of specific conventional control cigarettes should be based on study design and goals (e.g., researchers may want to use each subject’s brand of cigarettes as a control product). Conventional cigarettes and PRRTPs should be tested concurrently.

II.2.2 Mainstream Tobacco Smoke

A recent review by Borgerding and Klus (2005) summarized some principal issues associated with smoke chemistry analyses, and that review has guided development of this section.

II.2.2.1 Cigarette smoking machine parameters

The FTC developed a cigarette smoking machine protocol for comparing smoke yields when cigarettes are smoked using the same machine setting under the same prescribed conditions (Federal Trade Commission, 1967a, 1967b, 1980). The FTC method, implemented in 1967, stipulates how cigarettes are to be sampled and conditioned before being machine-smoked, defines the machine parameters to be used (puff volume = 35 ml, puff duration = 2 seconds, interpuff interval = 60 seconds), and specifies the butt length to which the cigarette should be smoked (the larger of 23 mm or the length of...
the filter and overwrap + 3 mm). The FTC method also stipulates methods for MS collection, analysis for smoke constituents, and chemical measurements to be obtained for nicotine, carbon monoxide (CO), and tar. The FTC later proposed using both the “lower tier” existing protocol and a new “upper tier” protocol (55 ml puff volume, 2 second puff duration, 30 second puff interval), but the proposal was not adopted (Federal Trade Commission, 1997).

The International Organization for Standardization (ISO) developed a standard method for assessing smoke yields that specifies the same puff volume, puff duration, and interpuff interval as the FTC method. The ISO 4387 method requires different smoking machine equipment, product sampling, and product conditioning methods than the FTC method (Borgerding & Klus, 2005).

The Commonwealth of Massachusetts and the State of Texas mandate a third cigarette smoking machine protocol with the intent to predict nicotine intake of the average smoker (Massachusetts Department of Public Health, 2005; Texas Administrative Code, 1998). These methods both define a larger puff volume (45 ml) and shorter puff interval (every 30 seconds) but the same puff duration (2 seconds) as the FTC method.

Cigarette ventilation is an engineering feature aimed at reducing tar yield. It can be achieved by using a porous wrapping paper on the cigarette so that smoke can be mixed with air, or by including a ring of ventilation holes in the cigarette's paper wrapper. However, some cigarette smokers negate or reduce smoke dilution by covering ventilation holes with their fingers during puffing (Kozlowski et al., 1996; Kozlowski & O'Connor, 2002; Zacny et al., 1986). Blocking these holes can lead to no differences in exposure to smoke toxicants between ventilated and unventilated cigarettes, or even to increased toxicant exposure for cigarettes with ventilation holes (Djordjevic et al., 2000; Kozlowski & O'Connor, 2004).

In an attempt to address potential alterations in smoke exposure related to this product use behavior, the Massachusetts-Texas method stipulates blockage of 50% of filter ventilation holes during machine smoking of cigarettes.

The Canadian federal government requires manufacturers of cigarettes sold in Canada to test smoke from their cigarettes according to both the ISO 4387 method and a “maximum emission” testing protocol intended to “provide data that reflects the emissions that are actually available to the consumer” (Health Canada & Losos, 1998). This maximum emission testing protocol
mandates a 55-ml puff volume and a 2-second puff every 30 seconds, with 100% blocking of cigarette ventilation holes (Health Canada, 2000a).

Bernstein (2004) reviewed studies that examined the effects of cigarette design and smoking behavior on smoke particle size. He concluded that the size range of particles in smoke from cigarettes with or without filters and with ventilation holes open or blocked are the same. While varying concentrations of smoke particles are taken into the mouth, the subsequent inhalation pattern does not seem to change significantly during smoking of different types of cigarettes and under different smoking conditions. As a result, the smoke particle deposition pattern within the lung is not likely to change significantly when comparing lower delivery or ventilated cigarettes to higher delivery or non-ventilated cigarettes.

Many studies have shown that regular smokers of cigarettes that yield lower levels of nicotine and tar when machine-smoked according to the FTC regimen, tend to take larger and more frequent puffs (Benowitz, 2001; Bridges et al., 1990; Djordjevic et al., 1997; U.S. Department of Health and Human Services, 1988). Kozlowski and O’Connor (2000) developed a “compensatory” approach to machine smoking of cigarettes that better approximates human smoking behavior. For example, light cigarettes would be machine-smoked more intensely than regular cigarettes, because many smokers compensate for lower levels of nicotine and tar in light cigarettes (Voncken et al., 1998) by more intense smoking (Kozlowski & O’Connor, 2000). Other attempts to develop more realistic cigarette smoking machine regimens involve studying human smoking behavior and then developing machine regimens that mimic average smoking behavior of one smoker or a group of smokers such as the “human mimic” protocol (Hammond et al., 2006).

A recent study by Hammond et al. (2006) compared the total puff volumes, and nicotine, tar, and CO yields from cigarettes when they were machine-smoked according to five testing regimens: ISO, Massachusetts/Texas, Canadian “intense”, compensatory, and human mimic. These investigators recorded smoke volumes and cigarette puffing behavior for 51 cigarette smokers during three smoking trials in a 2-month period. Each smoking trial lasted 1 week. During Trials 1 and 2, which were 6 weeks apart, subjects smoked their regular brand of cigarettes each day for 5 days through a Clinical Research Support System (CreSS) micro-smoking apparatus, which recorded puff volume, puff count, puff duration, peak flow, interpuff interval, time, and date. For machine-smoked cigarettes, investigators blocked 50% of cigarette ventilation holes to approximate the percentage blocked by
smokers in a study by Kozlowski and coworkers (1989). During Trial 3, which took place the week after Trial 2, subjects smoked either their usual brand or a lower yield cigarette with the same length and diameter as their usual brand. At the end of the week, investigators measured levels of cotinine, a major nicotine metabolite, in subjects’ saliva samples. This study found that smokers’ total puff volumes (product of the total number of puffs and mean puff volume) were significantly higher after the switch to the lower tar cigarettes compared with those found when subjects smoked their regular brand of cigarettes. Hammond et al. (2006) also used a linear regression model to study the association between smoking machine yields and smokers' salivary cotinine levels. They “adjusted for measure of intake and demographic variables” and found that the human mimic protocol was the only regimen that showed a significant association with salivary cotinine concentration ($p = 0.02, r^2 = 0.51$, where $r^2$ is the coefficient of determination).

The authors noted a number of limitations of the study. Only smokers whose regular brand of cigarettes fell within a specific tar range participated; therefore, the study sample did not represent all smokers. Also, the average number of puffs taken by smokers differed from the average number taken by the machine during the human mimic protocol. Finally, no information about ventilation hole blocking was obtained from human smokers (Hammond et al., 2006).

II.2.2.1.1 Emerging cigarette smoking machine protocols

The WHO Study Group on Tobacco Product Regulation (TobReg) recommends generating smoke according to the ISO/FTC protocols in addition to another protocol during which the cigarette is smoked more intensely (WHO Study Group on Tobacco Product Regulation (TobReg), 2004). The recommended intense protocol mandates a 55-ml puff volume, 30-second puff interval, and 2-second puff duration, with 100% of filter ventilation holes blocked while the cigarette is smoked down to the filter plus 3 mm (same as the Canadian intense method). However, TobReg acknowledged that changes in the more intense protocol may be necessary if smokers change their smoking patterns or the intense protocol no longer generates what is considered to be the maximum possible exposure for the smoker.
An ISO Working Group (ISO/TC126/WG9) considered developing a smoking machine regimen that will better reflect human smoking behavior (Hammond et al., 2007). They evaluated four alternative smoking regimens as detailed in Table II-2. The Working Group recently concluded that none of the potential alternative smoking regimens adequately predicted human exposure to tobacco smoke constituents and recommended additional work to identify alternative smoking machine regimens (Hammond et al., 2007).

**II.2.2.1.2 Recommendations for cigarette smoking machine protocols**

LSRO recommends using at least two smoking machine regimens to generate smoke from PRRTPs and control cigarettes. Use of multiple smoke generation methods approximates a range of human puffing behavior. Because of the availability of extensive historical data on cigarette smoke yields of substances generated by the FTC cigarette smoking machine protocol, LSRO recommends the inclusion of this method. A smoking machine protocol based on actual human smoking of PRRTPs (a smoking topography study) can provide additional information about potential tobacco smoke exposure (Djordjevic et al., 2000). PRRTPs containing significantly higher or lower levels of nicotine than control cigarettes could prove especially appropriate for such a study because of potentially altered cigarette puffing behavior and resulting differences in exposure to smoke constituents. However, as stated by the FTC (1967b), no standard method of machine-smoking cigarettes can reflect exposure of an individual smoker or population of smokers because of variations in intra- and inter-individual smoking behavior.

### Table II-2. Alternative Smoking Regimens Considered by the ISO Working Group (ISO/TC126/WG9)

<table>
<thead>
<tr>
<th>Possible regimen&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Puff volume (ml)</th>
<th>Puff frequency (s)</th>
<th>Blocked filter percentage</th>
<th>Flow rate (ml/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Option A</td>
<td>55</td>
<td>30</td>
<td>50</td>
<td>27.5</td>
</tr>
<tr>
<td>Option B</td>
<td>60</td>
<td>30</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>Option C</td>
<td>45</td>
<td>30</td>
<td>100</td>
<td>22.5</td>
</tr>
<tr>
<td>Option D</td>
<td>55</td>
<td>30</td>
<td>100</td>
<td>27.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>Each smoking machine regimen has a puff duration of 2 seconds, and a butt length of filter + 8mm. (Adapted from Hammond et al. 2007).
II.2.2.2 Selection of smoke analytes

Fresh MS contains more than 4,000 constituents (Baker, 1999), some of which are categorized as toxic to humans (Smith et al., 1997, 2000c, 2001; World Health Organization, 2004). Lists of smoke toxicants have been published in the scientific literature (Hoffmann & Hoffmann, 1998, 2001; Rodgman & Green, 2002; Smith et al., 1997; U.S. Consumer Product Safety Commission, 1993; U.S. Department of Health, Education, and Welfare, 1964; World Health Organization, 1986). Various groups of investigators have measured selected smoke toxicants from these lists (ECLIPSE Expert Panel, 2000; Hoffmann & Hoffmann, 1998).

The specific chemicals or classes of chemicals responsible for cigarette smoking-related diseases are not known, and lists of toxicants are likely incomplete (Rodgman & Green, 2002). Furthermore, available analytical methodology does not allow for detection and quantification of all identified smoke analytes. Aging of tobacco smoke and type of tobacco used also affect smoke composition.

II.2.2.2.1 Recommendations for selection of smoke analytes

Because the specific toxicants in cigarette smoke causing smoking-related diseases have not been identified, LSRO recommends measuring as many gas and particulate phase smoke constituents as possible from PRRTP and control cigarette smoke to determine whether analyte yields for these products differ significantly. LSRO considered the advantages and disadvantages of developing a list of required smoke analytes. A list of recommended smoke analytes may allow for better comparison between smoke constituent yields of PRRTPs and control cigarettes. The Centre de Coopération pour les Recherches Scientifiques Relatives au Tabac (CORESTA), an organization founded to “promote international cooperation in scientific research relative to tobacco,” or a similar organization could develop such a list. LSRO also considered recommending that investigators measure the “Hoffmann analytes,” an evolving list of smoke constituents developed by Dietrich Hoffmann and his colleagues (Hoffmann & Hoffmann, 1998, 2001). However, some regulatory authorities consider only a subset of these analytes to play a role in diseases associated with cigarette smoking. Although the Hoffmann analytes may be important to smoking-related diseases, they represent only a fraction of toxic smoke components, and methods do not exist for measuring all these analytes (Baker, 2006). A potential disadvantage of any list is that investigators may interpret it as representing the only smoke analytes that require analysis. Thus, LSRO considers it best to recommend measurement of as many smoke constituents as possible.
Product design and composition should guide the prioritization of smoke analytes, and investigators should provide the rationale for selection or exclusion of analytes. LSRO does not recommend using selected smoke constituents as surrogate compounds to reflect changes in levels of entire classes of compounds because such compounds may not accurately reflect changes for all members of a class. For example, formaldehyde levels in smoke from an electrically heated cigarette were higher than those in smoke from a conventional cigarette, but levels of other aldehydes (acetaldehyde, acrolein, and propionaldehyde) were significantly lower (Stabbert et al., 2003). Therefore, formaldehyde would not be a good representative of other aldehydes.

If substances not previously detected in smoke from cigarettes are identified in smoke from the PRRTP, an approach to addressing the novel compounds may involve determining the identity of the substance, measuring levels of the substance in smoke, considering structure-activity relationships, and determining whether the change in levels of the substance is likely to be of biological concern. Structure-activity relationships reflect associations between chemical (sub)structures of compounds or classes of compounds and biological effects of chemicals with that substructure (Simon-Hettich et al., 2006). Information from in vitro and in vivo studies and clinical trials is the basis of these relationships (Simon-Hettich et al., 2006). It is important to weight structure-activity relationships appropriately, because similarity of chemical structure does not necessarily indicate similar toxicity (Simon-Hettich et al., 2006).

II.2.2.3 Selection of analytical methods

Standardization of analytical methods can address concerns that differences in smoke analyte yields may be due to variations in analytical methods rather than to differences in product design. ISO methods undergo extensive intra- and inter-laboratory validation and are updated periodically (International Organization for Standardization, 2000c). ISO methods for determining tar, nicotine, and CO levels are the only standard methods that have been published to date (International Organization for Standardization, 1995, 1999, 2000a, 2000b). However, ISO is developing methods for measuring additional Hoffmann analytes in smoke, and CORESTA has published recommended methods for quantifying benzo[a]pyrene and tobacco-specific nitrosamines (TSNAs) in MS (CORESTA, 2004, 2005).

Official methods for cigarette smoke analysis were developed by Health Canada. In contrast to CORESTA/ISO methods, Health Canada methods did not undergo inter-laboratory validation and therefore were not assessed for repeatability or
reproducibility when instituted in 2000 (Borgerding & Klus, 2005; Rickert & Wright, 2002). Rickert and Wright (2002), using the Kentucky reference 1R4F cigarette, assessed the variability of Health Canada methods for 45 Hoffmann analytes. This 8-month single-laboratory study revealed that the coefficient of variation for Hoffmann analytes ranged from 5% to 15%, with a few analytes ranging between 20% and 80%. Considerable variability in measurements was reported for smoke from the 1R4F cigarette when different laboratories measured smoke constituents with their various in-house methods (Baker et al., 2004b; Purkis et al., 2003).

LSRO recommends publication, in appropriate peer-reviewed journals, of new or modified methods for assessing smoke chemistry at the same level of detail as that used in a typical standardization process. Such methods can serve to evaluate smoke yields from PRRTPs and conventional cigarettes.

II.2.2.3.1 Recommendations for selection of analytical methods

Standardized analytical methods are ideal for comparing cigarette smoke yields within and between laboratories, but validated standardized methods do not exist for all smoke analytes. One disadvantage of internationally standardized procedures is that they are slow and focus on only a few selected compounds. In the absence of standard methods for measuring smoke constituents, LSRO recommends use of any validated method to measure smoke constituent yields from control cigarettes and PRRTPs. The International Conference on Harmonisation is one of a number of entities that have developed guidelines for validation of analytical methodologies (International Conference on Harmonisation, 1996). The use of reference standards and controlling for blanks and spikes in recoveries are important features of such studies. Investigators should note whether they used standard or non-standard methods and provide the rationale for method selection. LSRO further recommends publication, in peer-reviewed literature, of non-standardized analytical methods used to analyze smoke constituent yields in extensive detail.

II.2.2.3.2 Emerging analytical methods and research gaps

The most common smoke analysis techniques are gas and liquid chromatography. With development of new methods and modification of existing methods, it is likely that, in the future, substances that are not now detectable in smoke will become measurable. Assay of such additional smoke constituents is encouraged as technology improves and new and better validated analytical methods become available.
Analytical methods for measuring tobacco and smoke chemistry in near real-time have been reported. Faster analytical methods, higher throughput methods, and techniques that allow more detailed analyses are being developed (Adam et al., 2006; Mitschke et al., 2005). Mitschke et al. (2005) described using time-of-flight mass spectrometry with laser-based photoionization methods for time-resolved on-line analysis of MS, which allows puff-by-puff analyses of smoke constituent yields and can measure a large number of smoke constituents. The study showed that yields of smoke constituents vary throughout the smoking of a cigarette. Yields of some constituents gradually increase at a constant rate. Others are produced in large amounts during the first puff, with the second puff containing a markedly lower yield, and successive puffs having gradually increasing yields. A third group of smoke constituents shows an inconsistent pattern of yields. Such compounds may, for example, decrease slowly in the initial 3 puffs and then increase slowly in the following puffs.

Methods for examining large arrays of compounds (e.g., principal component analysis and hierarchical cluster analysis) and instruments with increased sensitivity are now in use and have been reported (Adam et al., 2007; Crispino et al., 2007). One challenge associated with expanding technology is the interpretation of results. LSRO recommends emphasizing the constituents present in the largest amounts in smoke that are known to have biological activity.

II.2.2.4 Other mainstream tobacco smoke exposures

Tobacco product users are potentially exposed to various types of particles such as hardened adhesives, flakes of packaging and cigarette tipping inks, hardened casings, fibers from cigarette papers and cigarette packs, and tobacco fines (e.g., stem fibers, lamina, and reconstituted tobacco) (Agyei-Aye et al., 2004). Investigators should describe potential exposures to particles or shards during use of PRRTPs. LSRO recommends both physical and chemical characterizations of the tobacco smoke aerosol. A comparison of the amount and composition of the TPM from the PRRTP and control cigarettes would also provide useful information.

II.2.3 Sidestream Tobacco Smoke

Because SS is the major contributor to ETS, investigators have measured SS to provide information about ETS composition. However, SS should not be taken to reflect ETS because the composition and physical characteristics of SS changes as the smoke ages, is diluted by air, and is mixed with exhaled MS. A major challenge associated with SS studies is collection of SS. Drafts
and convection can disperse SS from the site of smoke generation as the cigarette burns (Proctor et al., 1988).

II.2.3.1 Sidestream smoke collection

Many devices for SS collection have been described in the literature (Brunnemann & Hoffmann, 1974; Johnson et al., 1973; Neurath & Ehmke, 1964; Proctor et al., 1988; Sakuma et al., 1983). Proctor et al. (1988) described design criteria for an SS collection apparatus. The most useful collection methods do not alter the normal combustion of the cigarette during puffing or smoldering. To accomplish this, the apparatus must permit the cigarette to burn at ambient temperature in an atmosphere of air. With the ISO method for smoke generation, the airflow range should be 4–7 linear feet per minute. The sampling device should allow easy recovery of escaped smoke components and should be readily adaptable to a standard cigarette-smoking machine. The apparatus should permit multiple measurements of MS and SS at an appropriate rate and allow collection of both phases of smoke. Automation of insertion and lighting of the cigarette should be accomplished easily. In addition, measurement of cigarette butt length is recommended. If yields of more than one cigarette are measured, the total yield should be the sum of yields for several cigarettes (Proctor et al., 1988).

Proctor et al. (1988) designed a fishtail chimney for SS collection, which served as the basis for the chimney for SS collection reported by Shi et al. (2003). Shi et al. (2003) collected smoke from two different cigarettes and measured ammonia, ethylene, and nitric oxide. The chimney described by Shi et al. (2003) was positioned around the cigarette with the open bottom of the container approximately 3 mm above the surface of a table to minimize smoke escaping into the environment. The collection device was connected to a fast-response infrared spectrometer using pulsed quantum cascade lasers. This method permitted real time measurements of gases in SS and MS and showed that levels of these substances differ prior to, during, and after puffs.

II.2.3.2 Selection of sidestream smoke analytes

Health Canada recommended measurement of specific SS analytes and published recommended analytical methods (Health Canada, 2000b). Distribution of gas and particulate phase smoke constituents can change depending on product characteristics (Guerin, 1987). Although filters may reduce MS yields of smoke constituents, SS yields may be unaltered (Adams et al., 1987). Because SS is the major contributor to ETS, a PRRTP that produces significantly lower levels of SS has the potential to reduce ETS exposure.
II.2.4 Environmental Tobacco Smoke

Current technology allows MS and SS chemistry analyses. SS analyses can be performed after analysis of MS, if the results of the MS studies indicate the potential for reduced exposure or reduced risk in smokers. Room levels of some ETS tracers such as ultraviolet-absorbing particulate matter, fluorescent particulate matter, solanesol, nicotine, and CO have been measured (Daisey, 1999). Environmental chambers that allow control over temperature, relative humidity, and air circulation, and workplace offices have been used for ETS studies (Jenkins et al., 2001; Suarez et al., 2005). Studies providing evidence are necessary to support a claim of reduced ETS exposure.

II.2.4.1 Human-smoker-generated ETS

Chemical exposure from human-smoker-generated ETS has been assessed in various unpublished studies; these are discussed in cited references in Baker and Proctor (1990) and Roethig et al. (2005). Using methods based on light scattering of particles and infrared spectrometry of gases, Roethig et al. (2005) measured respirable suspended particulates (RSP), CO, and total volatile organic compounds (TVOC) for 15 minutes before, 1 hour during, and 1 hour after smoking. Measurements were made at two locations in the room after smokers exited the room. No smoking and electrically heated cigarette smoking systems (EHCSSs) which are marketed as Accord® and Oasis®, produced levels of RSP 90% lower than those produced by conventional cigarettes. RSP measurements in the no-smoking group location were 95% lower than levels in the room where conventional cigarettes were smoked. Room air where no smoking occurred or EHCSS were smoked contained 40–50% lower levels of TVOCs compared with levels found after smoking conventional cigarettes (Roethig et al., 2005). CO was not detected in the no smoking and EHCSS rooms.

II.2.5 Relative Contributions of Tobacco Product and Smoke Chemistry to Assessing PRRTPs

Smoke chemistry analysis is an important early step in evaluation of a PRRTP and can be useful for characterizing differences between PRRTPs and control cigarettes. Because of individual variation in human puffing behavior, a reduction in the cigarette smoking machine yield of smoke analytes for the PRRTP compared with that for conventional cigarettes will not necessarily translate into either reduced exposure for users of the PRRTP or a reduction in risk of cigarette smoking-related diseases. Although smoke chemistry alone cannot predict internal exposure of individuals to substances in smoke, it can guide further testing by, for example, indicating which biomarkers may be important to measure.
Constituent analyses can determine whether yields of smoke analytes in smoke from control cigarettes differ significantly from yields for a PRRTP and whether a PRRTP exposes people to substances not measured in smoke from control cigarettes. LSRO recommends conducting and describing appropriate statistical analyses to determine whether smoke constituent yields from PRRTPs and control cigarettes differ significantly. The power of the study is an important consideration. Investigators should evaluate, in terms of possible increases in exposure to users, any significant increase in PRRTP smoke constituent yields compared with appropriate reference and commercially available cigarettes. Analysis of exposure to particles or shards is a component of a PRRTP evaluation.
III.1 SELECTION OF BIOLOGICAL SAMPLES

An important consideration is selection of the appropriate biological sample for biomarker measurement. Biomarkers have been measured in urine, saliva, blood, hair, exhaled breath condensate (EBC), toenail, exhaled air, and other biological materials. Measurement of biomarkers in each type of biological matrix has advantages and disadvantages, some of which are discussed below (Al Delaimy et al., 2002; Al Delaimy, 2002; American Conference of Governmental Industrial Hygienists, 2005).

III.1.1 Blood

According to the American Conference of Governmental Industrial Hygienists (2005), blood sampling has the advantage of low inter-individual variation in blood constituents that affect the amounts of most blood determinants. Other advantages include simplicity of the sampling technique and relatively few chances for contamination. Disadvantages include invasiveness of the sampling method, requirement for trained medical personnel, and rapid sample deterioration under inadequate sample storage and transportation conditions (American Conference of Governmental Industrial Hygienists, 2005). Blood sampling may also be more expensive than other biological sampling methods.

III.1.2 Urine

Urine sampling has the advantage of being non-invasive and likely to produce sufficient sample volume for biomarker measurement. Urine levels of some biomarkers (e.g., nicotine) are typically higher than levels in plasma and saliva (Benowitz et al., 2002). Sample clean up is simpler than that for other biological specimens. A disadvantage of urine sampling is variability in urine production because of behavioral factors, such as fluid intake and meat consumption.
consumption, and biological factors, such as secretion and absorption by renal tubules and urine pH.

Twenty four-hour urine collection is a highly regarded method for collecting urine, because it correlates better with the intensity of exposure than do spot or grab urine samples. Concerns related to 24-hour urine sampling include sample integrity, inconvenience of collection, and subject non-compliance. Spot urine samples, such as first-morning voids or other samples collected at the study participants’ convenience, are also subject to variability in sample volume and concentration of endogenous and exogenous urine components (Barr et al., 2005).

Urine output is expressed as concentration (mg/L), excretion rate (mg/h) or in relation to constituents whose levels change less than water excretion (e.g., total solids, expressed as specific gravity, or creatinine). Creatinine-adjusted measures reportedly correlate better with serum or plasma samples (Barr et al., 2005).

### III.1.3 Saliva

The saliva collection method can influence biomarker levels. For example, cotinine levels in unstimulated saliva samples are higher than levels in stimulated saliva samples (Schneider et al., 1997). Differences in saliva flow rate can alter pH and biomarker levels. Biomarker levels are also influenced by the last meal and the time when the last cigarette was smoked (Stevens & Munoz, 2004).

### III.1.4 Hair

Hair sampling is a non-invasive method that is generally less subject to variability compared with sampling urine, saliva, and blood, because of the slow rate of hair growth (~1 ± 0.3 cm/month) (Al Delaimy, 2002). Hair samples can be stored easily without rapid degradation. Hair sampling is not considered reliable, however, because chemical and physical processing can cause loss of nicotine from the hair shaft. Furthermore, some cultures are against cutting hair, and some individuals have little scalp hair available.

### III.1.5 Exhaled Air

Exhaled air is a potential biological sample matrix for measurement of volatile smoke constituents. The method of measurement is convenient, and chemical analysis is generally simple (American Conference of Governmental Industrial Hygienists, 2005). However, careful sampling is required, and changes in biomarker concentration during exhalation can occur.

For internal use of Philip Morris personnel only.
III.1.6 Toenail

Toenail samples are easy to store and have the advantage of being obtained via non-invasive means. Growth rates of toenails are approximately 0.1 cm/month (Palmeri et al., 2000). Toenail biomarker levels reflect nicotine exposure during 3–5 months and have several advantages to hair samples (Stepanov et al., 2006). Toenails have a lower limit of detection for nicotine than hair and are less likely to be contaminated with the analyte of interest from substances in the environment. Because toenails also grow more slowly than hair, biomarker values reflect cumulative levels and are less subject to irregular growth and variable axial distribution of the biomarker (Stepanov et al., 2006). Stepanov et al. (2006) reported measuring nicotine and the tobacco-specific nitrosamine (TSNA) metabolite 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), in human toenails.

III.2 STUDY PARTICIPANT SELECTION

The goal of the study should be well defined. The effect of criteria used to select study participants on applicability of information from that study to the general population is an important consideration. In some cases, the study objective may be to include a range of smokers; in other cases, to develop and get a representative sample. Studies should include individuals most likely to use the product.

All studies should follow good clinical practice guidelines. An appropriate Institutional Review Board should review the protocol and informed consent documents. Study participants should provide informed consent before the study begins and they should be told that they may withdraw from the study at any time.

III.3 APPROPRIATE CONTROL TOBACCO PRODUCTS

LSRO considers commercially available, conventional cigarettes to be the most appropriate control tobacco products in biomarker studies involving smoked tobacco products. Investigators should describe their rationale for selecting specific control cigarettes. LSRO does not consider Kentucky reference cigarettes to be appropriate control products for biomarker studies of PRRTPs because they are not commercially available and people generally do not enjoy smoking reference cigarettes. LSRO recommends developing smoke chemistry data for control cigarettes and PRRTPs.
Clinical studies to assess exposure include those evaluating biomarkers, tobacco product use behavior, filter analysis, and deposition and retention of particles from smoke. LSRO evaluated and reviewed these methods for assessing exposure, as detailed below.

### III.4 BIOMARKERS OF TOBACCO PRODUCT AND SMOKE EXPOSURE

Exposure to compounds related to tobacco products and smoke is influenced by the level of toxicants in the tobacco product or smoke from the product, the metabolism and absorption of these toxins, and the pattern of product use (Hatsukami et al., 2004a). Numerous studies have shown that smoke chemistry studies are inadequate for predicting exposure of individuals to smoke toxins (Djordjevic et al., 1998, 2000). Because biomarkers reflect integration of exposure-determining factors, LSRO considers them to be most useful for assessing internal exposure to smoke constituents.

LSRO has adapted definitions for different types of biomarkers from those used by the National Research Council (1987), the Institute of Medicine (IOM) Clearing the Smoke Committee (2001), and Hatsukami et al. (2006a) (Figure III.1).

Biomarkers are biological response variables that are measured in biological fluids, tissues, cells, and subcellular components and are indicative of exposure and/or effect. A “biomarker of exposure” is a constituent or metabolite that is measured in a biological fluid or tissue, or that is measured after it has interacted with critical subcellular, or target tissues (“biologically effective dose”). Examples of biomarkers of a biologically effective dose include carboxyhemoglobin (COHb), DNA adducts, and protein adducts. A “biomarker of effect” is a measured effect including an early subclinical

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**Figure III.1** Biomarkers of exposure and effect. Broken lines indicate that the biomarkers used may or may not be directly related to the final disease or condition.

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biological effect; alteration in morphology, structure, or function; or clinical symptom(s) consistent with the development of health impairment or disease. Figure III.1 shows the relationships between exposure, biomarkers, and disease. Biomarkers of exposure are discussed in this report and biomarkers of effect are discussed in the Biological Effects Assessment in the Evaluation of Potential Reduced-Risk Tobacco Products report (Life Sciences Research Office, 2007a).

Various biomarkers have been used to assess exposure to tobacco product and smoke constituents (Benowitz et al., 2002, 2005; Hecht, 2002; Phillips, 2002; Scherer, 2005). LSRO considered biomarker characteristics and analytical methods of greatest value in measuring exposure to such constituents and developed the list of desirable biomarker characteristics given below.

Biomarkers of greatest value in assessing exposure would have the following attributes:
- Tobacco specificity or substantial difference between smokers and nonsmokers;
- Intra-individual variation that mirrors variation in smoking behavior;
- Existing database on its pharmacokinetics;
- Low analytical method variation;
- Sensitivity and chemical specificity of analytical method(s); and
- Existence of other biomarkers that can confirm the exposure.

LSRO also discussed the need for biomarker validation. Methods for systematic validation of biomarkers have been published (U.S. Food and Drug Administration, 2001; World Health Organization, 2000).

LSRO reviewed information in the scientific literature about biomarkers used to assess tobacco-product-related exposure and ranked them in terms of potential usefulness in PRRTP exposure assessment studies. In ranking biomarkers, LSRO considered whether the biomarker had the desirable characteristics, the invasiveness of the sample acquisition method, and the phase of smoke in which the biomarker or its precursor in smoke is found.

LSRO developed three categories of biomarkers, with different levels of utility for tobacco product or smoke exposure. LSRO considers “Category A” biomarkers to be sufficiently studied, reliable tools for estimating exposure (Table III-1). Category A biomarkers are mostly urinary metabolites of tobacco product and smoke constituents, but two adducts
of hemoglobin (Hb) (4-aminobiphenyl [ABP]-Hb and N-(2-cyanoethyl)valine-
Hb) are also included. Hb adducts are easier to measure than DNA adducts, and because their amounts are proportional to those of DNA adducts they are sometimes used as biomarkers of exposure. The mere presence of DNA adducts alone is not evidence of mutation. Furthermore, at present, no certain threshold or quantitative correlation exists between DNA adducts and production of tumors (Pottenger et al., 2004). Available evidence suggests that there is no threshold for DNA adduct formation but that a threshold for tumor formation exists (Waddell, 2006). This result may suggest that at some point DNA repair is overwhelmed and tumorigenesis begins. However, scientists almost universally agree that DNA adduct formation is necessary for tumorigenesis induced by alkylating chemical carcinogens.

LSRO considers “Category B” biomarkers to be promising but deficient in one or more desirable characteristics; therefore, LSRO has a lower level of confidence in Category B biomarkers than in Category A biomarkers (Table III-1). LSRO recommends consideration of Category A and B biomarkers for routine use in PRRTP studies.

LSRO has the lowest level of confidence in “Category C” biomarkers and does not recommend their routine use in PRRTP exposure assessment (Table III-2). However, if smoke chemistry or other studies provide reason to suspect elevated levels of biomarkers for a specific chemical after use of the PRRTP or exposure to smoke from the PRRTP, LSRO recommends measurement of the relevant biomarker(s), regardless of category.

LSRO acknowledges that the biomarkers discussed do not constitute a comprehensive collection and that there is flexibility to add to the list of useful biomarkers. If necessary, analytical methods and techniques should be developed and validated. These rankings reflect LSRO’s judgment based on the current state-of-the-science. Identification of new validated biomarkers and advances in analytical methodology could affect biomarker rankings.

This report organizes biomarkers of exposure according to their precursors. Discussions of the utility of using biomarkers to assess cigarette smoke exposure, including selected studies comparing biomarker levels after use of PRRTPs with levels after use of conventional cigarettes are found below.
<table>
<thead>
<tr>
<th>Category A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomarker</td>
</tr>
<tr>
<td>Nicotine and at least 5 major nicotine metabolites, including cotinine</td>
</tr>
<tr>
<td>Carbon monoxide (CO); carboxyhemoglobin (COHb)</td>
</tr>
<tr>
<td>Urine mutagenicity</td>
</tr>
<tr>
<td>1-Hydroxypyrene (1-HOP)</td>
</tr>
<tr>
<td>3-Hydroxypropyl mercapturic acid (3-HPMA)</td>
</tr>
<tr>
<td>trans,trans-Muconic acid (t,t-MA)</td>
</tr>
<tr>
<td>S-Phenyl mercapturic acid (SPMA)</td>
</tr>
<tr>
<td>Monohydroxybutenyl mercapturic acids (MHBMA, or MII)</td>
</tr>
</tbody>
</table>
Table III-1. Biomarkers of Tobacco Smoke Exposure Recommended for Routine Use in Clinical Studies (continued)

<table>
<thead>
<tr>
<th>Category A</th>
<th>Biomarker</th>
<th>Precursor</th>
<th>Biological matrix</th>
<th>Comments/ selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biomarker</strong></td>
<td><strong>Precursor</strong></td>
<td><strong>Biological matrix</strong></td>
<td><strong>Comments/ selected references</strong></td>
<td></td>
</tr>
<tr>
<td>4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide</td>
<td>Tobacco-specific nitrosamines (TSNAs)</td>
<td>Urine</td>
<td>(Breland <em>et al.</em>, 2003; Hughes <em>et al.</em>, 2004)</td>
<td></td>
</tr>
<tr>
<td>4-Aminobiphenyl-hemoglobin (Hb) adducts</td>
<td>4-Aminobiphenyl</td>
<td>Blood</td>
<td>(Bartsch <em>et al.</em>, 1990)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category B</th>
<th>Biomarker</th>
<th>Precursor</th>
<th>Biological matrix</th>
<th>Comments/ selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>Acetonitrile</td>
<td>Exhaled breath</td>
<td>(Houeto <em>et al.</em>, 1997; Lirk <em>et al.</em>, 2004)</td>
<td></td>
</tr>
<tr>
<td>Anatabine, anabasine</td>
<td>Minor tobacco alkaloids</td>
<td>Urine</td>
<td>(Jacob, III <em>et al.</em>, 1999, 2002)</td>
<td></td>
</tr>
<tr>
<td>Dihydroxybutyl mercapturic acid (DHBMA, or MI)</td>
<td>1,3-Butadiene</td>
<td>Urine</td>
<td>(Urban <em>et al.</em>, 2003; van Sittert <em>et al.</em>, 2000)</td>
<td></td>
</tr>
<tr>
<td>3-Hydroxybenz[a]anthracene</td>
<td>Benz[a]anthracene</td>
<td>Urine</td>
<td>(Gündel &amp; Angerer, 2000; Simon <em>et al.</em>, 2000)</td>
<td></td>
</tr>
<tr>
<td>3-Hydroxybenzo[a]pyrene</td>
<td>Benzo[a]pyrene</td>
<td>Urine</td>
<td>(Gündel &amp; Angerer, 2000; Simon <em>et al.</em>, 2000)</td>
<td></td>
</tr>
</tbody>
</table>

**Category A**: Biomarkers that have been sufficiently studied and provide reliable exposure measurements. **Category B**: Biomarkers that have sufficient data to support their use in exposure studies but also have limitations related to one or more desirable biomarker characteristics. **PRRTPs**: potential reduced-risk tobacco products (Life Sciences Research Office, 2007b).
<table>
<thead>
<tr>
<th>Category C</th>
<th>Biomarker</th>
<th>Precursor</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N-Acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA) and N-(R,S)-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA)</td>
<td>Acrylamide</td>
<td>(Boettcher et al., 2005b; Boettcher &amp; Angerer, 2005)</td>
</tr>
<tr>
<td></td>
<td>1-, 2-, 3-, and 4- Aminonaphthalenes</td>
<td>Aromatic amines</td>
<td>(Grimmer et al., 2000; Riffelmann et al., 1995)</td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td>Benzene</td>
<td>(Jordan et al., 1995)</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>Cadmium</td>
<td>(Järup et al., 1998; Satarug et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>2,5-Dimethylfuran</td>
<td>2,5-Dimethylfuran</td>
<td>(Ashley et al., 1996; Perbellini et al., 2003)</td>
</tr>
<tr>
<td></td>
<td>1- and 2-Naphthols</td>
<td>Naphthols</td>
<td>(Nan et al., 2001; Yang et al., 1999)</td>
</tr>
<tr>
<td></td>
<td>Thiocyanate</td>
<td>Hydrogen cyanide</td>
<td>(Degiampietro et al., 1987; Torano &amp; van Kan, 2003)</td>
</tr>
<tr>
<td></td>
<td>1-, 2-, 3-, and 4- Hydroxyphenanthrenes</td>
<td>Phenanthrenes</td>
<td>(Heudorf &amp; Angerer, 2001; Jacob et al., 1999)</td>
</tr>
<tr>
<td></td>
<td>Thioethers</td>
<td>Electrophilic compounds</td>
<td>(Feng et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>3-Methyladenine in urine</td>
<td>Methylating substances</td>
<td>(Prevost et al., 1990)</td>
</tr>
</tbody>
</table>
### Table III-2: Biomarkers of Tobacco Smoke Exposure Not Recommended for Routine Use in Clinical Studies (continued)

<table>
<thead>
<tr>
<th>Category C</th>
<th>Biomarker</th>
<th>Precursor</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3-Ethyladenine in urine</td>
<td>Ethylating substances</td>
<td>Kopplin et al., 1995; Prevost &amp; Shuker, 1996</td>
</tr>
<tr>
<td></td>
<td>5-Hydroxymethyluracil</td>
<td>Reflects DNA damage by oxygen free radicals</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td></td>
<td>Nitrosated proteins</td>
<td>1,3-Butadiene adducts</td>
<td>Fustinoni et al., 2002</td>
</tr>
<tr>
<td></td>
<td>1,3-Butadiene adducts</td>
<td>B[a]P</td>
<td>Hoffmann &amp; Brunennmann, 1983; Ladd et al., 1984</td>
</tr>
<tr>
<td></td>
<td>Benzo[a]pyrene (B[a]P)-albumin adducts</td>
<td>B[a]P</td>
<td>Fusimoni et al., 2002 (Aturup et al., 1995; Crawford et al., 1994; Sherson et al., 1990; Tas et al., 1994)</td>
</tr>
<tr>
<td></td>
<td>Bulky DNA adducts</td>
<td>B[a]P-hemoglobin (Hb) adducts</td>
<td>Grimmer et al., 1987; Hoffmann &amp; Hoffmann, 1997</td>
</tr>
<tr>
<td></td>
<td>Aromatic amine-DNA adducts</td>
<td>Large, primarily apolar compounds i.e., polycyclic aromatic hydrocarbons (PAH), aromatic amines, and others</td>
<td>Bulky DNA adducts</td>
</tr>
<tr>
<td></td>
<td>Etheno-DNA adducts</td>
<td>F_{2}-Isoprostanes</td>
<td>Vinyl chloride, lipid peroxidation-derived products</td>
</tr>
<tr>
<td></td>
<td>Aromatic amine-DNA adducts</td>
<td>Arachidonic acid peroxidation</td>
<td>Aromatic amines</td>
</tr>
</tbody>
</table>
Table III-2. Biomarkers of Tobacco Smoke Exposure Not Recommended for Routine Use in Clinical Studies (continued)

<table>
<thead>
<tr>
<th>Category C</th>
<th>Biomarker</th>
<th>Precursor</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4-Hydroxy-1-(3-pyridyl)-1-butano (HPB)-DNA adducts</td>
<td>Tobacco-specific nitrosamines (TSNAs)</td>
<td>(Foiles et al., 1991)</td>
</tr>
<tr>
<td></td>
<td>HPB-Hb adducts</td>
<td>TSNAs</td>
<td>(Falter et al., 1994; Hecht et al., 1991)</td>
</tr>
<tr>
<td></td>
<td>8-Hydroxy-2'-deoxyguanosine</td>
<td>Marker of hydroxyl radical damage to DNA</td>
<td>(Daube et al., 1997)</td>
</tr>
<tr>
<td></td>
<td>N-(2-Hydroxyethyl)valine-Hb adducts</td>
<td>Ethylene or ethylene oxide</td>
<td>(Fennell et al., 2000; Schettgen et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>7-Methylguanine adducts</td>
<td>Methylating agents such as 4-[(Methylnitrosamino)-1-(3-pyridyl)-1-butano (NNK) and Nitrosodimethylamine (NDMA)</td>
<td>(Mustonen et al., 1993; Mustonen &amp; Hemminki, 1992)</td>
</tr>
<tr>
<td></td>
<td>N-(2-Carbamoylethyl)valine-Hb adducts</td>
<td>Acrylamide</td>
<td>(Bergmark, 1997; Hagmar et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>PAH-DNA adducts</td>
<td>PAH</td>
<td>(Kriek et al., 1998; Mooney et al., 1995; Tang et al., 1995)</td>
</tr>
<tr>
<td></td>
<td>N-Ethylvaline-Hb adducts</td>
<td>Not known, possibly nitrosation of ethylamine, acetonitrile</td>
<td>(Carmella et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>N-Methylvaline-Hb adducts</td>
<td>NDMA, NNK methyl halides, and other substances</td>
<td>(Bader et al., 1995; Carmella et al., 2002)</td>
</tr>
</tbody>
</table>

**Category C**: Biomarkers that are considered insufficiently reliable for routine use in exposure studies. (Life Sciences Research Office, 2007b).
III.4.1 Nicotine and Metabolites

III.4.1.1 Nicotine

Nicotine is the primary alkaloid in tobacco and is found in mainstream smoke (MS) at levels of 1–2 mg/cigarette (Dhar, 2004). Nicotine levels differ in different parts of a single plant and within and among tobacco strains (Benowitz et al., 1990). The desire to maintain a certain level of nicotine is thought to be the key motivation for continuing the smoking habit (Benowitz et al., 1994; U.S. Department of Health and Human Services, 1988), although reinforcing motor and non-nicotine sensory components of the smoking process, packaging, and other factors also influence maintenance of the habit (Rose, 2006). Nicotine is a highly specific marker of tobacco smoke exposure and has been widely used as a measure of tobacco product or smoke exposure. The major disadvantage of using nicotine is its short half-life. Because of nicotine’s pharmacokinetic properties, many investigators instead measure cotinine, its primary metabolite (Heinrich-Ramm et al., 2002). LSRO recommends routine measurement of nicotine in PRRTP evaluations but advises concurrent measurement of at least five major nicotine metabolites, including cotinine, to better estimate nicotine exposure.

Smokers’ nicotine levels are influenced by cigarette design, cigarette puffing and smoke inhalation behaviors, pregnancy, mucosal membrane pH, and genetic polymorphisms in nicotine-metabolizing enzymes (Byrd et al., 1995). Genetic polymorphisms can contribute to large inter-individual variations in nicotine metabolism (Benowitz & Jacob, III, 1997). Certain vegetables and other foods also serve as non-tobacco nicotine sources (Davis et al., 1991). Tomatoes, potatoes, and cauliflower contain nicotine, as do black tea and instant tea. However, the contribution of dietary sources of nicotine to body levels is considered to be negligible.

Smokers absorb approximately 1–1.4 mg nicotine/cigarette (Benowitz & Jacob, III, 1984, 1994; Fagerström, 2005), and their arterial blood nicotine levels typically range from less than 1 ng/ml to more than 50 ng/ml (Byrd et al., 2005). However, some studies reported arterial blood levels of 100 ng/ml (Henningfield et al., 1993). Approximately 5–9% of nicotine taken into the body is excreted unmetabolized in urine (Benowitz et al., 1994). Because of its relatively short half-life (1–2 hours), blood nicotine levels fluctuate throughout the day. As a result, these levels depend to a great degree on time of sampling and are often measured in the afternoon, when they are somewhat more stable (Benowitz, 1983; Benowitz & Jacob, III, 1994).
A smoker’s nicotine dose depends on cigarette smoking behavior and, potentially, nicotine dependence level, gender, race, level of nicotine sought, pulmonary condition, and characteristics of the specific cigarette (Bridges et al., 1986; Clark et al., 1998; McCarthy et al., 1992; Patterson et al., 2003; Zacny et al., 1987).

Many analytical methods have been used to assess nicotine exposure, each with associated advantages and disadvantages. Radioimmunoassays (RIAs), which had historically been used, have been replaced by other methods because of limitations such as the potential for cross-reactivity with matrix components, the measurement of only one component at a time, the requirement for radioactive materials and antisera for each metabolite, and the need to dispose of radioactive material (Byrd et al., 2005). An enzyme-linked immunosorbent assay (ELISA) does not require costly equipment and reagents or radioactive material. Furthermore, ELISA requires a shorter time for analysis (less than 5 hours) compared with the more than 48 hours required for the RIA (Dhar, 2004).

Various chromatography methods with different detection limits have been used to measure nicotine and its metabolites. Rapid liquid chromatography (LC)-tandem mass spectrometry permits simultaneous measurement of nicotine and cotinine. According to Byrd et al. (2005), this technique requires very little sample, is based on solid phase extraction in a 96-well plate format, and uses normal phase chromatography that increases sample throughput. The method is also more accurate and precise and has fewer matrix effects than does RIA. LC-tandem mass spectrometry has increased accuracy and precision compared with RIA, but instrumentation is more expensive and more complicated (Byrd et al., 2005).

High-performance liquid chromatography (HPLC) allows measurement of nicotine and cotinine in plasma and saliva (Dhar, 2004). Gas chromatography (GC)-mass spectrometry has been used to measure nicotine in urine, saliva, and hair. This method has been described as relatively simple, quick, and capable of simultaneous assay of nicotine and cotinine (James et al., 1998). LC-tandem mass spectrometry with atmospheric pressure chemical ionization in the positive ion mode was used to measure nicotine, cotinine, trans-3′-hydroxycotinine, and their respective glucuronides (Meger et al., 2002). Nicotine-N-oxide, cotinine-N-oxide, and norcotinine, which account for 12% of nicotine metabolites, were also measured. This method is fast, allows for high sample throughput, is less expensive and more precise than nitrogen-phosphorus GC, does not require sample extraction, and measures glucuronides directly, which reduces analytical variation.
Colorimetry is a simple, inexpensive procedure that can estimate total nicotine metabolites (Dhar, 2004). A disadvantage is its non-specificity, because drugs with a pyridine nucleus, such as isoniazid and nicotinamide, can affect measurement (Dhar, 2004).

### III.4.1.2 Cotinine

Approximately 70–80% of nicotine taken into the body is converted to cotinine (Benowitz & Jacob, III, 1994). Cotinine is further metabolized into trans-3′ hydroxycotinine, cotinine glucuronide, cotinine-N-oxide, norcotinine, and other substances. Approximately 10–15% of cotinine is excreted unchanged in urine (Benowitz et al., 1983). LSRO recommends routine measurement of cotinine concurrently with nicotine and at least four major nicotine metabolites.

Cotinine has been measured in blood, serum, saliva, urine, amniotic fluid, cervical mucus, and hair (Binnie et al., 2004; Chetiyanukornkul et al., 2004; Dahlström et al., 1990; Thomas et al., 2006). Its half-life is approximately 16 hours. It would take approximately 80 hours for an abstaining smoker with a starting cotinine level of 300 ng/ml to reach the cotinine level of a nonsmoker (Benowitz et al., 2002). Ethnicity and pregnancy influence cotinine’s half-life (Benowitz et al., 2002). Higher concentrations of cotinine than nicotine occur in blood after a few hours (Benowitz et al., 1983). Saliva cotinine and nicotine concentrations are approximately 25% higher than the plasma cotinine and nicotine concentrations (Jarvis et al., 2003), but the saliva cotinine concentration can vary with age and method of saliva sample collection. Stimulated saliva samples yield lower levels of cotinine than unstimulated samples (Schneider et al., 1997). Urinary cotinine concentration is generally twice as high as salivary and plasma cotinine concentrations (Watts et al., 1990).

Smokers’ cotinine levels rise incrementally throughout the day, reach their highest levels at the end of the day, and remain high overnight. A typical cutoff level for cotinine in smokers is any value higher than 15 ng/ml in plasma and saliva and higher than 50 ng/ml in urine (Benowitz et al., 2002).

Large inter-individual differences occur in the rate of renal cotinine clearance (19–75 ml/min) and in the percentage of nicotine metabolized to cotinine (Benowitz et al., 1983; Benowitz & Jacob, III, 1994). The half-life of cotinine in Black American and Chinese American individuals is longer than in White American individuals (Benowitz et al., 1999). Cotinine clearance during pregnancy is faster than clearance in the non-pregnant state (Dempsey et al., 2002). Clearance of nicotine, both total and non-renal, is reduced in individuals with kidney failure or liver cirrhosis (Molander et al., 2000).
Methods used to measure cotinine include enzyme immunoassays, HPLC, RIA, IMMULITE®, GC-, LC-mass spectrometry (Dhar, 2004). Using rapid LC-mass spectrometry-mass spectrometry Byrd et al. (2005) measured nicotine and cotinine. Nicotine and cotinine levels for non-smokers were below the limit of quantitation for the assay. LC-tandem mass spectrometry has increased accuracy and precision compared with RIA. Disadvantages are that the instrumentation is more expensive and more complicated than that for RIA (Byrd et al., 2005).

### III.4.1.3 Nicotine + metabolites

Whereas cotinine alone accounts for 15% or less of total nicotine intake, the sum of nicotine, cotinine, and trans-3′-hydroxycotinine and their respective glucuronides accounts for 80–85% of the total nicotine taken into the body (Benowitz et al., 1994). Simultaneous measurement of these substances (nicotine and at least five major metabolites, including cotinine) more accurately reflects total nicotine exposure and metabolism than measurement of only one metabolite, and this assay can be done non-invasively, by using urine (Roethig et al., 2005; Xu et al., 2004).

LSRO therefore recommends that nicotine and at least five of its major metabolites, including cotinine, be measured routinely in PRRTP evaluations (Category A biomarker).

### III.4.2 Carbon Monoxide

Carbon monoxide (CO) is an incomplete combustion product found in the gas phase of tobacco smoke. CO binds to Hb to form COHb with an affinity 240-fold greater than that of oxygen binding to Hb (Douglas et al., 1912). Although environmental sources (e.g., motor vehicle emissions, gas heaters, and cookers) and endogenous sources of CO compromise its usefulness as a biomarker of exposure to tobacco smoke, CO is particularly useful as a measure of a gas phase constituent of cigarette smoke and has been used as such a biomarker in PRRTP studies. These studies showed differences in CO levels after subjects smoked conventional cigarettes and after they smoked some PRRTPs. LSRO recommends routine measurement of CO in PRRTP studies (“Category A”).

CO exposure has been measured as both COHb levels and CO in exhaled air. Cunnington and Hormbrey (2002) reported a positive dose-response relationship between the number of cigarettes smoked per day and exhaled CO levels ($p = 0.01$). Wald et al. (1981) showed a strong correlation (correlation coefficient, $r = 0.97$) between CO in exhaled air and COHb levels.
Measurement of CO in exhaled breath is simple and non-invasive and yields immediate results (Deveci et al., 2004). Exhaled breath CO can be measured by using a monitor that determines the rate of conversion of CO to carbon dioxide as CO passes over a catalytically active electrode (Benowitz et al., 2002; Wald et al., 1981). The monitors are inexpensive and portable and do not require trained medical personnel for operation (Togores et al., 2000). CO levels in exhaled air are less than 3 ppm for nonsmokers in a smokeless environment and higher than 8 ppm for active smokers (Benowitz et al., 2002). Measurement of exhaled CO is more likely to be acceptable to subjects than measurement of COHb (World Health Organization, 2003a) because it is less invasive.

COHb values depend on the time of day of sampling, number of cigarettes smoked before sampling, extent of exposure to CO from sources other than cigarette smoking, medical status and demographics of study participants, and assay methods (Smith et al., 1998). Other factors influencing COHb levels are exercise, ambient temperature, and individual rates of CO metabolism (World Health Organization, 2003a).

Use of CO as a biomarker of exposure to tobacco smoke involves certain considerations. As mentioned, the timing of CO measurement is critical because of the short half-life of the biomarker. Physical activity also influences CO levels: the half-life of CO is as short as 1 hour for active individuals, 2–3 hours for sedentary individuals, and 4–8 hours during sleep (Benowitz et al., 2002; Coburn et al., 1965). Blood samples for COHb measurement are typically obtained by standard venipuncture, with COHb usually measured soon after sample acquisition (Smith et al., 1998). Samples of blood plus an anticoagulant should be housed in a closed container and can be kept, at 4°C in the dark, for a number of days before analysis. COHb can be measured via spectrophotometry (Leone, 2005). COHb levels in nonsmokers are between 0.3% and 0.7% (World Health Organization, 2003a). Active smokers have mean levels of 4% COHb, with a range of 3–8% (World Health Organization, 2003a).

CO has been used as a biomarker of tobacco smoke exposure in a number of PRRTP studies. Sutherland et al. (1993) measured expired air CO levels for 20 smokers who switched from their usual cigarette brand to a cigarette-like PRRTP, brand name Premier® that heats but does not burn tobacco. Mean expired air CO levels increased by 19% ($p = 0.018$) after subjects smoked Premier® for 3 days. Smith et al. (1998) measured COHb and expired air CO levels for 14 smokers who used their usual brand of cigarette for 5 days after which 12 of these subjects smoked a research cigarette prototype
that heats rather than burns tobacco (TOB-HT) for 5 days. Although subjects smoked the same numbers of usual brand and TOB-HT cigarettes, use of the TOB-HT increased levels of COHb by 24.4% and expired air CO by 30.6% compared with the smokers’ usual cigarette.

The Omni® cigarette is a type of PRRTP that contains tobacco treated with a palladium catalyst to increase burning efficiency. In a crossover study, Hughes et al. (2004) measured the difference between expired air CO levels before and after smoking for 19 light/ultra-light cigarette smokers and 15 regular cigarette smokers. Subjects used their usual brand or Omni® cigarettes for 6 weeks and then smoked the other type of cigarette for 6 weeks. Omni® cigarettes produced a significantly larger CO increase (21% higher; 95% confidence interval [CI 3–38% higher]) than did the subjects’ own brand of cigarettes ($F$ [variance ratio] = 5.74, $p = 0.02$). Cigarette machine smoking showed that Omni® cigarettes produced 16% less to 10% more CO than conventional cigarettes with which they were compared. This finding may have been due to differences between Omni® and conventional cigarette filters, type of tobacco, or vent blocking (Hughes et al., 2004).

Breland et al. (2002a) compared exhaled air CO levels after subjects smoked their own cigarette brand, denicotinized cigarettes, Eclipse®, or Accord®. Eclipse® and Accord® are cigarette-like PRRTPs that primarily heat, instead of burning tobacco. Twenty smokers abstained from smoking overnight, and the following day puffed 1 type of cigarette 8 times within a 30-minute period. Smoking sessions for each type of cigarette were separated by 24 hours, and all subjects smoked each type of cigarette. Smoking Eclipse® cigarettes increased CO levels in air exhaled by smokers by an average of 33% compared with their own brand of cigarettes, whereas smoking Accord® cigarettes reduced these levels compared with their own brand. In another study, Breland et al. (2002b) measured expired air CO levels when subjects smoked their own brand of cigarettes or Advance® (a PRRTP) or when they sham-smoked unlit conventional cigarettes. Filter ventilation holes of all cigarettes were blocked. Twenty subjects abstained from smoking the night before the study began. In this crossover study, subjects participated in 2.5-hour sessions during which they took 8 puffs of 1 type of cigarette in 4 different blocks of time. Different kinds of cigarettes were smoked on different days. Advance® cigarettes delivered 11% less CO than their own brand of cigarettes.

In a later study, Breland et al. (2003) measured expired air CO levels for 12 individuals after they smoked their own brand of light or ultra-light cigarettes.

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4 Hughes et al. (2004) cited www.omni.cigs.com as the source of this information, but this web site is no longer active.

For internal use of Philip Morris personnel only.
Lee et al. (2004) reported an increase in exhaled CO for 10 smokers after they smoked Eclipse® or their own brand of cigarettes. The mean FTC yields of their own brand of cigarettes were 1.1 mg of nicotine and 14.5 mg of tar. Testing sessions were held 24 hours apart. Study participants smoked the cigarette while holding it in their hand, or they smoked it through a mouthpiece connected to a smoking topography device. Cigarettes were smoked in random order. Smoking Eclipse® cigarettes resulted in statistically non-significant increases in exhaled breath CO (6.6 ± 0.6 ppm) compared with the level of 4.5 ± 0.6 ppm obtained after smoking their own brand of cigarettes (Lee et al., 2004).

Fagerström et al. (2000) studied the effects of smokers switching from their own brand of cigarettes to Eclipse® cigarettes or a nicotine inhaler on CO levels. Subjects were randomly assigned either to smoke Eclipse® or to use the Nicotrol® nicotine inhaler for 2 weeks and then to switch to the other method. Subjects were asked to smoke as few of their own brand of cigarettes as possible during the study and use the substitute product as needed. Expired air CO values were determined prior to switching to the first product. Smoking Eclipse® cigarettes increased the exhaled breath CO value to 33 ppm compared with 21 ppm when subjects smoked their own cigarettes (p = 0.001; n = 40). CO levels after smokers used the inhaler were significantly reduced (12.7 ppm; p = 0.001; n = 40) compared with when subjects smoked their own brand.

In another study of Eclipse®, 38 subjects from the study just described were asked to use Eclipse® or the Nicorette® inhaler for 8 weeks to help them lower the number of cigarettes they smoked per day (Fagerström et al., 2002). Subjects were allowed to choose between using Eclipse® (n = 10) and the inhaler (n = 15), or, if they liked neither product, to continue smoking their own brand of cigarettes (n = 13). When subjects used Eclipse®, their exhaled breath CO levels increased by 45% (32.5 ppm) compared with baseline (22.2 ppm). Use of the inhaler resulted in a 47% decrease in exhaled breath CO levels (12.5 ppm). Exhaled breath CO levels after smoking Eclipse®
were significantly higher than levels after using the inhaler \((p = 0.05)\), but they were not significantly higher than levels for those who continued to smoke cigarettes. Subjects who used Eclipse\textsuperscript{®} also had significantly higher levels of COHb (7.7\%) than subjects who used the inhaler (3.5\%; \(p = 0.05)\).

Roethig et al. (2005) measured expired air CO and blood COHb levels in 110 smokers divided into 5 groups. Subjects switched from smoking their usual brand of cigarette to smoking an electrically heated cigarette (EHC) either Accord\textsuperscript{®} or Oasis\textsuperscript{®} or a conventional cigarette (Marlboro Lights\textsuperscript{®} or Marlboro Ultra Lights\textsuperscript{®}). Twenty subjects smoked each type of cigarette; 30 subjects abstained from smoking. CO yields of these EHCs have reportedly been approximately 90\% lower than the yield of the University of Kentucky 1R4F reference cigarette (Stabbert et al., 2003). Subjects were allowed to acclimate to the clinical setting for 1 day, during which their smoking was monitored. Subjects were not permitted to smoke more cigarettes than they smoked on their acclimation day plus 20\%. The CO value was obtained within 1 minute of cigarette consumption. The exhaled CO level decreased by 93\% for people who abstained from smoking, by 80\% for smokers of the electrically heated cigarettes, and by 18\% in the afternoon and 30\% in the evening for individuals who smoked Marlboro Ultra Lights\textsuperscript{®} cigarettes. The COHb level decreased by 95\% for subjects who abstained from smoking, by 93\% for smokers of Accord\textsuperscript{®} and Oasis\textsuperscript{®}, and by 39\% for smokers of Marlboro Ultra Lights\textsuperscript{®} cigarettes (Roethig et al., 2005).

III.4.3 Urine Mutagenicity

Cigarette smoke contains many carcinogenic and mutagenic substances. Urine mutagenicity has been used as an integrated indicator of genotoxic exposure. The mutagenicity of smokers’ urine has reportedly been higher than that of nonsmokers’ urine (Bartsch et al., 1990; Granella et al., 1996; Pavanello et al., 2002a; Yamasaki & Ames, 1977). Urine mutagenicity is not a specific biomarker of tobacco exposure, because occupational exposure to carcinogens (Dolara et al., 1981), chemotherapy (Speck et al., 1976), and diet can increase it (Baker et al., 1982; Baker et al., 1986). The half-life of mutagenic activity in the 

\textit{Salmonella} urine mutagenicity assay is approximately 7 hours (Kado et al., 1985; Scherer, 2005). PRRTP studies have assessed urine mutagenicity, which has shown significant differences between PRRTPs and conventional cigarettes. LSRO ranks urine mutagenicity as a Category A biomarker.

Mure et al. (1997) compared the mutagenicity of urine of 32 smokers with that of urine of 37 nonsmokers. Smokers’ urine showed significantly higher levels of mutagenic activity than nonsmokers’ urine \((p < 0.05)\). Although one
study reported a dose-response relationship between number of cigarettes smoked and urine mutagenicity (Mure et al., 1997), other studies have not demonstrated such a relationship (Einstö et al., 1990; Kado et al., 1985).

Bowman et al. (2002) compared urine mutagenicity for nonsmokers (n = 31) with that for regular smokers of ultra-low-tar (n = 11), full-flavor low-tar (n = 41), and full-flavor tar cigarettes (n = 15) who switched to smoking Eclipse® cigarettes for 1 week and then returned to smoking their own brand of cigarettes. The study participants avoided consuming pan-fried, broiled, and pre-cooked meats because mutagenic pyrolysis products are present in such foods (Doolittle et al., 1989; Nagao et al., 1977a; Nagao et al., 1977b; Smith et al., 1996). Subjects consumed cigarettes ad libitum. Nonsmokers’ urine samples were significantly less mutagenic (approximately 15-fold) than smokers’ urine samples (p < 0.05) and had 4-fold lower mutagenicity than urine samples from Eclipse® smokers. All smokers’ urine samples were significantly less mutagenic after switching to Eclipse® cigarettes (p < 0.05). Reductions in urine mutagenicity ranged between 70% and 77% across the usual brand tar categories when the Salmonella typhimurium strains TA98 and YG1024 were used in the test.

Smith et al. (1996) reported reduced urine mutagenicity when smokers used cigarettes that mostly heat tobacco compared with using their usual brand of conventional cigarette. In this crossover study, each subject smoked both their own brand of cigarette and the cigarette that primarily heats tobacco. The 24-hour urine samples, collected twice weekly from smokers, were tested for urine mutagenicity and compared with mutagenicity of urine of 14 nonsmokers. Subjects were not permitted to eat pan-fried and broiled meats. Urine mutagenicity decreased by 74% after subjects smoked the cigarette that mostly heats tobacco when the test utilized the bacterial strain YG1024 and by 72% when the strain was TA98. The YG1024 strain is more sensitive than its parent TA98 strain to aromatic nitro, amino, and hydroxylamino compounds (Watanabe et al., 1990).

Roethig et al. (2005) compared urine mutagenicity for regular smokers of Marlboro Lights® cigarettes who switched to smoking Accord®, Oasis®, or Marlboro Ultra Lights® cigarettes with that of subjects who continued to smoke Marlboro Lights® or who stopped smoking for 8 days. On Day 8 of the study, urine mutagenicity declined by 53% for those who switched to Accord® (n = 20), by 66% for those who switched to Oasis® (n = 18), by 26% for those who switched to Marlboro Ultra Lights® cigarettes (n = 19), and by 58% for the no-smoking group (n = 29), but urine mutagenicity for subjects who continued to smoke Marlboro Lights® did not decrease (n = 20).
Urine mutagenicity has been assessed by using tools such as C\textsubscript{18} resin columns (Yamasaki & Ames, 1977), XAD-2 resin (Mohtashamipur \textit{et al.}, 1985), HPLC (Rannug \textit{et al.}, 1988), blue rayon extraction (Einistö \textit{et al.}, 1990), and liquid/liquid extraction of mutagens with dichloromethane or chloroform. The XAD-2 method was described as time-consuming and laborious. Furthermore, high background mutagenicity of XAD-2 water sample concentrates has been reported (Curvall \textit{et al.}, 1987).

Diet and occupational and environmental exposures influence urine mutagenicity. Elevated cytochrome P450 1A2 (CYP1A2) enzyme activity has been associated with increased mutagenicity (Pavanello \textit{et al.}, 2002b). Pavanello \textit{et al.} (2002a) assessed mutagenic activity of urine samples from 118 healthy smokers who had been asked to avoid consuming charcoal-grilled and pan-fried meat for 24 hours prior to beginning the study. They reported that although various investigators have found differences in urine mutagenicity between smokers and nonsmokers, they have not always shown a relationship between urine mutagenicity and daily cigarette consumption or the amount of nicotine or tar in the smoke from cigarettes (Einistö \textit{et al.}, 1990). Pavanello \textit{et al.} (2002a) also noted that studies with a small sample size and not controlled for diet have not shown a relationship between daily cigarette consumption and/or nicotine content of cigarettes.

\section*{III.4.4 \textit{N}-Nitrosamine Biomarkers}

\subsection*{III.4.4.1 Tobacco-specific nitrosamines}

TSNAs are present only in tobacco products but can be formed in products that contain nicotine. They are produced during tobacco drying, fermentation, and pyrolysis (Hoffmann \textit{et al.}, 1994; Spiegelhalder & Bartsch, 1996). Nitrate levels during growth, curing, fermenting, and aging of tobacco influence TSNA levels (Burton \textit{et al.}, 1992).

The TSNA 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is formed by nitrosation of nicotine or another tobacco alkaloid, pseudonicotine 4-(methylamino)-1-(3-pyridyl)-1-butanone (Hecht, 2002). \textit{N}'-Nitrosonornicotine (NNN), another TSNA present in tobacco smoke, is formed via nitrosation of nicotine, nornicotine, and myosmine (Hecht \textit{et al.}, 1977; Klus & Kuhn, 1975; Zwickenpflug, 2000). The International Agency for Research on Cancer (IARC) categorized NNN and NNK as human carcinogens (in Group 1) (International Agency for Research on Cancer, 2007). \textit{N}'-Nitrososanabasine (NAB), a weak carcinogen, and \textit{N}'-nitrosoanatabine (NAT), which is not a known carcinogen, have also been measured as biomarkers of tobacco product exposure (Hecht, 1998). Some tobacco companies modified their
curing processes to reduce TSNA levels, for example, by applying a microwave curing process (Star Scientific Inc., 1999).

NNK is found in tobacco, MS, and sidestream smoke (SS) (World Health Organization, 2004). Tobacco contains 100–960 ng of NNK/cigarette (Tricker et al., 1991). MS yields of NNK were 100–200 ng/cigarette (Chepiga et al., 2000; Hoffmann et al., 1994, 1995; Spiegelhalder & Bartsch, 1996), whereas SS yields of NNK were 386–1444 ng/cigarette (Adams et al., 1987). NNK and NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol) induce lung adenocarcinoma in rodents, and NNK has been proposed as a cause of human lung cancer (Hecht & Hoffmann, 1988, 1989). NNN is present in tobacco at 400–5340 ng/cigarette and in tobacco MS at 19–855 ng/cigarette (Tricker et al., 1991). It is not known whether reduced exposure to TSNA would lower tobacco-related morbidity and mortality (Breland et al., 2002b).

III.4.4.1.1 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol and metabolites

NNK undergoes extensive metabolism and is not present in urine (Hecht et al., 1999). Its metabolites NNAL, NNAL glucuronides (NNAL-N-Gluc and NNAL-O-Gluc), and total NNAL (the sum of NNAL and its glucuronide metabolites), have been well studied as biomarkers of tobacco product and smoke exposure. Consumption of cruciferous vegetables, such as watercress (Nasturtium officinale), inhibits oxidative metabolism of NNK. TSNA are highly specific biomarkers of tobacco and nicotine exposure, are directly relevant to carcinogen uptake, and can be measured consistently in persons who are exposed to tobacco smoke or who use tobacco products (Carmella et al., 2003). Given their specificity and ability to show differences between PRRTPs and conventional cigarettes, NNAL and NNAL-Gluc are highly useful biomarkers of exposure to NNK and LSRO recommends them for routine measurement in PRRTP studies (Category A biomarkers).

The elimination half-life of NNAL and NNAL-Gluc is 40–45 days and the distribution life is 3–4 days (Hecht et al., 1999). Carmella et al. (1993) first reported higher TSNA metabolites levels for smokers than for nonsmokers. Byrd and Ogden (2003) determined that the mean free NNAL concentration ranges from 101–256 pg/ml and that of NNAL-Gluc, from 247–566 pg/ml.

Carmella et al. (1995) assessed intra-individual variation in urine levels of NNAL and NNAL-Gluc and found relatively constant excretion from day to day and over the long term. They measured levels of NNAL and its NNAL-O-Gluc in the urine of 61 smokers and discovered a 16-fold variation in the NNAL-Gluc:NNAL ratio (Carmella et al., 1995). Inter-individual variability in these measures was much higher than intra-individual variability. They
categorized study participants into two phenotypes based on the NNAL-Gluc:NNAL ratio, which they proposed may reflect an ability to metabolize NNK. NNAL-Gluc is probably a detoxification product of NNAL and NNK, although this has not been proved. Eighty-five percent of subjects had NNAL-Gluc:NNAL ratios lower than 6, whereas the remaining subjects had ratios of 6–11. Carmella et al. (1995) suggested that individuals with higher ratios may be better protected against cancer than individuals with lower ratios.

Richie, Jr., et al. (1997) showed that Black smokers were more likely than White smokers to fall into the “poor metabolizer” group (NNAL-Gluc:NNAL ratio < 6) but believed this phenotype unlikely to be due to dietary or socioeconomic differences or differences in exposure. Furthermore, absolute levels of NNAL and NNAL-Gluc were higher for Black smokers than for White smokers, although the former tended to smoke fewer cigarettes (Richie, Jr., et al., 1997).

Breland et al. (2003) measured TSNAs in urine of individuals who smoked their own brand of cigarettes, Advance® cigarettes, or no cigarettes. The Advance® cigarette, which has a 3-part ionic filter, was developed by collaboration of Brown and Williamson Tobacco Company and Star Scientific Tobacco Company. Marketing information in the Advance® Lights Kings pack onsert included a statement that tobacco in Advance® was manufactured by using a “patented tobacco curing process [that] significantly inhibits the formation of tobacco specific nitrosamines” (Brown & Williamson Tobacco, 2002). In this crossover study, 15 light and ultra-light cigarette smokers smoked their own brand of cigarettes, switched to Advance®, or abstained from smoking for 5 days. Urinary NNAL levels decreased by 51% when they smoked Advance® and 70% when smokers abstained from smoking compared with when they smoked their own brand.

In another crossover study, a group of 10 men and 7 women (group A) initially smoked Advance® cigarettes for 4 weeks and then switched to their usual brand of light cigarette. Another group of 10 men and 6 women (group B) first used their usual brand for 4 weeks and then switched to Advance® cigarettes (Melikian et al., 2003). Twelve-hour urine samples were collected at baseline (week 0), at crossover time (week 4), and at the final visit (week 8). Urine samples were analyzed for levels of NNAL, S-phenylmercapturic acid (S-PMA), and trans,trans-muconic acid (t,t-MA) (metabolites of volatile benzene); 1-hydroxypyrene, a marker for polycyclic aromatic hydrocarbons; thiocyanate, a metabolite of hydrogen cyanide (HCN); cotinine; and creatinine. After 4 weeks of smoking the Advance® cigarette, levels of urinary NNAL were reduced by approximately 60%, those of S-PMA and t,t-MA were
respectively about 46% and 40% lower, that of 1-hydroxypyrene was reduced by 45%, and that of thiocyanate was reduced by 20% (Melikian et al., 2003).

Omni® cigarettes yield 53% lower TSNA values than conventional cigarettes when smoked using the FTC cigarette machine-smoking regimen. Hughes et al. (2004) conducted a 12-week randomized crossover study in which 19 smokers of light or ultra-light cigarettes smoked either their own cigarettes or Omni® cigarettes for 6 weeks and then switched to smoking the type of cigarette they had not smoked in the first part of the study. Total NNAL levels showed no significant reduction when subjects used Omni® cigarettes compared with their own brand. The authors noted that the small sample size (34 study participants) may have contributed to this finding.

Hatsukami et al. (2004b) reported significant decreases in urine TSNA levels for 38 individuals who switched from their own brand of conventional cigarettes to Omni® cigarettes ($p = 0.003$) or the nicotine patch ($p < 0.001$) for 4 weeks. The mean NNAL concentration before switching to Omni® cigarettes for 4 weeks was 2.4 pmol total NNAL/mg creatinine (95% CI = 1.8 to 3.0). After 4 weeks, the level decreased to 1.5 pmol/mg creatinine (95% CI = 1.1 to 1.9) (Hatsukami et al., 2004b). Since the number of cigarettes smoked per day changed with use of the Omni® cigarettes, ratios of total NNAL/cigarettes per day and total NNAL/cotinine level were calculated and showed significant reductions ($p < 0.001$ and $p = 0.009$).

Levels of NNAL and NNAL-Gluc for individuals attempting to quit smoking have also been analyzed. Hecht et al. (1999) measured urine levels of free NNAL and NNAL-O-Gluc in 27 smokers before and after smoking cessation. NNAL means ± SD and ranges were, respectively, 944 ± 517 and 180 ± 2080 pmol/ml; those for NNAL-O-Gluc were 2200 ± 1130 and 280 ± 4970 pmol/ml. At 1, 3, and 6 weeks after smoking cessation, NNAL levels (expressed as a percentage of levels before smoking cessation) were 34.5%, 15.3%, and 7.6%, respectively. At 281 days after quitting, NNAL and NNAL-Gluc levels were not detectable in the urine of 3 of 5 former smokers who were called back; however, urine NNAL concentrations for 2 of 5 subjects remained above those of 2 nonsmokers. Mean ± SD urine NNAL levels for the two former smokers and 21 nonsmokers were 0.025 ± 0.029 pmol/ml and 0.016 ± 0.029 pmol/ml, respectively (Hecht et al., 1999).

The utility of NNAL and NNAL-Gluc as measures of decreased daily cigarette consumption has also been assessed. Hecht et al. (2004b) quantified NNAL and NNAL-Gluc for individuals who reduced their daily cigarette consumption by 40–70% for up to 26 weeks. They reported overall modest decreases in
NNAL and NNAL-Gluc that were sometimes not maintained. The authors proposed that smokers may have compensated for fewer cigarettes per day by smoking cigarettes differently, thereby changing NNK delivery.

Interindividual differences in metabolism and exposure intensity may also contribute to a lower than expected reduction in exposure to tobacco smoke carcinogens for given changes in cigarette design. Melikian et al. (2007a) measured yields of NNK, nicotine, and benzo[a]pyrene (B[a]P) in MS that was generated by using cigarette smoking machine settings that mimicked individual puffing behavior of each of 257 smokers of low-, medium-, and high-yield cigarettes and compared them to metabolite levels. Although the delivered dose of smoke toxins to smokers of low-yield cigarettes was lower than that to smokers of high-yield cigarettes, the amount of metabolite per parent smoke constituent was higher for smokers of low-yield cigarettes. Gender and race may also modify urinary biomarker levels (Benowitz et al., 2004, 2006; Melikian et al., 2004, 2007b).

Exposure to ETS is consistently associated with increased urine levels of NNAL and NNAL-Gluc in nonsmokers (Anderson et al., 2001; Hecht et al., 1993, 2001; Meger et al., 2000; Parsons et al., 1998). The mean ± SD of total NNAL concentration for nonsmokers exposed to ETS (n = 18) was 0.042 ± 0.20 pmol/mg creatinine (Carmella et al., 2003). Hecht et al. (2001) measured NNAL and NNAL-Gluc levels in urine samples from elementary school age children and reported significantly higher levels (mean = 0.05 pmol/ml urine) of these substances with ETS exposure (reported by children’s caregivers) than without it.

Tulunay et al. (2005) analyzed NNAL and NNAL-Gluc in 24-hour urine samples collected from hospital workers who were nonsmokers. The mean total NNAL was 0.066 pmol/ml urine, and most study participants had levels of total NNAL above 0.01 pmol/ml; a concentration generally seen in non-exposed individuals. Women whose husbands smoked had approximately 6 times higher levels of total NNAL than women with non-smoking husbands: 0.045 pmol/mg creatinine (95% CI = 0.027–0.073) versus 0.007 pmol/mg creatinine (95% CI = 0.004–0.010) (Anderson et al., 2001). (See Table 4 in Hecht et al. (2001) for a summary of NNAL and NNAL-Gluc levels in various non-smoking populations.)

Various methods can quantitate TSNA levels. Different groups have used GC coupled to a thermal energy analyzer (GC-TEA) (Benowitz et al., 2002; Carmella, 1995, 2003; Meger, 2000; Muscat, 2005; Parsons, 1998). The GC-TEA method used by Carmella and others (2003) has twice the analytical
speed of the older method (GC-tandem mass spectrometry), is more sensitive, produces cleaner samples, and includes an internal standard. However, Carmella et al. (2003) also found that GC-tandem mass spectrometry, with mass spectrometry operating in the positive ion chemical ionization mode, a transition of m/z 282→162, and resolution set at 0.5 amu, used to measure the urine analytes had a lower limit of detection for NNAL (30 fmol) compared with that for GC-TEA (120 fmol).

LC-tandem mass spectrometry involves one solid-phase extraction step and tandem mass spectrometry monitoring after electrospray ionization and has been used for simultaneous analysis of NNAL and its glucuronides in urine (Byrd & Ogden, 2003). Byrd and Ogden (2003) described this method as being simpler to perform than GC-TEA but having adequate specificity for measuring TSNA metabolites. Compared with GC, the method is faster, requires less sample analysis time, has greater sample throughput capacity, and requires a small sample volume. The method was validated using the Food and Drug Administration, Center for Drug Evaluation and Research validation criteria (U.S. Food and Drug Administration, 2001).

Carmella et al. (2005) also used an LC-based technique (LC-electrospray ionization-tandem mass spectrometry) to measure total NNAL (NNAL + NNAL-Gluc) in plasma. This method required a 1-ml plasma sample, whereas the Hecht et al. (1999, 2002) method needed a 5- to 10-ml sample.

NNAL + NNAL-Gluc levels and cotinine excretion are correlated [correlation coefficient (r) = 0.90] but not NNAL + NNAL-Gluc levels and the number of cigarettes smoked on the day of urine collection (Meger et al., 1996). NNAL + NNAL-Gluc concentrations are a most useful measure of tobacco product and smoke exposure.

III.4.4.1.2 4-Hydroxy-1-(3-pyridyl)-1-butanone-DNA adducts in lung

Nitrosation of NNN and NNK leads to creation of an intermediate that can form adducts with DNA and Hb. Adducts of 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) and DNA in lung reflect exposure to NNK and are biomarkers of particulate phase exposure (Carmella & Hecht, 1987). HPB-DNA adducts reflect an exposure time of more than 1 month (Scherer, 2005). Given the ease of sampling and established differences between smokers and nonsmokers and PRRTP studies that exist for TSNA urinary metabolites, LSRO considers measurement of urinary TSNA metabolites the preferred method and therefore does not recommend routine analysis of HPB-DNA adduct formation in PRRTP studies (Category C biomarker).
Levels of HPB-DNA adducts in smokers are approximately 10-fold the levels in nonsmokers (Scherer, 2005). Foiles et al. (1991) measured HPB-DNA adducts in autopsy samples from the peripheral lung and tracheobronchial tree of smokers and nonsmokers. Mean ± SD adduct levels in peripheral lung tissue were 11 ± 16 fmol/mg DNA for 9 smokers and 0.9 ± 2.3 fmol/mg DNA for 8 nonsmokers. Corresponding values of adduct levels in tracheobronchi were 16 ± 18 fmol/mg DNA for 4 smokers and 0.9 ± 1.7 fmol/mg DNA for 8 nonsmokers. Considerable inter-individual variation exists in HPB-DNA adduct levels, which limits the usefulness of this indicator as a measure of exposure to tobacco smoke (Foiles et al., 1991).

In a more recent study, Hölzle et al. (2007) used GC-mass spectrometry in the negative ion chemical ionization mode, based on the method used by Foiles et al. (1991), to evaluate HPB-releasing DNA adducts in peripheral lung tissue samples. They reported that adduct levels in 21 smokers’ lungs (404 ± 258 fmol HPB/mg DNA) were significantly higher than levels in 11 nonsmokers’ lungs (59 ± 56 fmol HPB/mg DNA) ($p < 0.0001$). Although the GC-mass spectrometry method allows detection of approximately 7-fold differences in levels between smokers and nonsmokers, analysis of NNAL and NNAL-Gluc in urine, with reported 100-fold differences between smokers and nonsmokers, is considered a better measure of TSNA exposure.

**III.4.4.1.3 4-Hydroxyl-1-(3-pyridyl)-1-butanone-hemoglobin adducts**

HPB-Hb adducts are biomarkers of particulate phase exposure. These adducts persist over the lifetime of the erythrocyte. Levels in smokers are approximately twice as high as those in nonsmokers.

Carmella et al. (1990) measured HPB-Hb adduct levels in smokers ($n = 40$) and nonsmokers ($n = 21$). Mean adduct levels were 0.080 pmol/g Hb for smokers, and 0.0293 pmol/g Hb for nonsmokers.

Analytical methods for HPB-Hb adducts include GC-mass spectrometry (Hecht et al., 1991) and capillary column GC with detection by negative ion chemical ionization (Carmella et al., 1990). The small difference (2- to 3-fold) in adduct levels between smokers and nonsmokers is a major disadvantage of this biomarker as a measure of tobacco smoke exposure (Hölzle et al., 2007; Wilp et al., 2002).

Because of the better quantification of urinary metabolites of TSNA (Category A biomarkers), LSRO ranks HPB-Hb adducts as Category C biomarkers.

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III.4.4.2 N′-Nitrosonornicotine adducts

NNN is metabolized via two mechanisms to produce electrophiles that form adducts with DNA (Hecht, 1998). Upadhyaya et al. (2006) recently reported the identification of deoxyguanosine and DNA adducts from the 5′-hydroxylation pathway of NNN metabolism and noted that these adducts are potential biomarkers of metabolic activation for smokers. These biomarkers will require additional study and LSRO does not recommend their routine measurement as biomarkers of exposure to TSNA in PRRTP studies.

III.4.4.3 N′-Nitrosonornicotine, N′-Nitrosoanatabine, N′-Nitrosoanabasine, and their pyridine N-Glucuronides in urine

NNN, NAT, NAB, and their pyridine N-glucuronides in urine are newer measures of TSNA exposure. NAT and NAB are formed via nitrosation of anatabine and anabasine, respectively. Specific analysis of NNN uptake was previously difficult, because NNN metabolites in urine are also metabolites of alkaloids found at higher levels in tobacco than NNN. Stepanov and Hecht (2005) first measured these substances in urine samples from smokers (n = 14). The mean ± SD values for NNN, NNN-N-Gluc, NAT, and NAB were 0.086 ± 0.12 pmol/mg creatinine, 0.096 ± 0.11 pmol/mg creatinine, 0.19 ± 0.20 pmol/mg creatinine, and 0.04 ± 0.039 pmol/mg creatinine, respectively (Stepanov & Hecht, 2005).

These biomarkers may have future application, but at present, LSRO does not recommend these biomarkers for routine use in PRRTP studies and categorizes them as Category C biomarkers.

III.4.4.4 N-Nitrosoproline and other nitrosamino compounds

N-Nitrosoproline (NPRO), N-Nitrosarcosine (NSAR), N-Nitrosothiazolidine-4-carboxylic acid (NTCA), and N-Nitroso-2-methylthiazolidine-4-carboxylic acid (NMTCA) are the major non-volatile nitrosamines found in urine. Endogenous nitrosation of proline has been studied in smokers and nonsmokers after ingestion of nitrate and proline. Hoffmann and Brunnemann (1983) reported that NPRO levels in smokers’ urine was significantly higher than levels in nonsmokers’ urine when study participants ate a restricted diet.

Ladd et al. (1984) reported no significant difference between baseline levels of NPRO in urine of smokers and nonsmokers when study participants had not ingested nitrate or proline. Ingestion of proline alone did not produce different NPRO levels, but consumption of nitrate and proline resulted in approximately 2.5 higher levels of NPRO in smokers’ 24-hour urine samples.
NTCA and NMTCA have been detected in urine of smokers and nonsmokers (Ohshima et al., 1984). Although some studies reported an effect of smoking on levels of total non-volatile nitrosamines (NSAR, NPRO, NTCA, and NMTCA) (Malaveille et al., 1989; Tsuda et al., 1986), others showed no effect of smoking (Knight et al., 1991; Nair et al., 1986; Ohshima et al., 1984). Nitrosodimethylamine (NDMA) levels in urine are significantly higher for individuals who consume alcohol but do not smoke and individuals who consume alcohol and smoke than for non-smoking non-drinkers (Cooney et al., 1986).

LSRO does not recommend routine measurement of these N-nitroso compounds (Category C biomarkers) and, as noted previously, recommends that NNAL and its metabolites be used as markers of TSNA exposure from tobacco smoke.

### III.4.5 Aromatic Amine Biomarkers

#### III.4.5.1 Aromatic amine-hemoglobin adducts

#### III.4.5.1.1 4-Aminobiphenyl-hemoglobin adducts

IARC categorized the aromatic amine 4-ABP as a human bladder carcinogen (Group 1). The most widely used method for analysis is GC-mass spectrometry in the negative ion chemical ionization mode (Richter & Branner, 2002). 4-ABP is found in MS at levels of 1–100 ng/cigarette (Patrianakos et al., 1979), but levels in ETS are 20–30 times higher than those in MS. 4-ABP-Hb adducts reflect exposure to the particulate phase of tobacco smoke. Most studies have reported higher 4-ABP-Hb adduct levels in smokers than in nonsmokers (Bryant et al., 1987; Perera et al., 1987; Weston & Bowman, 1991), in fact by 3- to 8-fold (Bryant et al., 1987; Coghlin et al., 1991; Dalilinga et al., 1998; Falter et al., 1994; Hammond et al., 1993; Perera et al., 1987; Stillwell et al., 1987). A representative ratio of 4-ABP-Hb adducts in smokers compared with nonsmokers is 5.5 (Scherer, 2005). The half-life of 4-ABP-Hb adducts is 7–9 weeks (Maclure et al., 1990; Mooney et al., 1995). They reflect a cumulative 4-month exposure.

Various studies reported a dose-response relationship between smoking and 4-ABP-Hb adducts (Bartsch et al., 1990; Vineis et al., 1990; Yu et al., 1994). These adducts have been used as biomarkers of exposure in PRRTP studies and have revealed differences between conventional cigarettes and PRRTPs. Because of the relative consistency of 4-ABP-Hb adducts in distinguishing between smokers and nonsmokers, LSRO ranks them Category A biomarkers.

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Cigarette tobacco type influences 4-ABP levels (Bryant et al., 1988). Smokers of black tobacco have 40–50% higher 4-ABP-Hb levels than smokers of blond tobacco. Vineis et al. (1996) measured 4-ABP adducts in exfoliated bladder cells from 50 nonsmokers, 31 flue-cured tobacco smokers, and 16 air-cured tobacco smokers. Means ± SE for these adducts were 28 ± 3, 103 ± 9.9, and 146 ± 11 pg/g Hb, respectively.

Mooney et al. (1995) measured 4-ABP-Hb adducts in 16 individuals who smoked more than 20 cigarettes/day and who were trying to quit smoking. Mean ± SD adduct levels measured before quitting and at 10 weeks, 8 months, and 14 months after smoking cessation were 18.9 ± 8.7 nM/mHb (n = 16), 8.1 ± 5.0 nM/mHb (n = 16), 4.7 ± 3.5 nM/mHb (n = 16), and 4.8 ± 2.3 nM/mHb (n = 15), respectively. Adduct levels were significantly different from values before quitting at every time point measured after baseline, but no significant difference was found between adduct levels at 8 and 14 months.

Maclure et al. (1990) showed that smoking cessation reduces 4-ABP-Hb adduct levels. They measured adduct levels for 34 smokers on the day that they quit smoking, at 3 weeks after quitting, and at 2 months after quitting. Adduct levels decreased from a mean ± SD at baseline of 120 ± 7 to a 3-week value of 82 ± 6 pg/g Hb. For 15 subjects who continued to refrain from smoking, levels declined further to 34 ± 5 pg/g Hb at 2 months after cessation.

4-ABP-Hb adducts have also been measured in individuals who reduced daily cigarette consumption. Smokers of more than 40 cigarettes/day were provided with nicotine inhalers and asked to decrease the number of cigarettes smoked to 30 cigarettes/day by the end of week 4 of the study, to 20 cigarettes/day by the end of week 8, and to 10 cigarettes/day by the end of week 24 (Hurt et al., 2000). Mean ± SD values for 4-ABP-Hb adducts measured at baseline and at weeks 12 and 24 were, respectively, 10.4 ± 2.7 nmol/mol Hb; (range = 7.0 17.2 nmol/mol Hb; n = 16), 11.3 ± 4.4 nmol/mol Hb (range = 5.6 22.0; n = 15), and 9.5 ± 2.5 (range = 5.3 14.3; n = 16). In this study, 4-ABP-Hb adduct levels at weeks 12 and 24 showed no significant reduction from baseline.

There are limitations to the use of 4-ABP as a measure of tobacco smoke exposure. One such limitation is potential confounding from trace amounts of aromatic amines in the environment (Richter & Branner, 2002). Non-tobacco sources of 4-ABP exposure include diesel fuel exhaust, heated cooking fuels, hair dyes, and diet. 4-Nitrobiphenyl, a product of incomplete combustion of diesel fuels, can be metabolized to 4-ABP. 4-ABP-Hb adduct
levels are also influenced by N-acetyltransferase 2 and CYP1A2 status. Individuals who are slow acetylators and extensive N-hydroxylators have elevated adduct levels (Bartsch et al., 1990; Riffelmann et al., 1995; Vineis et al., 1990; Yu et al., 1994). Ethnic and racial differences may be accounted for by differences in numbers of slow acetylators between groups (Vineis et al., 1990; Yu et al., 1994). Slow acetylators were reported to have higher mean levels of 4-ABP-Hb adducts (Vineis et al., 1990; Yu et al., 1994). Yu et al. (1994) studied the prevalence of the slow acetylator phenotype in 47 Black men, 44 White men, and 42 Asian men; 34%, 54%, and 14%, respectively, were slow acetylators.

III.4.5.1.2 Other aromatic amine-hemoglobin adducts

Stillwell et al. (1987) reported that levels of Hb adducts of o-toluidine, p-toluidine, 2-naphthylamine, and 4-ABP were elevated in smokers (n = 12) as compared with nonsmokers (n = 10). Although smokers had 2- to 3-fold higher values for Hb adducts of o-toluidine, p-toluidine, and 2-naphthylamine compared with nonsmokers, 4-ABP-Hb adduct levels were 6-fold higher than those of nonsmokers. Levels of Hb adducts of m-toluidine, another aromatic amine, were not different for smokers and nonsmokers. LSRO recommends 4-ABP-Hb adducts for assessing aromatic amine exposure, and does not recommend routine measurement of other aromatic amines in Category C.

Richter et al. (1995) compared Hb adduct levels of the aromatic amines aniline, o-toluidine, m-toluidine, p-toluidine, 2-ethylaniline, 2,4-dimethylaniline, o-anisidine, 3-aminobiphenyl (3-ABP), and 4-ABP in smoking women (n = 27) and non-smoking women who were pregnant (n = 73) in Germany. Compared with nonsmokers, smokers had significantly elevated levels of adducts of Hb with o-toluidine (p < 0.0001), p-toluidine (p < 0.001), 2,4-dimethylaniline (p < 0.018), 3-ABP (p < 0.001), and 4-ABP (p < 0.0001).

Riffelmann et al. (1995) also measured urinary aromatic amines and 4-ABP-Hb adducts for men with occupational exposure to aromatic amines and men who were not known to have such exposure. They reported significant differences in urinary 2-aminonaphthalene for 22 exposed smokers and 21 exposed nonsmokers (mean ± SD = 3.9 ± 2.2 versus 2.1 ± 2.8 ug/L, respectively; p = 0.0116), as well as for 8 non-occupationally exposed smokers and 8 nonsmokers (mean ± SD = 3.1 ± 2.1 versus 0.5 ± 0.7 ug/L, respectively; p = 0.0128). Significant differences (p = 0.0001) existed in means ± SD for 4-ABP-Hb adducts for 22 occupationally exposed smokers (19.9 ± 7.1 ng/L) and 21 occupationally exposed nonsmokers (7.3 ± 3.6 ng/L).
III.4.5.2 4-Aminobiphenyl-DNA adducts

4-ABP exposure has been measured as 4-ABP-DNA adducts. Analytical methods include immunoassays, $^{32}$P-postlabeling–HPLC, and GC-mass spectrometry (Skipper & Tannenbaum, 1994). Hsu et al. (1997) analyzed immunoperoxidase to quantify 4-ABP-DNA adduct levels in oral mucosa and exfoliated urothelial cells of smokers. Mean ± SD relative staining intensity of urothelial cells was significantly greater (~1.7-fold) for 20 smokers than for nonsmokers (517 ± 137 versus 313 ± 79; $p < 0.0005$). The relative staining intensity of oral mucosa cells was also significantly higher for smokers than for nonsmokers (552 ± 157 versus 326 ± 101; $p < 0.0005$). A 3-fold variation in staining intensity for smokers and nonsmokers was observed.

Flamini et al. (1998) measured 4-ABP-DNA adduct levels in laryngeal tissues by using 3C8 monoclonal antibody, which is highly specific for 4-ABP. For smokers and nonsmokers, a significant difference was found in adduct levels in polyps and surrounding tissue but not tumor tissues. The variation in 4-ABP levels was approximately 6-fold for smokers and 5-fold for nonsmokers (Flamini et al., 1998).

4-ABP-Hb adducts are more useful than 4-ABP-DNA adducts for PRRTP studies. 4-ABP-Hb adducts are the best studied aromatic amine-Hb adduct and reflect exposure to a Group 1 carcinogen. LSRO recommends measurement of 4-ABP-Hb adducts rather than 4-ABP-DNA adducts.

III.4.5.3 Urinary aromatic amines

Grimmer et al. (2000) reported that mean ± SD values for total aromatic amines (1-aminonaphthalene, 2-aminonaphthalene, 2-aminobiphenyl, and 4-ABP) in urine of 12 smokers were more than 2-fold higher than levels in urine of 14 nonsmokers (736 ± 911 versus 303 ± 516 ng/24 hours) and 22 passive smokers (272 ng/24 hours). This difference was mainly due to increased 1-aminonaphthalene. The authors reported no significant differences in levels of urinary aromatic amines for nonsmokers and passive smokers and in levels of 2-aminonaphthalene and 4-ABP between groups. LSRO recommends measurement of 4-ABP-Hb adducts rather than urine levels of aromatic amines.
III.4.6 Benzene Biomarkers

III.4.6.1 Benzene

Benzene is a component of the gas phase of tobacco smoke and is a biomarker of exposure to aromatic hydrocarbons. Benzene is a known human carcinogen (IARC Group 1) that is found in MS at 54–73 μg (47–67 ppm) per cigarette (Wallace et al., 1987). Vehicular travel, pumping gasoline into automobiles, gas vapors from from attached garages, and emissions from materials such as latex paint, tapes, marking pens, and other consumer products are other sources of benzene (Wallace, 1989). Benzene is also produced as a component of vehicle exhaust, by petrochemical industry processes, and by organic material pyrolysis (Scherer et al., 1998). Benzene has been measured in blood, breath, and urine. LSRO considers benzene in biological fluids to be a Category C biomarker because other benzene-related biomarkers (S-PMA and t,t,-MA) are more reliable for measuring benzene exposure. Measurement of benzene and other volatile organic compounds in breath is a promising technique that may be used routinely for PRRTP studies, however.

Wallace and Pellizari (1986) showed that breath benzene concentrations for 198 smokers were significantly higher than those for 322 nonsmokers (16 versus 2.5 μg/m³; p < 0.001). They found a significant dose-response relationship between the number of cigarettes smoked and breath benzene levels (p < 0.01). Breath benzene levels decreased to those of nonsmokers within an hour of smoking a cigarette (Jordan et al., 1995). Gordon et al. (2002) used a real-time approach to measure breath levels of volatile organic compounds as indicators of active and passive smoking. They reported the mean ± SD value of benzene in nonsmokers’ breath to be 25.7 ± 12.9 μg/m³ (n = 4) and the value in smokers’ breath to be 478 ± 144 μg/m³ (n = 5).

Brugnone et al. (1998) measured blood benzene levels for 167 individuals who had occupational exposure to benzene and 243 individuals who did not have such exposure (general population). Smokers in the general population had significantly higher blood benzene concentrations (264 ± 178 ng/L) than nonsmokers (123 ± 74 ng/L). Smokers who had occupational exposure to benzene also had significantly higher blood benzene values (584 ± 372 ng/L) than occupationally exposed nonsmokers (289 ± 307 ng/L).

Perbellini et al. (2002) also measured benzene levels in blood and urine of 10 individuals who smoked 3–20 cigarettes/day and 15 nonsmokers. All subjects were woodworkers who lived in a rural area. Smokers’ urine samples
had significantly higher amounts of benzene than did nonsmokers’ urine samples (median = 125 versus 77 ng/L; \( p < 0.05 \)). Blood levels of benzene were also significantly higher for smokers than for nonsmokers (246 versus 87 ng/L). Benzene levels in blood were approximately 2.7-fold higher than levels in urine (Perbellini et al., 2002). Other researchers found higher levels of benzene in urine than in blood, however, and benzene levels vary considerably when measured by different investigators (Perbellini et al., 2002). Benzene levels are typically higher in smokers than in nonsmokers; however, urinary benzene levels vary widely for similar benzene exposures (Ong et al., 1995, 1996).

The headspace technique with GC-mass spectrometry has been used to quantify benzene in blood and urine (Perbellini et al., 2002). Toluene, ethylbenzene, and xylene can also be measured. Advantages of this method include easy sample preparation, a requirement for small volumes, and good repeatability and linearity in the range of interest (Perbellini et al., 2002). Urine samples are thought to have slightly higher sensitivity than blood samples. Solid phase microextraction for sampling chemicals from the headspace of urine with GC-mass spectrometry for selective analysis of chemicals has also served to measure benzene levels (Fustinoni et al., 1999).

III.4.6.2 trans,trans-Muconic acid (trans,trans-2,4-hexadienedioic acid)

Approximately 2–25% of total benzene is found as \( t,t\)-MA in urine (Boogaard & van Sittert, 1995; Inoue et al., 1989; Scherer et al., 1998). The elimination half-life of \( t,t\)-MA is estimated at 5 ± 2.3 hours (Scherer et al., 1998). Ong et al. (1994) showed a dose-response relationship for cigarette smoking and \( t,t\)-MA levels (\( r = 0.47 \)). LSRO recommends routine measurement of \( t,t\)-MA in PRRTP evaluations (Category A biomarker).

Sorbic acid (trans,trans-2,4-hexadienoic acid), a preservative found in food products, can be converted to \( t,t\)-MA, which contributes to background levels of \( t,t\)-MA and reduces its specificity as a biomarker of tobacco smoke exposure (Hecht, 2002; Scherer et al., 1998). Lee et al. (2005) reported that this interference was not significant, however. Coexposure to toluene inhibits formation of \( t,t\)-MA from benzene in rats and humans. Genetic polymorphisms may influence conversion of benzene to \( t,t\)-MA (Gobba et al., 1997; Rossi et al., 1999).

Melikian et al. (1993) measured \( t,t\)-MA levels in urine of 42 smokers and 42 nonsmokers and found approximately 3-fold higher levels for smokers (mean ± SE = 0.29 ± 0.04 mg/g creatinine, range = 0.02–1.3) than for
nonsmokers (mean ± SE = 0.09 ± 0.02 mg/g creatinine, range = not detectable [ND]–0.52; p = 0.0001). Ong et al. (1994) reported higher t,t-MA levels in urine for smokers (mean ± SD = 0.19 ± 0.09 mg/g creatinine; n = 46) than for nonsmokers (mean ± SD = 0.14 ± 0.07 mg/g creatinine; n = 40, p = 0.04), but this difference was not significant.

Liquid chromatography has been used for spectrophotometric determination of t,t-MA. The authors describe this method as being more cost-effective, easy to perform, and convenient than mass spectrometry.

III.4.6.3 S-Phenylmercapturic acid (N-acetyl-S-phenyl-L-cysteine)

S-Phenylmercapturic acid (S-PMA) or phenylhydroxyethylmercapturic acid (PheMA), another benzene metabolite, is thought to form via enzymatic degradation of the glutathione adduct (Farmer et al., 2005). A relatively low percentage of benzene (0.005–0.3%) is converted to S-PMA (Boogaard & van Sittert, 1995; Melikian et al., 2002). The half-life of S-PMA is approximately 9 hours (Boogaard & van Sittert, 1995, 1996). As noted previously, multiple sources of benzene exposure exist; therefore, benzene is not a specific biomarker of tobacco smoke exposure. However, significantly higher levels of S-PMA, which is considered a highly specific marker of benzene exposure (Ghittori et al., 1995), were reported in smokers than in nonsmokers (Boogaard & van Sittert, 1996; Scherer et al., 2001). Melikian et al. (2002) considered S-PMA to be a more sensitive biomarker of benzene exposure compared with t,t-MA. LSRO ranks S-PMA as a Category A biomarker.

Boogaard and van Sittert (1996) determined the urinary S-PMA concentration for 38 nonsmokers and 14 smokers. Mean ± SE concentrations of S-PMA for smokers and nonsmokers were significantly different (1.71 ± 0.27 versus 0.94 ± 0.15 mol/mol creatinine, respectively; p = 0.013).

S-PMA levels in nonsmokers and individuals who do not have occupational exposure to benzene range between 1 and 2 μg/g creatinine (Farmer et al., 2005).

Rossi et al. (1999) showed that differences in genetic polymorphisms of CYP2D6, glutathione S-transferase 1, and NAD(P)H:quinone oxidoreductase may play a role in variation in metabolism of benzene. (See Farmer et al. (2005) for a summary of analytical methods for S-PMAs.)

For benzene levels less than 1 ppm, S-PMA does not indicate benzene exposure as effectively as do other benzene biomarkers (Kivistö et al., 1997).
Farmer et al. (2005) did not consider S-PMA to provide a valid measure of exposure to low benzene concentrations.

Feng et al. (2006) measured urinary S-PMA and t,t-MA levels for 110 regular smokers of a conventional cigarette that delivered approximately 11 mg tar/cigarette. Thirty subjects were assigned to a stop smoking group; 80 smokers were randomly divided into 4 smoking groups of 20 each: to continue smoking their own brand of conventional cigarette, to switch to smoking a conventional cigarette that delivered 3 mg tar/cigarette, or to change to using one of two electrically heated smoking systems (EHCSSs) for 8 days. Both t,t-MA and S-PMA distinguished between benzene exposure from smoking conventional cigarettes and the different EHCSSs. Although S-PMA was more variable than t,t-MA, Feng et al. (2006) considered it to be a better biomarker of benzene exposure than t,t-MA because of higher background levels of t,t-MA from sorbic acid.

III.4.7 1,3-Butadiene Biomarkers

III.4.7.1 Monohydroxybutenyl mercapturic acids and dihydroxybutyl mercapturic acid

Monohydroxybutenyl mercapturic acids (MHBMA, or MII) and dihydroxybutyl mercapturic acid (DHBMA, or MI) are the major urinary metabolites of 1,3-butadiene. 1,3-Butadiene is classified by IARC as “probably carcinogenic to humans” (Group 2A) (International Agency for Research on Cancer, 1999), and by the US National Toxicology Program as a “known human carcinogen” (National Toxicology Program, 2005). The range of 1,3-butadiene in MS is 16–75 μg/cigarette; that in SS is 205–361 μg/cigarette (Brunnemann et al., 1990). 1,3-Butadiene is found in the gas phase of cigarette smoke and is not a specific biomarker of tobacco smoke exposure because of occupational and other exposures. 1,3-Butadiene is used for rubber resin and latex manufacturing and is a component of motor vehicle exhaust (Fustinoni et al., 2002). LSRO categorizes MHBMA as a Category A biomarker and DHBMA as a Category B biomarker.

Half-lives of DHBMA and MHBMA are approximately 12 hours (Scherer, 2005). Urban et al. (2003) reported a significant difference in urine levels of MHBMA of 10 nonsmokers and 10 smokers: mean ± SE values (μg/24 hours) were 12.5 ± 1.0 (range = 7.0–18.0) in nonsmokers and 86.4 ± 14.0 (range = 15.2–145.1) in smokers (p < 0.001). No significant difference in excreted DNBMA was found between nonsmokers and smokers: mean ± SE values (μg/24 hours) were 459 ± 72 (range = 209–898) and 644 ± 90 (range = 116–1084), respectively.
GC tandem mass spectrometry with negative ion chemical ionization (van Sittert et al., 2000) has been utilized to analyze MHBMA and DHBMA in urine. A strength of this assay is the small sample volume required (1 ml). Rapid LC-tandem mass spectrometry has been used for the same purpose (Urban et al., 2003). This method is faster than the GC method and still requires only a small sample volume (5 ml).

A weak correlation has been noted between MHBMA and DHBMA levels and other biomarkers of exposure (e.g., exhaled air and salivary cotinine) possibly because of the small sample size (Urban et al., 2003). van Sittert et al. (2000) determined that MHBMA was better than DHBMA at measuring lower exposures to 1,3-butadiene. They reported relatively high background concentrations of DHBMA in urine.

### III.4.7.2 1,3-Butadiene adducts

Other biomarkers of 1,3-butadiene exposure include Hb adducts of 1,3-butadiene and 1- and 2-hydroxy-3-butenyl valine (MHBVal). MHBVal adducts have been measured in blood samples via GC-tandem mass spectrometry (Richardson et al., 1996). In unexposed men, MHBVal levels ranged between 0.1 and 1.2 pmol/g Hb; levels in exposed men ranged from 0.6 to 3.8 pmol/g Hb. MHBVal has been described by van Sittert et al. (2000) as a sensitive tool for measuring cumulative environmental exposure to 1,3-butadiene but they reported no effect of smoking on MHBVal levels.

Cigarette smokers had significantly higher levels of N-(2,3,4-trihydroxybutyl) valine-Hb adducts than nonsmokers (Fustinoni et al., 2002).

LSRO does not recommend routine measurement of these adducts in PRRTP studies because assays of MHBMA and DHBMA are simpler. LSRO ranks 1,3-butadiene adducts as Category C biomarkers.

### III.4.8 Acrolein Biomarker

Acrolein is found in the gas phase of cigarette smoke. Sources of acrolein exposure other than tobacco smoke include motor vehicle exhaust, burning fatty food, occupational exposure, and treatment with the drug cyclophosphamide (Linhart et al., 1996; Mascher et al., 2001; Sanduja et al., 1989). Acrolein is also a product of endogenous lipid peroxidation (Kaye, 1973; Linhart et al., 1996; Sanduja et al., 1989). Its half-life is approximately 12 hours (Scherer, 2005).
III.4.8.1 3-Hydroxypropylmercapturic acid

3-Hydroxypropylmercapturic acid (3-HPMA) is a biomarker of exposure to acrolein in tobacco smoke. HPLC-tandem mass spectrometry as been used to measure urine levels of 3-HPMA, as an indicator of acrolein. 3-HPMA is stable in urine at 37°C for 24 hours. This analytical method is said to be fast, sensitive, and specific (Mascher et al., 2001). Mascher and co-workers (2001) compared urinary 3-HPMA levels for 27 smokers (11 of whom smoked more than 20 cigarettes per day) with levels for 41 nonsmokers. 3-HPMA excretion was significantly higher for smokers (2809 ± 385 μg/24 hours) than for nonsmokers (812 ± 123 μg/24 hours). Other studies showed that smoking leads to elevated levels of 3-HPMA (Esterbauer et al., 1990; International Agency for Research on Cancer, 1995).

Measurement of 3-HPMA is considered to be particularly useful for PRRTP studies because of an ability to distinguish between smokers and nonsmokers and LSRO ranks 3-HPMA as a Category A biomarker.

III.4.9 Acrylonitrile Biomarkers

Acrylonitrile, a chemical used in manufacturing plastics, resins, fibers, and rubber, is a “possible human carcinogen” (Group 2B) (International Agency for Research on Cancer, 1999). Levels in MS are 3–19 μg/cigarette (Smith et al., 2001). SS acrylonitrile levels are approximately 4-fold higher than MS levels (World Health Organization, 1986).

III.4.9.1 N-(2-Cyanoethyl)valine-hemoglobin adducts

N-(2-Cyanoethyl)valine-Hb adducts, which are biomarkers of exposure to acrylonitrile, reflect gas phase exposure and persist for the lifetime of an erythrocyte (120 days) but are not specific for tobacco smoke exposure (Scherer, 2005). An analytical method used is GC-mass spectrometry, in electron impact mode with a limit of detection of 4 pmol/g globin (Schettgen et al., 2002). LSRO ranks these adducts as Category A biomarkers.

Pérez et al. (1999) measured levels of N-(2-cyanoethyl)valine-Hb adducts in nonsmokers, ex-smokers, and current smokers. Mean ± SD adduct levels (pmol/g Hb) for nonsmokers who reported no exposure to ETS (n = 18) and nonsmokers who reported ETS exposure (n = 4) were 0.76 ± 0.36 (range = 0.32–1.6) and 1.1 ± 0.6 (range = 0.6–1.7), respectively. The mean ± SD value (pmol/g Hb) for ex-smokers who had stopped smoking 4 months before the study (n = 2) was 1.2 ± 0.5 (range = 0.7–1.7). Mean adduct levels for a 10–15 cigarettes/day smoker and for a 20 cigarettes/day smoker before smoking cessation were 64 and 91 pmol/g Hb, respectively.
One smoker of 1 cigarette/day before smoking cessation had a value of 8.3 pmol/g Hb (Bergmark, 1997).

Fennell et al. (2000) reported that consumption of 1 cigarette/day raised the level of these adducts by 9 pmol/g Hb. Means ± SD for adduct levels in nonsmokers and active smokers of 1 pack of cigarettes/day were 4.9 ± 1.9 pmol/g Hb and 252 ± 22 pmol/g Hb, respectively (p < 0.0001). The mean ± SD value for active smokers of 2 packs of cigarettes/day was 364 ± 34 pmol/g Hb, which was significantly higher than levels for smokers of 1 pack/day (p < 0.016) and nonsmokers (p < 0.0001).

Schettgen et al. (2002) reported the median value of N-(2-cyanoethyl)valine-Hb adducts for smokers (n = 38) as 131 pmol/g globin (range = 12–256, 95th percentile = 14 pmol/g globin) while that for nonsmokers (n = 24) was less than 4 pmol/g globin (range = 4–71, 95th percentile = 24 pmol/g globin). The correlation between daily cigarette consumption and adduct levels was 0.68. The authors estimated that smoking 1 cigarette/day raised the level of adducts by approximately 6.1 pmol/g Hb (Schettgen et al., 2002). Other studies also demonstrated elevated adduct values in smokers compared with nonsmokers (Bergmark, 1997; Calleman et al., 1994; Osterman-Golkar et al., 1994; Tavares et al., 1996).

III.4.10 Polycyclic Aromatic Hydrocarbon Biomarkers

Polycyclic aromatic hydrocarbons (PAHs) are considered to be likely human carcinogens and are ubiquitous. Major sources are coal tar and coal tar products (Jongeneelen, 2001). Individuals who work in aluminum plants, iron and steel plants, plants that produce creosol, mechanic shops, and meat-smoking facilities generally have higher exposures to PAHs than the general population (Levin, 1995). Exposure can also result from consumption of foods that are grilled, smoked, or stored in charred containers.

III.4.10.1 1-Hydroxypyrene

1-Hydroxypyrene (1-HOP) is the major urinary metabolite of pyrene (Harper, 1957; Jacob et al., 1982). Pyrene is a non-carcinogenic component of all PAH mixtures (e.g., exhaust fuels) and often accounts for a large proportion of PAHs (Dor et al., 1999). MS and SS ranges of pyrene are 50–270 and 390–1,010 ng/cigarette, respectively (Li et al., 2000). 1-HOP has been used extensively as a measure of tobacco smoke exposure and in PRRTP studies. The elimination half-life of 1-HOP is approximately 20 hours (Dor et al., 1999). LSRO ranks 1-HOP as a Category A biomarker.
Smokers’ 1-HOP levels are typically 2- to 3-fold higher than nonsmokers’ levels, and most studies have reported significantly elevated levels of 1-HOP in smokers compared with nonsmokers (e.g., (Hecht, 2002; Merlo et al., 1998)). (See Table 1 in Hecht (2002) for a summary of 1-HOP levels in smokers and nonsmokers.)

HPLC with fluorescence detection has been used to quantify PAHs (Carmella et al., 2004b; Li et al., 2000). Advantages of this assay include a low detection limit, sensitivity sufficient for small amounts of urine, rapid analysis of multiple samples, and use of an internal standard, 1-hydroxybenz[a]anthracene (Carmella et al., 2004b). HPLC with immunoaffinity columns and synchronous fluorescence spectrometry has been used to analyze pyrene in urine (Weston et al., 1993).

Li and others (Li et al., 2000; Merlo et al., 1998) reported a significant positive dose-response relationship for the number of cigarettes smoked per day and urinary 1-HOP levels ($p = 0.001$; coefficient of determination $[r^2] = 0.93$). In first-morning urine voids, Li et al. (2000) measured 1-HOP levels of $0.04 \pm 0.03 \, \mu\text{mol/mol creatinine}$ (nonsmokers), $0.20 \pm 0.18 \, \mu\text{mol/mol creatinine}$ (light smokers, <20 cigarettes/day), $0.46 \pm 0.36 \, \mu\text{mol/mol creatinine}$ (medium smokers, >20 to <40 cigarettes/day), and $1.16 \pm 0.73 \, \mu\text{mol/mol creatinine}$ (heavy smokers, >40 cigarettes/day).

Dor et al. (1999) reported background levels of 1-HOP of 0.03–0.79 $\mu\text{mol/mol creatinine}$ in a population without occupational exposure to 1-HOP. Increases in 1-HOP concentrations between the start and the end of the workday for occupationally exposed individuals are typically between 1 and 10 $\mu\text{mol/mol creatinine}$, but increases of up to 50 $\mu\text{mol/mol creatinine}$ have been reported (Dor et al., 1999; Heikkila et al., 1995). Exposure to ETS does not significantly raise 1-HOP levels (Scherer et al., 1992; Van Rooij et al., 1994).

Hatsukami et al. (2004b) measured concentrations of 1-HOP in the urine of 22 individuals who switched from their usual brand of conventional cigarette to Omni® cigarettes for 4 weeks. According to the Omni® cigarette website [cited by Hatsukami et al. (2004b)], these cigarettes yielded 15–20% less pyrene than a conventional cigarette when machine-smoked via the FTC method. Hatsukami et al. (2004b) reported 5% lower levels of 1-HOP levels in urine of individuals who switched to using Omni® cigarettes compared with levels when they smoked their usual brand of cigarettes.
Feng et al. (2006) measured urinary 1-HOP levels for groups of smokers who were either assigned to a no smoking group, or instructed to continue smoking their own brand of conventional cigarette, to switch to smoking a conventional cigarette that delivered 3 mg tar/cigarette, or to change to smoking one of two EHCSSs for 8 days. On day 8, the urinary 1-HOP value for subjects who smoked EHCSSs was significantly lower than that for subjects who smoked conventional cigarettes (p < 0.0001) and was not significantly different from that for the no-smoking group (p > 0.05).

Hecht et al. (2004a) studied the effect of reduced daily cigarette consumption on urinary 1-HOP levels. Smokers were asked to reduce their smoking by 25% during weeks 0–2, by 50% during weeks 2–4, and by 75% during weeks 4–6. They were asked to maintain reduced smoking through week 26 of the study. Urinary 1-HOP values were measured at baseline and at 4, 6, 8, 12, and 26 weeks after smoking reduction began. Reductions in 1-HOP levels ranged from 14% to 35% and were less than what might have been expected on the basis of the number of cigarettes smoked per day.

Murphy et al. (2004) measured 1-HOP in smoking subjects during 4 visits. Significant correlations were seen between visits (p < 0.0001 to p < 0.005). These authors estimated that 30–50% of pyrene derives from environmental non-tobacco sources. According to Van Rooij et al. (1994), food is the source of 53% of total PAHs for smokers and 99% of PAHs for nonsmokers without occupational exposure to PAHs. 1-HOP levels may be influenced by genetic polymorphisms of carcinogen-metabolizing enzymes (Alexandrie et al., 2000; Nan et al., 2001; Nerurkar et al., 2000; van Leeuwen et al., 2005). Dor et al. (1999) discovered a significant inter-individual variation in 1-HOP levels. However, levels were consistently elevated in smokers, and studies of PRRTPs detected reduced levels. Smoking generally increases 1-HOP values only 2- to 3-fold, so measuring reduced levels can prove challenging (Hecht, 2002). Sampling methods and analysis conditions are standardized (Dor et al., 1999).

III.4.10.2 3-Hydroxybenzo[a]pyrene and 3-hydroxybenz[a]anthracene

3-Hydroxybenzo[a]pyrene and 3-hydroxybenz[a]anthracene are the major metabolites of the PAHs B[a]P and benz[a]anthracene. The amount of B[a]P in cigarette smoke is approximately 9 ng/cigarette (Chepiga et al., 2000). 3-Hydroxybenzo[a]pyrene and 3-hydroxybenz[a]anthracene are not specific markers of tobacco smoke exposure because of dietary, occupational, and other environmental exposures. The mean value ± SD of 3-hydroxybenz[a]anthracene in urine of 19 occupationally exposed individuals was 376 ± 466 ng/g creatinine (range = 15–1,871). Corresponding values for the same individuals for 3-hydroxybenzo[a]pyrene...
were 37 ± 56 ng/g creatinine (range = 3–198) (Gündel & Angerer, 2000). LSRO considers 1-HOP to be a better measure of PAH exposure than these biomarkers; however, given the ease of their measurement in urine, 3-hydroxybenzo[a]pyrene and 3-hydroxybenz[a]anthracene are considered to have utility as indicators of tobacco smoke exposure (Category B biomarkers).

3-Hydroxybenzo[a]pyrene is primarily excreted in feces and is not generally measurable in urine (Hecht, 2002). The excreted amount is lower than that of other markers of PAH exposure, such as 1-HOP and hydroxyphenanthrenes. B[a]P is found at levels 2,500 times lower than the level of 1-HOP, the primary pyrene metabolite in mammals (Harper, 1957; Jacob et al., 1982). Few data are available on 3-hydroxybenzo[a]pyrene levels in smokers (Hecht, 2002). However, Simon et al. (2000) reported ranges of 0.1–0.8 ng/ml for smokers and ND–0.2 ng/ml for nonsmokers.

HPLC that utilizes an automated column-switching method has served for analysis of 3-hydroxybenzo[a]pyrene in urine (Simon et al., 2000). The column-switching approach, described as easy, allows the hydrolyzed urine sample to be injected directly into the LC apparatus after a fast, on-line sample treatment process (Simon et al., 2000). Capillary GC-mass spectrometry (Grimmer et al., 1997) has also been used to quantify as many as 25 PAHs including benz[a]anthracene and B[a]P. HPLC with an enriching precolumn and fluorescence detection measures both 3-hydroxybenzo[a]pyrene and 3-hydroxybenz[a]anthracene and is described as being simple and sensitive (Gündel & Angerer, 2000). Limitations of this assay include the presence of co-eluting compounds and resolution (Jacob & Seidel, 2002).

III.4.10.3 Benzo[a]pyrene-hemoglobin adducts
These adducts reflect exposure to B[a]P, a PAH occurring in the particulate phase of smoke. The ultimate carcinogen of B[a]P is B[a]P diol epoxide (BPDE). B[a]P adducts persist for the life of an erythrocyte (approximately 120 days). Seasonal variation in BPDE adduct levels was reported, with higher levels in the winter than in the summer (Pastorelli et al., 1999). A number of groups have used GC-mass spectrometry for analysis. (Day et al., 1991; Melikian et al., 1997; Pastorelli et al., 1996, 1998; Scherer et al., 2000).

Scherer et al. (2000) measured B[a]P-Hb adduct levels in smokers (n = 27) and nonsmokers (n = 69). Mean adduct levels for smokers and nonsmokers were 105 and 68 pmol/g Hb, respectively. Smokers’ B[a]P-Hb levels were approximately double that of nonsmokers; however, the difference in adduct
levels between smokers and nonsmokers was not significant ($p = 0.12$), nor were urinary cotinine concentration and $B[a]P$-Hb adduct levels significantly correlated ($p = 0.35$, $r = 0.19$).

LSRO ranks $B[a]P$-Hb adducts as Category C biomarkers.

**III.4.10.4 Benzo[a]pyrene-albumin adducts**

The $B[a]P$-albumin adduct has a half-life of approximately 21 days. Scherer (2005) reported smokers’ levels of $B[a]P$-albumin adducts as approximately 2-fold those of nonsmokers. Another study by Scherer et al. (2000) also found significantly higher levels of $B[a]P$-albumin adducts for smokers (mean ± SE = 0.042 ± 0.011 fmol/mg albumin, range = ND–0.254; $n = 27$) than for nonsmokers (mean = 0.020 ± 0.005 fmol/mg albumin, range = ND–0.192; $n = 42$) ($p < 0.05$). $B[a]P$-albumin adduct values were significantly correlated with cotinine levels ($p < 0.05$, $r = 0.44$). Other groups reported higher levels of $B[a]P$-albumin for smokers than for nonsmokers as well (Astrup et al., 1995; Crawford et al., 1994; Sherson et al., 1990; Tas et al., 1994).

Analytical methods include ELISA (Astrup et al., 1995; Crawford et al., 1994; Sherson et al., 1990), GC-mass spectrometry (Pastorelli et al., 1998; Scherer et al., 2000), and HPLC with fluorescence detection. ELISA showed adduct levels several orders of magnitude higher than those reported for the other 2 methods (Tas et al., 1994).

LSRO ranks these adducts as Category C biomarkers.

**III.4.10.5 Naphthols**

Naphthols are metabolites of naphthalene, a dicyclic aromatic hydrocarbon found in mixtures of PAHs. Naphthalene levels range from 2 to 4 $\mu$g/cigarette (World Health Organization, 1986). The half-life of naphthols is approximately 12 hours. These biomarkers are not specific to tobacco smoke; PAH exposure can result from food, occupational sources, some medications, and environmental sources. Analytical methods for naphthols in urine include HPLC (Kim et al., 1999), with a detection limit of 0.13 ng/ml, and GC-mass spectrometry with a detection limit of 0.27 $\mu$g/L (Yang et al., 1999).

Smokers’ urine naphthol concentrations are typically higher than nonsmokers’ naphthol concentrations (Hansen et al., 1994; Heikkila et al., 1995; Jansen et al., 1995; Kim et al., 1999; Nan et al., 2001; Yang et al., 1999). Nan et al. (2001) showed that 2-naphthol levels in 41 smokers’ urine samples were
approximately twice those of 87 nonsmokers (geometric mean\(^5 = 3.94\), geometric SD = 1.89 versus geometric mean = 1.55, geometric SD = 2.19). Yang et al. (1999) reported that levels of 1- and 2-naphthols in male Japanese smokers in a population not believed to have significant occupational exposure to PAHs were approximately 3- to 7-fold higher than those in nonsmokers. Kim et al. (1999) reported that the mean urinary 2-naphthol levels for Korean shipyard workers who were smokers (7.03 ± 6.16 ng/ml) were significantly higher than levels for nonsmokers (2.49 ± 3.92 ng/ml).

Genetic polymorphisms of carcinogen-metabolizing enzymes such as CYP2E1 and GSTM1 can cause urinary 2-naphthol levels to vary. Urine levels of 2-naphthol have better correlations with cotinine than do urine levels of 1-naphthol (Yang et al., 1999) and thus may be preferred as a biomarker of PAH exposure compared with levels of 1-naphthol (Kim et al., 1999).

LSRO does not recommend routine measurement of naphthols in PRRTP evaluations and ranks them as Category C biomarkers. LSRO considers 1-HOP to be a better biomarker for PAH levels.

III.4.10.6 Hydroxyphenanthrenes and phenanthrene dihydrodiols

The 1-, 2-, 3-, 4-, and 9-hydroxyphenanthrenes are metabolites of phenanthrene, a non-carcinogenic PAH (International Agency for Research on Cancer, 1983; LaVoie & Rice, 1988). The range of phenanthrene yields in MS is approximately 85–620 ng/cigarette (World Health Organization, 1986). The half-life of phenanthrenes is approximately 13 hours (Scherer, 2005).

Phenanthrenes are not specific measures of tobacco smoke exposure because of environmental and dietary exposures to PAHs. Individuals who work in coal and pitch tar production; aluminum plants; iron and steel plants; and industries producing creosote, rubber, mineral oil, soot, and carbon-black have an increased likelihood of PAH exposure (Jacob & Seidel, 2002). An argument against using phenanthrenes as biomarkers of exposure is that they are not biologically active metabolites and will thus not reflect exposure to carcinogenic PAHs (Jacob & Seidel, 2002). Phenanthrenes have not been studied extensively as measures of tobacco smoke exposure and LSRO does not recommend routine measurement of phenanthrenes in PRRTP evaluations (Category C biomarkers).

Individuals exposed to ETS do not have higher urine levels of phenanthrenes than unexposed individuals (Hoepfner et al., 1987; Martin et al., 1989). Certain

\(^5\) The geometric mean is the product of the numbers in a set divided by the \(n^{th}\) root of the product.
studies showed no significant differences in urine phenanthrene values for smokers and nonsmokers (Carmella et al., 2004a; Jacob et al., 1999). However, Heudorf and Angerer (2001), using HPLC and fluorescence detection, found significant differences between smokers and nonsmokers.

HPLC and fluorescence detection revealed a significant positive dose-response relationship between the number of cigarettes smoked per day and 2-, 3-, and 4- hydroxyphenanthrenes, but not a relationship for 1-hydroxyphenanthrene (Heudorf & Angerer, 2001). However, other analytical methods have not demonstrated such a dose-response relationship (Carmella et al., 2004a; Jacob et al., 1999). Analytical methods for phenanthrenes include HPLC (Gündel et al., 1996; Heudorf & Angerer, 2001; Hoepfner et al., 1987; Lintelmann et al., 1994; Mannschreck et al., 1996), GC (Grimmer et al., 1993; Hoepfner et al., 1987), GC-mass spectrometry (Gmeiner et al., 1998; Grimmer et al., 1997; Jacob et al., 1999; Serdar et al., 2003; Smith et al., 2002), and GC with positive ion chemical ionization (Carmella et al., 2004a). HPLC with fluorescence detection has been used to analyze phenanthrenes in spot urine samples (Heudorf & Angerer, 2001); GC has been used for measurements of phenanthrenes in urine (Grimmer et al., 1993; Hoepfner et al., 1987).

### III.4.10.7 Polycyclic aromatic hydrocarbon-DNA adducts

PAH-DNA adducts are biomarkers of exposure to PAHs found in the particulate phase of cigarette smoke. After metabolic activation, PAHs produce electrophilic species that bind covalently to DNA (Lodovici et al., 1998). The half-life of these adducts varies according to cell and kind of tissue, but these adducts are generally markers of long-term exposure (Scherer, 2005). LSRO does not recommend routine measurement of PAH-DNA adducts in PRRTP studies (Category C biomarkers).

PAH-DNA adduct levels in smokers are approximately twice as high as levels in nonsmokers (Scherer, 2005). Mooney et al. (1995) measured PAH-DNA adducts in smokers of more than 20 cigarettes/day who were trying to quit smoking. At 1 year after quitting, values of these adducts declined by 50% (Mooney et al., 1995).

B[a]P is activated by cytochrome P450 and epoxide hydrolase to form electrophilic and free radical species, such as BPDE (Gelboin, 1980). Lodovici et al. (1998) measured mean levels of anti-BPDE-DNA adducts in autopsy samples from smokers, ex-smokers, and nonsmokers who had lived in Italy. They reported mean levels of total BPDE-DNA adducts of 4.46 ± 5.76 per 10^8 bases in smokers, 4.04 ± 2.37 per 10^8 bases in
Melikian et al. (1999) observed that BPDE-DNA adduct concentrations in epithelial cervical tissues of smokers were approximately twice those in nonsmokers (mean ± SD = 3.5 ± 1.06 versus 1.8 ± 0.96 adducts/10^8 nucleotides, respectively; p = 0.02). Zenzes et al. (1999) reported that that smokers had significantly elevated mean levels of BPDE-DNA adducts in semen compared with nonsmokers.

Romano et al. (1999) used an immunohistochemical approach to evaluate B[a]P-DNA adducts in oral cells from 33 tobacco smokers and 64 nonsmokers. The mean relative staining intensity ± SD was significantly greater for smokers than for nonsmokers (smokers = 330 ± 98, nonsmokers = 286 ± 83; p = 0.013). Furthermore, as the number of cigarettes smoked increased, the PAH-DNA adduct level increased.

Nia et al. (2000c) compared levels of PAH-DNA adducts in smokers and nonsmokers via an antibody that recognizes a BPDE that cross-reacts with structurally similar PAH-diol epoxide adducts. PAH-DNA adduct levels (expressed in arbitrary units) in the mouth floor and buccal mucosa for 26 smokers and 22 smokers were significantly higher than those for nonsmokers (0.045 ± 0.022 versus 0.022 ± 0.016; p = 0.0008). Buccal mucosa adduct concentrations for smokers were also significantly higher than those for nonsmokers (0.058 ± 0.028 versus 0.028 ± 0.012; p = 0.001).

Godschalk et al. (2002) reported significantly higher mean ± SD levels of bulky DNA adducts in 13 lung cancer patients who smoked (11.2 ± 7.8 adducts/10^8 nucleotides, range = ND–25.9) compared with levels in 11 lung cancer patients who did not smoke (2.2 ± 2.2 adducts/10^8 nucleotides, range = ND–0.7, p = 0.001). Means ± SD (and ranges) of putative BPDE-DNA adduct levels for the same smokers and nonsmokers were 1.5 ± 1.0 adducts/10^8 nucleotides (range = ND–3.6) and 0.2 ± 0.2 adducts/10^8 nucleotides (range = ND–0.7, p < 0.001), respectively.

Significant inter-individual variability of levels of DNA adducts is observed. One disadvantage of the anti-BPDE–DNA adducts antibody is the potential for cross-reaction with other PAH-DNA adducts. High variation in PAH-DNA adducts reflects differences in exposure, response, nutrition, and genetic factors (Mooney et al., 1995). Furthermore, studies have not consistently shown that smokers’ levels of PAH-DNA adducts are higher than those of nonsmokers (Kriek et al., 1998; Tang et al., 1995).
III.4.11 Acetonitrile Biomarkers

Acetonitrile is found in the gas phase of MS at levels of 44–140 μg/cigarette. Acetonitrile is an organic solvent that is used in laboratories and various industries (Michaelis et al., 1991); it is also thought to be produced endogenously (Jordan et al., 1995). LSRO considers measurement of acetonitrile as useful for routine assessment of PRRTPs (Category B biomarker).

Acetonitrile has been measured in a number of biological matrices, including blood (Houeto et al., 1997), exhaled breath (Jordan et al., 1995; Lindinger et al., 1998), and urine (McKee et al., 1962; Pinggera et al., 2005). Its half-life is approximately 32 hours in blood and approximately 24 hours in breath (Michaelis et al., 1991). For one smoker it took approximately 1 week for breath acetonitrile levels to decline to those of nonsmokers (Jordan et al., 1995).

Houeto et al. (1997) measured blood acetonitrile levels in nonsmokers and in smokers whose daily cigarette consumptions were “minimal” (1–5 cigarettes/day), “low” (6–10 cigarettes/day), or “high” (>10 cigarettes/day). The mean ± SD blood acetonitrile concentration for 18 men who smoked more than 10 cigarettes/day (143 ± 18.0 μg/L) was significantly higher than that for the 6 individuals who smoked 1–10 cigarettes/day (43.3 ± 6.0 μg/L, p = 0.03).

Jordan et al. (1995) measured the acetonitrile concentration in exhaled breath samples of 75 smokers and 56 nonsmokers. The mean concentration for nonsmokers was less than 15 ppb (mean ± SD = 5.65 ± 1.90 ppb), and that for smokers ranged from 30 to 200 ppb (mean ± SD = 69.33 ± 33.34 ppb).

Acetonitrile levels were also measured in morning urine voids of 40 smokers of 0.25–2.5 packs of cigarettes/day and 20 nonsmokers (McKee et al., 1962). The lowest acetonitrile concentration measured for smokers of 3 or more cigarettes/day was 2.2 μg/100 ml urine, and the highest concentration found for smokers of less than 3 cigarettes/day was 0.74 μg/100 ml (McKee et al., 1962). Acetonitrile in urine appears to be stable.

Pinggera et al. (2005) measured urinary acetonitrile concentrations for 46 nonsmokers who were not exposed to ETS at home or at work, 10 “light smokers” (≤10 cigarettes/day), 23 “moderate smokers” (10–20 cigarettes/day), 9 “heavy smokers” (≤30 cigarettes/day), and 13 “very heavy smokers” (>30 cigarettes/day). They found a significant, positive dose-response relationship between number of cigarettes smoked per day and urine acetonitrile concentration (p = 0.001). They also reported that mean urinary...
acetonitrile concentrations for “passive smokers” (i.e., nonsmokers) exposed to ETS (8.2 ppbv) were almost as high as those for moderate smokers (12.95 ppbv) (Pinggera et al., 2005). Measurement at 4, 12, 24, and 36 hours after initial analysis showed no decline in acetonitrile levels.

Analytical methods for measuring acetonitrile include proton transfer reaction-mass spectrometry for urine samples (Jordan et al., 1995; Lirk et al., 2004; Pinggera et al., 2005), GC by the headspace technique (Houeto et al., 1997), and GC (Breland et al., 2003; McKee et al., 1962).

Analysis of breath acetonitrile concentration was described as a simple, non-invasive, cost-effective method for assessing cigarette smoking habits (Pinggera et al., 2005). Houeto (1997) described acetonitrile in blood as a moderately sensitive marker of cigarette smoking with a dose-effect relationship.

III.4.12 Minor Tobacco Alkaloids (Anatabine and Anabasine)

Anatabine and anabasine are minor tobacco alkaloids found in MS. A limitation of these substances as biomarkers of tobacco smoke exposure is that they are present in tobacco at 0.2–1% of nicotine levels (3–15 μg/cigarette) (Hatsukami et al., 2003; Schmeltz & Hoffmann, 1977). Anatabine and anabasine account for 2–3% and 0.3%, respectively, of total tobacco alkaloids, whereas nicotine accounts for 95% of this total (Saitoh et al., 1985). They are, however, specific markers of tobacco smoke exposure and appear to correlate well with nicotine intake measures (Benowitz & Jacob, III, 1984). The change in anatabine levels after tobacco exposure is greater than the change in anabasine levels. Although these substances have a number of desirable characteristics, nicotine and its metabolites are better measures of tobacco alkaloid exposure than are anatabine and anabasine. Quantification is easier because of their higher concentrations. LSRO considers anatabine and anabasine to be Category B biomarkers, because nicotine and its metabolites are better indicators of tobacco alkaloid exposure than are anatabine and anabasine.

The elimination half-lives of anatabine and anabasine are 10 and 16 hours, respectively (Jacob, III et al., 1999). These alkaloids are detectable in urine for 1–2 days after smoking cessation (Jacob, III, et al., 1999). Anatabine and anabasine are unlikely to be found in foods or other non-tobacco sources but do occur in nicotine-containing medications (Jacob, III, et al., 2002).

Jacob, III, et al. (2002) reported that cigarette smokers’ mean urine levels of anatabine were 22 ng/ml. They also measured the concentrations of...
anatabine and anabasine in urine samples of 35 individuals who did not use tobacco products or medicines containing nicotine (Jacob, III, et al., 2002). Only one subject had a urinary anatabine concentration above the detection limit for the assay. Three subjects had anabasine levels above the detection limit of the assay (1.7, 2.42, and 3.4 ng/ml).

Murphy et al. (2004) analyzed urinary anatabine levels of 46 smokers of 20–40 cigarettes/day who were asked to reduce their daily cigarette consumption by 75% during a 6-week period. The authors measured mean urinary anatabine levels for each subject in samples taken during weeks 1, 2, 3, and 8 of the study, and the mean of means was calculated. Anatabine levels were generally consistent over time and correlated significantly with free and total cotinine levels ($p < 0.001$, $r = 0.465$, and $p < 0.001$, $r = 0.514$, respectively), as well as free and total nicotine ($p < 0.0001$, $r = 0.753$ and 0.773, respectively). Murphy et al. (2004) could not analyze anabasine levels in some samples because of interference of a co-eluting peak, but they reported significant intra-individual correlation of anatabine samples across 4 visits ($p < 0.001$ to $p < 0.05$).

GC-mass spectrometry has been used to quantify anatabine and anabasine in urine (Jacob, III, et al., 1993, 2002). Jacob, III, et al. (2002) reported developing a more sensitive LC-tandem mass spectrometry assay that detected levels of anatabine and anabasine at 0.2 ng/ml.

### III.4.13 Cadmium

Cadmium is a heavy metal that is classified by IARC (1993) as a human carcinogen (Group 1) and is found in tobacco and the particulate phase of smoke. Cadmium yield in MS ranges from 0 to 6.67 μg/cigarette (Smith et al., 1997). Cadmium is not a specific biomarker of tobacco product and smoke exposure; other sources of cadmium include polyvinyl chloride products and food (Järup, 2003). The elimination half-life of cadmium has a fast component (75–128 days) and a slow component (7.4–16.0 years) (Järup et al., 1983). Urinary cadmium levels reflect long-term exposure (body burden); blood levels reflect more recent exposure (Satarug et al., 2004). A significant positive correlation exists between the number of cigarettes smoked per day and blood cadmium concentration ($p < 0.01$, $r = 0.296$) (Telisman et al., 1997). LSRO categorizes cadmium as a Category C biomarker.

Cadmium levels have been measured in urine by using inductively coupled plasma-mass spectrometry (Satarug et al., 2004). Simultaneous measurement of other metals such as lead is possible.
Individuals who are exposed to ETS have urine levels of cadmium that are twice as high as levels for individuals without such exposure (Järup et al., 1998). Järup et al. (1998) reported that blood cadmium levels of smokers were typically 4- to 5-fold higher than those of nonsmokers. Telisman et al. (1997) found that blood cadmium levels of 78 smokers were significantly higher than those of 42 nonsmokers (9 of whom were former smokers) who did not have occupational exposure to cadmium. The median values and ranges of blood cadmium values for smokers and nonsmokers were 4.33 (0.49–13.33) μg/L and 0.46 (0.19–1.49) μg/L, respectively.

### III.4.14 Acrylamide Biomarkers

#### III.4.14.1 \(N\)-Acetyl-\(S\)-(2-carbamoylethyl)-\(L\)-cysteine and \(N\)-(\(R\),\(S\))-Acetyl-\(S\)-(2-carbamoyl-2-hydroxyethyl)-\(L\)-cysteine

Acrylamide is a “probable human carcinogen” (Group 2A) (International Agency for Research on Cancer, 1994) that is produced during heating of foods that are high in starch (Tareke et al., 2000; 2002). Polymer production, cosmetics manufacturing, and textile production also generate acrylamide. MS yields of acrylamide range from 1.1 to 2.3 μg/cigarette (Smith et al., 2000c). Acrylamide is found in the vapor phase of smoke. \(N\)-Acetyl-\(S\)-(2-carbamoylethyl)-\(L\)-cysteine (AAMA) and its epoxide, \(N\)-(\(R\),\(S\))-Acetyl-\(S\)-(2-carbamoyl-2-hydroxyethyl)-\(L\)-cysteine (GAMA) are mercapturic acid metabolites of acrylamide. LSRO considers these adducts to have limited utility for PRRTP assessment and ranks them as Category C biomarkers.

Boettcher et al. (2005b) reported that levels of urinary AAMA were significantly higher (~4-fold) for 13 smokers as compared with levels for 16 nonsmokers (median = 127 μg/L, range = 17–338, versus median = 29 μg/L, range = ND–83). Median urinary concentrations of GAMA for smokers and nonsmokers were 19 μg/L (range = <limit of detection–83) and 5 μg/L (range = <ND–14), respectively.

Hagmar et al. (2005) also determined acrylamide adduct values in smokers and nonsmokers. The median concentration of Hb adducts for smokers (n = 72) was 0.152 nmol/g globin (range = 0.03 0.43). The corresponding value for nonsmokers (n = 70) was 0.0030 nmol/g globin (range = 0.02 0.1). Adduct levels for nonsmokers who did not have occupational exposure to acrylamide were similar to values for nonsmokers in previous studies (0.031 nmol/g globin) (Hagmar et al., 2005).

Boettcher & Angerer (2005) reported a high background level of AAMA and GAMA in German individuals who were not occupationally exposed to...
acrylamide. The median value of AAMA in urine for 13 smokers was 127 (range = 17–338 μg/L urine) and the median value for nonsmokers was 29 (range = ND–83 μg/L urine). The median value of GAMA in urine for 13 smokers was 19 (range = ND–45) and the median value for nonsmokers was 5 (range = ND–14 μg/L urine).

LC-electrospray ionization-tandem mass spectrometry has been used to measure AAMA and GAMA levels in urine (Boettcher et al., 2005a, 2005b). The detection limits for AAMA and GAMA were 1.5 μg/L urine, and the limit of quantitation for both analytes was approximately 5 μg/L. AAMA and GAMA can be quantified simultaneously. Acrylamide adducts in blood have been detected by using GC-tandem mass spectrometry in the negative ion/chemical ionization mode with single reaction monitoring (Hagmar et al., 2005).

III.4.14.2 N-(2-Carbamoylethyl)valine-hemoglobin adducts

N-(2-Carbamoylethyl)valine-Hb adducts are biomarkers of exposure to acrylamide. These adducts persist for the lifetime of an erythrocyte and reflect long-term exposure (Scherer, 2005). An analytical method applied to measure levels of this adduct is GC-tandem mass spectrometry in the negative ion/chemical ionization mode. LSRO ranks these biomarkers as Category C biomarkers.

Bergmark (1997) reported that mean levels of N-(2-carbamoylethyl) valine-Hb adducts in smokers and nonsmokers were 116 and 31 pmol/g globin, respectively. Laboratory personnel who worked with polyacrylamide gels had mean adduct levels of 54 pmol/g globin. Schettgen et al. (2002) reported median values of these adducts in 38 smokers as 89 pmol/g globin (range = 16–294) and in 24 nonsmokers as 22 pmol/g globin (range = <11–50).

Hagmar et al. (2005) also analyzed N-(2-carbamoylethyl)valine-Hb adducts in smokers and nonsmokers. The median value of these adducts for smokers (n = 72) was 152 pmol/g globin (range = 30–430). The corresponding value for nonsmokers (n = 70) was 31 pmol/g globin (range = 20–100). A 5-fold variation in acrylamide levels was reported for nonsmokers and a 10-fold variation was reported for smokers (Hagmar et al., 2005). Adduct levels for nonsmokers with no occupational exposure to acrylamide were similar to values for nonsmokers in previous studies (31 pmol/g globin) (Hagmar et al., 2005). Another study by Schettgen et al. (2003) reported background adduct levels of 0.012–0.05 nmol/g Hb.
III.4.15 Hydrogen Cyanide Biomarker

HCN is present in tobacco at trace levels. It transfers into cigarette smoke (Vogt et al., 1977) and is converted to thiocyanate in a reaction catalyzed by rhodanese (Westley, 1973). Thiocyanate is measured in plasma, urine, sweat, and saliva (Prue et al., 1981). Saliva flow rate can influence saliva levels of thiocyanate, however. Sensitivity and specificity of detection are enhanced when plasma serves as the specimen compared with urine and saliva (Bourdoux, 1995; Degiampietro et al., 1987). LSRO ranks thiocyanate as a Category C biomarker.

Thiocyanate is not a specific biomarker of cigarette smoke exposure because exposure can also come from foods containing thiocyanate such as cheese and milk (Bliss & O’Connell, 1984); vegetables containing glucosinolates (Weuffen et al., 1984); and industrial sources such as gas manufacturing and electroplating (Bark & Higson, 1963). Exposure can also arise through use of diuretics (Stookey et al., 1987).

The half-life of thiocyanate is 6–14 days. Junge (1985) reported a half-life of 6.4 (SD–0.9) days in 6 subjects who stopped smoking abruptly. In contrast, Pettigrew and Fell (1972) obtained a half-life of approximately 14 days for 1 subject. A half-life of 1 day has also been reported (Bliss & O’Connell, 1984). Thiocyanate levels in smokers and nonsmokers can overlap, particularly for light smokers (Vogt et al., 1977). Benowitz et al. (2002) recommended a cutoff level of 78–84 mmol/L for serum thiocyanate to distinguish between smokers and nonsmokers.

Hurt et al. (2000) studied blood thiocyanate levels for 23 heavy smokers who reduced their smoking from more than 40 cigarettes/day. Smoking reduction was unexpectedly associated with increased plasma thiocyanate values at weeks 8 and 24. The authors proposed that dietary sources of thiocyanate contributed to this finding.

Colorimetric analysis with ferric nitrate to produce red ferric thiocyanate has been used to quantify thiocyanate in serum and urine (Butts et al., 1974; Muscat et al., 2005; Westley, 1987). However, ferric thiocyanate can undergo photocatalyzed fading, so samples must be kept away from sunlight. Samples are stable for hours after color development. Complex biological samples may contain substances that interfere with the colored ferric complexes (Westley, 1987). Another technique, capillary zone electrophoresis (Glatz et al., 2001), has been used to measure thiocyanate in plasma, urine, and saliva. This method was described as being simple, rapid, and effective.
Furthermore, it could be automated, and only a small amount of sample was needed (Glatz et al., 2001).

Although multiple studies have utilized serum thiocyanate concentration to assess smoking status, control of dietary sources of exposure would be needed.

### III.4.16 Alkylation Biomarkers

#### III.4.16.1 3-Methyladenine

3-Methyladenine (3-Me-Ade) is a product of reactions between DNA and alkylating N-nitroso carcinogens (e.g., NDMA and NNK). The methylated base residue, 3-methyl-2′-deoxyguanosine, is released and excreted in urine as 3-Me-Ade. 3-Me-Ade reflects gas phase and particulate phase exposure and has a half-life of approximately 12 hours (Scherer, 2005). 3-Me-Ade levels in smokers’ urine are approximately 2-fold those in nonsmokers’ urine. LSRO does not recommend routine measurement of 3-Me-Ade in studies of PRRTPs (Category C biomarker).

In people whose diets are not controlled, Prevost et al. (1990) considered 3-Me-Ade to be of limited use as a measure of DNA methylation. 3-Me-Ade has not been used extensively as a biomarker of tobacco smoke exposure. One limitation of using 3-Me-Ade is high background levels. More than 90% of 3-Me-Ade is thought to come from dietary sources (Prevost et al., 1990). Feng et al. (2006) reported that 3-Me-Ade could distinguish between smokers who did not smoke for 8 days and continuing smokers but could not distinguish between smokers of conventional cigarettes and smokers of two types of EHCs.

#### III.4.16.2 3-Ethyladenine

3-Ethyladenine adducts in urine reflect exposure to ethylating substances in smoke. Their half-life is approximately 12 hours (Scherer, 2005). Diet has little influence on 3-ethyladenine levels, unlike its effect on 3-Me-Ade levels. Two studies demonstrated higher levels of 3-ethyladenine in urine of smokers than in urine of nonsmokers (Kopplin et al., 1995; Prevost & Shuker, 1996). Smokers have approximately 5-fold the levels of 3-ethyladenine measured in nonsmokers. An analytical method used for this substance is GC with negative ion chemical ionization–tandem mass spectrometry (Carmella et al., 2002). LSRO does not have confidence in this biomarker as a measure of tobacco product or smoke exposure studies and ranks it as a Category C biomarker.
Feng et al. (2006) reported mixed results for studies investigating the ability of 3-ethyladenine to differentiate between smokers who did not smoke for 8 days and continuing smokers, and between smokers of conventional cigarettes and smokers of two types of electrically heated cigarettes.

III.4.16.3 N-Ethylvaline-hemoglobin adducts

N-Ethylvaline-Hb adducts are reaction products of ethylating compounds in cigarette smoke and Hb. Mean ± SD values for 39 smokers (3.76 ± 2.77 pmol/g Hb) were significantly higher than those for 28 nonsmokers (2.50 ± 1.65 pmol/g Hb; \( p = 0.023 \)) (Carmella et al., 2002). Age and gender had no effect on N-ethylvaline-Hb adduct levels. These adducts have been measured via HPLC and GC with negative ion chemical ionization-tandem mass spectrometry (Carmella et al., 2002). LSRO ranks these adducts as Category C biomarkers.

III.4.16.4 N-(2-Hydroxyethyl)valine-hemoglobin adducts

N-(2-Hydroxyethyl)valine-Hb adducts reflect exposure to ethylene and ethylene oxide in the gas phase of cigarette smoke. Ethylene oxide, categorized by IARC as a human carcinogen (Group 1), is a commonly used gaseous sterilant and intermediate in chemical production (Bono et al., 1999). Non-tobacco smoke sources of ethylene oxide include ambient air and endogenous formation (Fennell et al., 2000). N-(2-Hydroxyethyl)valine-Hb adducts adducts have been analyzed via GC-mass spectrophotometry with a detection limit of 9 pmol/g globin (Schettgen et al., 2002). These adducts are promising biomarkers of tobacco smoke exposure but LSRO does not recommend routine measurement of them in PRRTP studies (Category C biomarkers).

N-(2-Hydroxyethyl)valine-Hb adducts persist for the life of the erythrocyte (120 days). Fennell et al. (2000) reported levels of N-(2-hydroxyethyl)valine-Hb adducts for smokers and nonsmokers of 242 and 13 pmol/g Hb, respectively. Bader et al. (1995) reported N-(2-hydroxyethyl)valine mean ± SD levels of 46 ± 12 pmol/g globin (range = 19–64) for nonsmokers (\( n = 37 \)) and 171 ± 93 pmol/g globin (range = 31–327) for smokers (\( n = 32 \)).

Schettgen et al. (2002) compared N-(2-hydroxyethyl)valine-Hb adduct levels for individuals working in a chemical plant that makes textile industry surfactants. Median N-(2-hydroxyethyl)valine-Hb adduct levels for nonsmokers and smokers were 77 pmol/g globin (range = 16–2353, 95th percentile = 988 pmol/g globin) and 175 pmol/g globin (range = 27–1653, 95th percentile = 1328 pmol/g globin) respectively.
III.4.16.5 N-Methylvaline-hemoglobin adducts

N-Methylvaline-Hb adducts reflect exposure to NDMA, NNK, methyl halides, and other methylating substances in gas and particulate phases of tobacco smoke. Another source is endogenous formation. N-Methylvaline-Hb adducts persist throughout the lifetime of the erythrocyte. LSRO ranks these adducts as Category C biomarkers.

Carmella et al. (2002) reported N-methylvaline-Hb adduct values in 29 nonsmokers as 904 ± 149 pmol/g globin and in 45 active smokers as 997 ± 203 pmol/g Hb, $p = 0.037$ (two-sided test). Bader et al. (1995) reported a significant difference between mean ± SD background levels of N-methylvaline in 37 nonsmokers, 1175 ± 176 pmol/g globin (range = 722–1516), and those in 32 smokers, 1546 ± 432 pmol/g globin (range = 938–2702) ($p < 0.001$). They also reported small inter-individual differences in levels (approximately 15%) for nonsmokers (Carmella et al., 2002). Scherer (2005) reported that levels in smokers were approximately 1.5 times greater than the levels in nonsmokers.

Endogenous levels of N-terminal N-ethylvaline were 250- to 350-fold lower than levels of N-terminal N-methylvaline (Carmella et al., 2002). Bader et al. (1995) reported that these adducts are not appropriate as a measure of cigarette smoke exposure.

III.4.17 Thioether Biomarkers

Thioethers are biomarkers of exposure to electrophilic compounds in the gas and particulate phases of smoke. The half-life is approximately 12 hours (Scherer, 2005). They are not specific markers of tobacco smoke exposure because diet influences their concentrations. LSRO does not recommend these biomarkers for routine measurement in PRRTP studies and ranks them as Category C biomarkers.

Thioethers can be measured by using alkaline hydrolysis and assays with Ellman’s reagent with modifications. Scherer et al. (1996) reported that smokers’ urine contained significantly elevated levels of thioethers compared with nonsmokers’ urine, with levels in smokers’ urine less than 50% higher than levels in nonsmokers’ urine. A significant dose-response relationship was reported for the number of cigarettes smoked per day and urinary thioether excretion (van Doorn et al., 1979).

Feng et al. (2006) compared urinary thioethers excretion levels of 19 smokers who smoked their own brand of cigarettes from before and after they had...
switched to smoking a conventional cigarette for 8 days. No significant difference was noted between levels before (mean ± SD = 157.0 ± 81.2 μmol/24hr urine) and after switching (mean ± SD = 159.9 ± 75.9 μmol/24hr urine) for 19 smokers. The authors reported that this assay was not adequately sensitive.

Exposure to artificially high levels of ETS led to increased thioether excretion (Scherer et al., 1990). However, in a later study, Scherer et al. (1996) found no significant difference between urine levels of thioethers for nonsmokers who were exposed to more realistic levels of ETS and those who were not exposed to ETS. Dietary sources of sulfur give rise to inter-individual variation in thioether levels, and minimization of consumption of grilled, fried, toasted, and roasted foods by study participants decreased dietary contribution of thioethers (Scherer et al., 1996).

### III.4.18 Other Biomarkers

#### III.4.18.1 7-Methylguanine-DNA adducts

7-Methylguanine-DNA adducts are products of DNA methylation by substances in the body, air, and both phases of tobacco smoke. The half-life of these adducts is approximately 60 hours (Scherer, 2005). Adduct levels in DNA for smokers and nonsmokers vary widely. Food contains precursors and factors that modify in vivo nitrosation (Bartsch & Montesano, 1984). These adducts can be detected by means of the $^{32}$P-postlabeling assay. LSRO does not recommend these adducts for routine measurement in PRRTP evaluations (Category C biomarkers).

Smokers’ 7-methylguanine DNA adduct levels are approximately 5-fold higher than nonsmokers’ levels. Mustonen and Hemminki (1992) measured 7-methylguanine values in total leukocyte, granulocyte, and lymphocyte DNA of 10 smokers and 10 nonsmokers and found significant differences between total lymphocyte and leukocyte values and between granulocyte and lymphocyte values. Lymphocytes have half-lives for up to years and granulocytes have half-lives up to days (Mustonen & Hemminki, 1992).

In another study, Mustonen et al. (1993) measured 7-methylguanine adducts in bronchial specimens and peripheral blood lymphocytes of cancer patients who had undergone pulmonary surgery. Mean bronchial adduct levels were significantly elevated ($p < 0.01$) for smokers (17.3 adducts/10$^7$ nucleotides; $n = 11$) compared with nonsmokers (4.7 adducts/10$^7$ nucleotides; $n = 6$). Mean 7-methylguanine adduct levels in lymphocyte DNA for smokers and nonsmokers ($n = 3$) were of borderline significance ($p = 0.055$).
III.4.18.2 8-Hydroxy-2′-deoxyguanosine

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is produced by reactive oxygen species, which are compounds with partially reduced oxygen that are highly reactive with biological molecules (Nia et al., 2001). Diet, antioxidant levels, physical activity, and exposure to motor vehicle exhaust affect 8-OHdG levels (Daube et al., 1997). The preferred analytical methods to analyze 8-OHdG are HPLC and GC-mass spectrometry (Pilger & Rüdiger, 2006). Smokers and individuals with chronic hepatitis have elevated 8-OHdG concentrations. LSRO ranks 8-OHdG as a Category C biomarker.

Oral cells from 38 healthy smokers contained significantly higher ($p < 0.01$) levels of 8-OHdG (mean ± SD relative absorbance = 111 ± 55) than cells from 71 healthy nonsmokers (mean ± SD relative absorbance = 78 ± 48) (Romano et al., 1999). Nia et al. (2001) observed no significant difference between urinary excretion of 8-OHdG for 21 smokers (mean ± SE = 197 ± 31 ng/BMI, range = 78–449) and 24 nonsmokers (mean ± SE = 240 ± 33 ng/BMI, range = 60–472). Nia et al. (2001) also reported reduced levels of 8-OHdG in smokers’ lymphocytes compared with nonsmokers’ lymphocytes, which was reported in a previous study (Zwingmann et al., 1999), and another group reported similar findings (van Zeeland et al., 1999). In contrast, other investigators measured leukocyte and lymphocyte levels of 8-OHdG but showed higher levels for smokers than for nonsmokers (Asami et al., 1996, 1997). Another study did not find a significant difference in lymphocyte 8-OHdG levels for smokers and nonsmokers (Loft et al., 1992).

Feng et al. (2006) reported an apparent lack of association between 8-OHdG levels and smoking in a study that evaluated levels in smokers of two types of conventional cigarettes, and smokers who abstained from smoking for 8 days.

III.4.18.3 2,5-Dimethylfuran

The volatile organic compound, 2,5-dimethylfuran, is found in smoke at an approximate concentration of 58 μg/cigarette (Baggett et al. 1974). Other sources are coffee (Wang et al., 1983) and occupational exposure to n-hexanes and its metabolites (Perbellini et al., 1981). 2,5-Dimethylfuran can be measured in breath (Gordon, 1990), blood, urine, and alveolar air (Ashley et al., 1996; Perbellini et al., 2003). Analysis of volatile organic compounds in breath holds promise, in particular of 2,5-dimethylfuran, but at this time LSRO does not recommend routine measurement of this biomarker in PRRTP evaluations (Category C biomarker).
Analytical methods include GC-mass spectrometry (Perbellini et al., 2002, 2003), which has a sensitivity of 1 ng/L in alveolar air and 5 ng/L in blood and urine (Perbellini et al., 2003). Blood 2,5-dimethylfuran concentrations can be measured along with those of other volatile organic compounds, which makes additional analytical procedures unnecessary (Ashley et al., 1996). Gordon (1990) found 2,5-dimethylfuran in the breath of 92% of cigarette smokers and blood of 96% of cigarette smokers but not in nonsmokers. In a later study, Gordon et al. (2002) used a real-time breath measurement approach to obtain levels of 2,5-dimethylfuran and volatile organic compounds as indicators of active and passive smoking. They reported 2,5-dimethylfuran mean ± SD values of 27.1 ± 18.1 μg/m³ in nonsmokers' breath (n = 4) and 340 ± 71 μg/m³ in smokers' breath (n = 5).

Mean 2,5-dimethylfuran blood levels were significantly higher for 15 smokers (100.2 ng/L; SD = 103.4) than for 46 nonsmokers 5.7 ng/L; SD = 7.7). The mean 2,5-dimethylfuran alveolar air levels for smokers was 3.6 ng/L (SD = 3.3) and that for nonsmokers was 0.6 ng/L (SD = 0.3) (Perbellini et al., 2003).

### III.4.18.4 5-Hydroxymethyluracil

5-Hydroxymethyluracil, a product of uracil oxidation, is excreted in urine. Pourcelot et al. (1999) reported no significant difference between 5-hydroxymethyluracil urine levels for smokers and nonsmokers. No dose-response relationship between 5-hydroxymethyluracil levels and the number of cigarettes smoked was found (Collier & Pritsos, 2003). Therefore, 5-hydroxymethyluracil better reflects DNA damage by oxygen free radicals than it reflects cigarette smoke exposure (Bianchini et al., 1998). LSRO ranks this substance as a Category C biomarker.

### III.4.18.5 F₂-isoprostanes

Oxidation of biomolecules is thought to be a component of the pathological processes of atherosclerosis and other diseases. F₂-isoprostanes (F₂-IsoPs) are prostaglandin-like substances produced by peroxidation of arachidonic acid. F₂-IsoPs have been described as the best-characterized biomarkers of oxidative stress. LSRO ranks F₂-IsoPs as a Category C biomarker.

F₂-IsoP levels are elevated in cigarette smokers (Chehne et al., 2002; Pilz et al., 2000; Reilly et al., 1996), as well as in individuals with diabetes, chronic obstructive pulmonary disease, hypocholesterolemia, and scleroderma (Ridker et al., 2004).
Smoking cessation results in a rapid decline in F$_2$-IsoP levels. Within 2 weeks of quitting, levels are close to those measured in healthy nonsmokers without the above-mentioned risk factors (Pilz et al., 2000). Levels increase on resumption of smoking (Chehne et al., 2002).

F$_2$-IsoPs are measured in plasma and urine by methods such as GC-mass spectrometry and ELISA (Ridker et al., 2004). Although highly specific and sensitive, certain analytical methods are labor-intensive, and the instrumentation is expensive (Morrow, 2005). ELISA methods have been described as having promise but have not been validated. Furthermore, the value of plasma measurements of F$_2$-IsoP is still in question (Ridker et al., 2004).

### III.4.18.6 Etheno-DNA adducts

Vinyl chloride is classified by the US Environmental Protection Agency as a known human carcinogen and by IARC as a Group 1 human carcinogen (Albertini et al., 2003). Chloroethylene is the reactive metabolite of vinyl chloride that forms etheno adducts with DNA. Information about adduct formation and DNA repair after vinyl chloride exposure is lacking. Godschalk et al. (2002) reported no significant difference in pulmonary etheno-DNA (ε-DNA) adduct levels between smokers and nonsmokers. They observed considerable inter-individual variations in levels of εdA and εdT adducts (80- and 250-fold differences, respectively). LSRO ranks these adducts as Category C biomarkers.

### III.4.18.7 Bulky DNA adducts

Bulky DNA adducts are likely to be biomarkers of exposure to large, primarily apolar compounds such as PAHs and aromatic amines (Phillips, 2002). They reflect particulate phase smoke constituent exposure. Bulky DNA adducts have been measured in a wide range of tissues (e.g., lung and bronchus, larynx, sputum, oral and nasal cavities, bladder, cervix, breast, pancreas, colon, stomach, placental and fetal tissue, sperm, cardiovascular tissue, blood cells, and anal tissue) (World Health Organization, 2004). LSRO does not recommend routine measurement of these adducts as indicators of tobacco smoke exposure (Category C biomarker).

Bulky adducts indicate exposure for more than 1 month, but the half-life varies for different cell and tissue types (Scherer, 2005). They can also arise from dietary, ambient air, workplace, and possibly endogenous sources (Scherer, 2005). Most studies reported significantly higher values of these adducts in smokers than in nonsmokers, with smokers’ levels approximately double those of nonsmokers (Scherer, 2005).
Degawa et al. (1994) reported that hydrophobic adducts in the larynx (which the authors considered likely to be PAH adducts) were found in smokers but not in nonsmokers. Szyfter et al. (1996) showed that smokers of more than 40 cigarettes/day had levels of adduct 2.5-fold higher than those of smokers of approximately 20 cigarettes/day and 6-fold higher than levels of nonsmokers and ex-smokers.

Nia et al. (2000b) analyzed DNA in induced sputum from 20 smokers and 24 nonsmokers by using the $^{32}$P-postlabeling method with nuclease P1 digestion or with butanol enrichment. For the nuclease P1 digestion method, DNA of all smokers and one nonsmoker showed a diagonal radioactive zone, and the number of adducts in smokers' DNA was significantly higher ($3.1 \pm 1.4/10^8$ nucleotides) than that in nonsmokers' DNA ($0.6 \pm 0.8/10^8$ nucleotides, $p = 0.0007$). The butanol enrichment method also showed a significant difference between smokers and nonsmokers. However, the authors considered the nuclease P1 digestion approach to be preferred because it demonstrated larger differences between smokers and nonsmokers (Nia et al., 2000b). The butanol extraction method is thought to enrich various adducts, including some that are not specific for tobacco smoke (Gallagher et al., 1989).

In another study, Nia et al. (2000a) measured lipophilic DNA adducts in induced sputum and peripheral blood lymphocytes from 9 smokers and 9 nonsmokers. They reported the presence of diagonal radioactive zones in adduct maps of induced sputum of all smokers but no nonsmokers. Adduct maps of peripheral blood lymphocytes of 5 smokers contained this zone, but no such zones were observed for nonsmokers. Adduct levels in induced sputum from smokers were significantly higher ($p = 0.0005$) than those for nonsmokers ($3.7 \pm 0.9$ versus $0.7 \pm 0.2/10^8$ nucleotides, respectively). Adduct values for peripheral blood lymphocytes for smokers were also significantly higher ($p = 0.0001$) than those for nonsmokers ($2.1 \pm 0.3$ versus $0.6 \pm 0.1/10^8$ nucleotides, respectively). These authors believed induced sputum to be a better biological matrix for analysis than peripheral blood lymphocytes (Nia et al., 2000a).

Vineis et al. (1996), by means of the $^{32}$P-postlabeling assay, measured 2 of these DNA adducts in exfoliated bladder cells from 21 nonsmokers, 11 smokers of flue-cured tobacco, and 7 smokers of air-cured tobacco. Mean $\pm$ SE values of numbers of DNA adduct 2 were $1.4 \pm 0.7$, Relative Adduct Labeling (RAL)$^6$ for nonsmokers, $1.9 \pm 1.1$ RAL for flue-cured tobacco smokers, and

\[ \text{RAL} = \frac{\text{cpm adducts} \times \text{cpm non-adducted nucleotides}}{10^8} \]

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22.5 ± 12.3 RAL for air-cured tobacco smokers. Corresponding values for DNA adduct 4 were 4.8 ± 1.6 RAL, 7.7 ± 4.0 RAL, and 8.8 ± 5.0 RAL, respectively.

Methods used to quantify these adducts include the $^{32}$P-postlabeling assay (Culp et al., 1997; Vineis et al., 1996) and immunoassays (Culp et al., 1997). Exposure to substances that give rise to bulky DNA adducts can derive from environmental, dietary, occupational, and endogenous sources. Certain studies indicated either an effect of gender on DNA adduct levels (Mollerup et al., 1999; Ryberg et al., 1994) or no gender effect (Schoket et al., 1998). The $^{32}$P-postlabeling assay does not identify specific adducts.

**III.4.19 Analysis of Exhaled Breath Condensate as a Measure of Tobacco Smoke Exposure**

EBC analysis is described as a simple, non-invasive method of sampling the lining of the lower respiratory tract (Mutlu et al., 2001) and of monitoring inflammation. Exhaled breath comprises a gaseous phase that contains volatile compounds and a water vapor-saturated phase that contains aerosol particles. Cooling of exhaled breath results in precipitation of the aerosol particles, which contain non-volatile substances such as proteins, lipids, oxidants, and nucleotides (Mutlu et al., 2001; Scheideler et al., 1993).

EBC contains several markers of oxidative stress including leukotrienes, prostaglandins, hydrogen peroxide, and nitric oxide-derived products such as S-nitrosothiols, nitrate, nitrite, and 3-nitrotyrosine (Montuschi & Barnes, 2002). Although EBC analysis holds promise, significant methodological issues related to this technique remain to be resolved. One challenge is the presence of biomarkers at very low concentrations, so highly sensitive assays are needed. Also, substances measured tend to be markers of biological effect, not of exposure per se. A recent report by the American Thoracic Society/European Respiratory Task Force on EBC analysis proposed guidelines for sample collection and biomarker analysis (Horvath et al., 2005). This approach is not currently recommended for routine use in PRRTP studies.
III.4.20 LSRO Recommendations for Biomarkers

III.4.20.1 Biomarkers recommended for inclusion in assessment of all PRRTPs

On the basis of the review of tobacco product and smoke exposure biomarkers reported in the literature, the sum of nicotine and at least five of its major metabolites, including cotinine, is considered to be the most reliable marker of exposure to tobacco products and tobacco smoke and should be included in evaluation of all PRRTPs. Measurement of additional metabolites (e.g., nicotine-$N'$-oxide, cotinine-$N$-oxide, and demethylcotinine (norcotinine) can improve estimates of nicotine exposure (Benowitz & Jacob, III, 1994; Byrd et al., 1992).

III.4.20.2 Additional biomarkers

Various PRRTPs exist, including products made from tobacco containing reduced nicotine or TSNA levels and PRRTPs that heat but do not burn tobacco (Institute of Medicine, 2001). Because of the array of possibilities for modification of manufacturing conditions, product design, and composition and the potential for effects on internal exposure to tobacco product- and smoke-related substances, measuring additional biomarkers to assess exposure to other substances of biological concern related to PRRTP use has value. LSRO recommends flexibility in selection of additional biomarkers to assess exposure because various PRRTPs are likely to have different product characteristics, which would signal a need for evaluation of different biomarkers. Therefore, other than nicotine and at least five of its major metabolites, including cotinine, LSRO does not recommend a defined set of biomarkers for assessment of every PRRTP. LSRO advises use of a battery of biomarkers that reflect exposure to both particulate and vapor phases of smoke.

Data from smoke chemistry analyses can provide an additional rationale for biomarker selection. Machine smoking of the PRRTP and control cigarettes using at least two protocols can reveal whether yields of particular smoke constituents differ between PRRTPs and control cigarettes, and biomarkers can be used to evaluate internal exposure to smoke constituents of interest. For example, if an increase in yields of acrolein in smoke from the PRRTP compared with control cigarettes is observed, analysis of the acrolein biomarker 3-HPMA could indicate whether a smoker’s internal exposure to acrolein also increased after PRRTP use. A specific list of biomarkers to measure for all PRRTPs is less important than the criteria used to select biomarkers and the determination that smoke chemistry results are consistent with biomarkers of exposure.
Other factors likely to influence the selection of biomarkers include a targeted interest in assessing exposure to specific smoke constituents that are believed to be of biological concern. However, analytical methods for all biomarkers for all substances of biological concern are not currently available, and all methods for quantifying biomarkers have not been validated. Regulatory authorities also influence the biomarkers measured. The Massachusetts Department of Public Health requires that urine samples of 30 female smokers and 30 male smokers be tested, using federal regulatory methods, for two metabolites of NNK and for NNAL and its glucuronide. Measurement of the benzene metabolite $t,t$-MA according to federal regulatory methods is also required (45CFR46, 2001; Massachusetts Department of Public Health, 2001).

Confidence in exposure reduction can increase when smoke chemistry studies show lower yields of smoke constituents from PRRTPs compared with control cigarettes and when biomarkers that measure exposure to those smoke constituents are lower after PRRTP use compared with levels after cigarette use. It is important to address any inconsistencies between changes in smoke chemistry and biomarker levels. PRRTP evaluation is an iterative process, so a battery of tests including other kinds of systems (e.g., biological effect studies) should be utilized to answer questions about reduced risk. Unlike consistent results across test systems, inconsistencies in test results that cannot be resolved reduce confidence that risk is likely to decrease.

### III.4.20.3 Relative contribution of biomarkers of exposure to assess PRRTPs

Many factors should determine selection of biomarkers to quantify tobacco-product-related exposure. Information from product characteristics and tobacco product and smoke chemistry data, as well as a targeted interest in specific substances and regulatory requirements, can influence the choice of biomarkers. Biomarker characteristics are an important consideration, and analytical methods should be sufficiently accurate, sensitive, and selective (American Conference of Governmental Industrial Hygienists, 2005). Study design and study participant selection methods should receive careful consideration. Confidence in data from biomarker studies is enhanced by a quality assurance program. Given the possible variations in product characteristics, some PRRTPs, compared with conventional cigarettes, may reduce levels of some compounds but elevate others (Hatsukami et al., 2004a). Such a possibility should be weighed as a component of a risk assessment process.
Exposure to ETS is influenced by the number of smokers, volume of the space in which smoking is occurring, room ventilation, and many other factors. The types of evaluations to be performed might include chamber studies, clinical studies, and studies in natural settings. Many studies have utilized biomarkers to assess exposure to ETS (Benowitz, 1999; Bianchini et al., 1998; Coulta et al., 1987; Cummings et al., 1990; Hecht, 2004; Jarvis et al., 1984; Scherer et al., 1996; Smith et al., 2000a; Tsai et al., 2003). LSRO considers nicotine and at least five its major metabolites, including cotinine, to be the most reliable biomarkers of ETS exposure.

Although LSRO has confidence in analysis of smoke constituents as a measure of ETS exposure, LSRO has less confidence in other methods. LSRO therefore recommends that smoke chemistry studies of ETS be conducted as a routine screen for modified products. If a claim concerning ETS exposure is planned, sufficient studies are required to support the claim, including human exposure and other studies.

III.5 BEHAVIOR RELATED TO TOBACCO PRODUCT USE

III.5.1 Smoking Topography

Smoking topography is described as a method for external exposure assessment (Institute of Medicine, 2001). Assessment parameters include puff volume, puff duration, inter-puff interval, maximum puff velocity, number of puffs per cigarette, inhalation depth, inhalation volume, time to smoke a cigarette, and total inhalation time (Lee et al., 2003). A US Department of Health and Human Services report indicated wide variability in cigarette puffing behavior for conventional cigarettes (U.S. Department of Health and Human Services, 1988). A number of studies have used smoking topography to determine external exposure to smoke for PRRTP users (Buchhalter et al., 2001; Lee et al., 2003, 2004).

A concern associated with using smoking topography for this purpose is whether the smoking topography apparatus alters normal smoking behavior (Watson et al., 2004). Lee et al. (2003) determined that cigarette-smoking behavior does not change when CreSS Plowshare smoking topography equipment was used on a single occasion or repeatedly. A disadvantage of the apparatus is that it blocks filter ventilation holes during smoking (Watson et al., 2004).

LSRO considered the value of using smoking topography studies to measure smoke exposure but was concerned about the value and reliability of these
data compared with measurement of validated, reliable biomarkers of exposure. However, smoking topography can provide insight into the mechanism by which exposure to smoke constituents is altered. One potential application of these studies is to assess the way in which the PRRTP is smoked and use this information to set cigarette smoking machine parameters (Djordjevic et al., 2000). Smoking topography that imitates the human cigarette smoking profile is a method of evaluating human smoker yield (Baker et al., 2004a).

III.5.2 Measures of Cigarette Consumption

These measures include the number of cigarettes smoked per day, pack-years, and dose-years, evaluated by means of questionnaires and interviews.

Epidemiological studies showed a dose-response relationship between daily cigarette consumption and duration of cigarette consumption and diseases associated with cigarette smoking (U.S. Department of Health and Human Services, 2004). Joseph et al. (2005) examined the relationship between the number of cigarettes smoked per day and CO, NNK metabolites, 1-HOP, and total cotinine (cotinine plus its N-glucuronide) for 400 smokers of 1–100 cigarettes/day. Twelve subjects smoked more than 45 cigarettes/day. The authors reported that daily cigarette consumption is “not necessarily a reliable measure of toxin exposure” because it potentially underestimates exposure for individuals who smoke few cigarettes per day and overestimates exposure for individuals who smoke more cigarettes per day (Joseph et al., 2005). As mentioned previously, exposure to tobacco smoke is influenced by cigarette puffing and smoke inhalation behaviors as well as subjective and physiological factors, which are not evaluated via the number of cigarettes smoked per day parameter.

III.6 FILTER ANALYSIS

Filter analysis is used to evaluate smoker yield (Baker et al., 2004a). This method involves analysis of part of the filter tip from a smoked cigarette and provides information about possible maximum tar and nicotine exposures of the cigarette smoker. Watson et al. (2004) described a method for estimating nicotine and tar intake by analyzing deposition of solanesol, a high-molecular-weight, nonvolatile alcohol in tobacco leaves and tobacco products, in smoked cigarette filters. Disadvantages of this method include its being tested only with cellulose acetate filters, that underestimating solanesol deposition is possible because a filter may become saturated if a cigarette is smoked very intensely, and that it would be necessary to calibrate each type of
cigarette because variations in product characteristics (such as tobacco or filler blend) can lead to different relationships between solanesol content and nicotine and tar delivery (Watson et al., 2004). Filter analysis does not provide information about exposure to vapor phase components of tobacco smoke, which pass through the filter, or about depth and duration of inhalation and internal exposure to smoke constituents. However, filter analysis can supply useful ancillary information, especially for PRRTPs in which a filter is designed to retain specific toxicants or classes of toxicants.

III.7 DEPOSITION AND RETENTION OF TOBACCO SMOKE PARTICLES

Cigarette smoking is typically a 2-step process (Bernstein, 2004; Dixon & Baker, 2003). First, a smoker puffs on the cigarette and draws smoke into the mouth as the soft palate closes. Within 1 second of completing the puff, the smoker relaxes the soft palate. Second, the smoker inhales, taking in smoke and air that has not escaped from the mouth, holds the mixture of smoke in the lung for 1–2 seconds, and then exhales (Baker & Dixon, 2006).

After a smoker exhales, lungs retain some smoke constituents. Smoking behavior characteristics such as depth of inhalation, time for which smoke is held in lungs, and exhalation volume contribute to the extent to which particles are retained. Coughing, mucociliary transport, endocytosis, and other mechanisms clear some of these aerosol particles from the respiratory tract. Particles that remain can have toxic effects, which are a function of the amount and deposition pattern of the aerosol particles (Schleshinger, 1995). Smoke particle characteristics (such as particle size, shape, density, hygroscopicity, and electrical charge), respiratory tract geometry, ventilation characteristics, respiratory tract disease, and other factors affect deposition (Schleshinger, 1995). The aerodynamic diameter of the particle influences respirability and lung deposition.

In a recent review of respiratory tract retention of cigarette smoke aerosol constituents, Baker and Dixon (2006) reported that between 60% and 80% of particulate matter in MS is retained by lungs after inhalation. Lungs also retain 90–100% of nicotine, 55–65% of CO, and 100% of nitric oxide. Individuals who are exposed to ETS retain approximately 71–91% of nicotine and 11–59% of particulate matter (Baker & Dixon, 2006). Differences between MS and ETS retention in the respiratory tract result from the kind of tobacco in the product, filter ventilation, and some added ingredients that affect growth of the smoke aerosol particles in the respiratory tract and retention of particulate matter (Baker & Dixon, 2006).
Feng et al. (2007) reported a newer method of estimating lung retention of particulate and vapor smoke constituents by estimating the difference between delivery of smoke constituents to the respiratory tract and exhalation of the constituents. An advantage of this method is that it monitors respiratory patterns while the subject smokes. Estimates of delivered smoke constituents were obtained by analyzing the linear relationship between solanesol levels in a cigarette filter and cigarette smoking machine-generated yields of the selected constituents. The amount of exhaled constituent was determined by passing exhaled breath through a Cambridge filter pad and then measuring particulate phase components on the pad. Smoke vapor phase constituents were measured by using an infrared spectrophotometer that was attached to the Cambridge filter pad via stainless steel tubing (Feng et al., 2007). With this method, Feng et al. (2007) determined levels of nicotine, NNK, NNN, CO, isoprene, acetaldehyde, and ethylene.

LSRO recommends considering particle deposition and retention in evaluation of PRRTPs.

III.8 “OMICs”

DNA microarrays have allowed profiles of differential gene expression after exposure to cigarette smoke to be obtained (Shah et al., 2005; Spira et al., 2004a, 2004b; Yoneda et al., 2001). Jorgensen et al. (2004) studied effects of exposure to 12-hour cigarette smoke condensate on global gene expression profiles for short-term cultures of normal human bronchial epithelial cells. However, use of information obtained from functional genomics studies is premature, because the degree of correlation between genomic expression and protein expression continues to evolve. At present, this approach is a research tool. LSRO encourages the use of this approach to acquire additional data and to correlate results with other measures; however, microarrays are not sufficiently understood to serve as definitive exposure assessment tools.
Comparison of exposure to toxicants arising from the use of potential reduced-risk tobacco products (PRRTPs) with toxicant exposure arising from conventional cigarette use is a critical component of assessment of PRRTPs. The Life Sciences Research Office (LSRO) considered methods and approaches used to assess exposure and concluded that the current state-of-the-science is adequate for measuring reductions in exposure related to PRRTP use. Multiple methods such as smoke chemistry analysis, smoking topography, biomarker studies, and other approaches can contribute to exposure assessment. However, of the available techniques, LSRO weighted biomarker evaluation most heavily for demonstration of the exposure impact of a PRRTP on product users. LSRO identified biomarkers that have been sufficiently studied and validated so that, when used in adequately designed and executed clinical studies, investigators can obtain reliable estimates of exposure.

A number of analyses (preclinical studies) are necessary to provide initial information for design of biomarker studies, beginning with a scientifically informed consideration of likely effects of the design and composition of the PRRTP compared with those of conventional cigarettes. Smoke chemistry studies should be as comprehensive as possible to ensure that significant levels of newly documented compounds and increases and decreases in previously detected compounds are measured and to provide the necessary information to identify the appropriate biomarkers for use in exposure assessment studies. Exposure assessment is considered an iterative process; therefore, as new studies provide additional information, it should be used to reexamine data obtained earlier.

Although LSRO focused on the effect of PRRTP use on the tobacco product user, environmental tobacco smoke (ETS) chemistry and, if necessary, biomarker studies are also important components of a full characterization of the potential effects of a PRRTP on all individuals exposed to its smoke emissions. A decision tree approach to assess the potential for ETS exposure reduction may be most useful. Components of such an approach could include, in addition to other considerations, discussion of product design and composition. A demonstration that ETS is quantitatively reduced and...
that smoke constituents are not quantitatively increased could follow. If one or more smoke constituents show a quantitative increase, toxicity information indicating that risk would not increase should be collected and, if necessary, developed. Data from emerging technologies and other more standard techniques may also contribute to confidence in the conclusions concerning assessment results.

The key to interpretation of all studies described is a consistent trend in data across studies. Inconsistencies across studies should be specifically addressed and reconciled with overall conclusions about the relative effects of the PRRTP and conventional cigarettes on tobacco product and smoke exposure. A subsequent step is to weigh the importance of increases and decreases in exposure measures.

The amount of exposure reduction required to lead to reduction in harm is not known. Although exposure assessment is an important step in PRRTP evaluation, ultimately decreased toxicity of the complex mixture of smoke is critical to risk reduction.


Committee on Biological Markers & Subcommitteee on Reproductive and Developmental Toxicology. (1989) Biologic Markers in Reproductive Toxicology. Washington, DC: National Academy Press.


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Richard N. Dalby, Ph.D.

Richard Dalby is a professor in the Department of Pharmaceutical Sciences at the University of Maryland, School of Pharmacy. He 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). Dr. Dalby’s aerosol research, which encompasses novel pulmonary and nasal formulation development, device design, and product testing, is founded on his Ph.D. work on sustained-release metered-dose inhalers, and industrial experience as a Formulation Scientist with Fisons (now Aventis). He has published more than 40 papers and 90 abstracts related to aerosol technology, authored several book chapters, and spoken at many national and international meetings. He holds 3 patents concerned with novel metered-dose inhaler formulations, is a reviewer for many international journals, and is a frequent consultant to industry and the FDA. Dr. Dalby is the director of the Inhalation Aerosol Technology Workshop that is taught annually in Baltimore and at company facilities worldwide and is co-organizer and editor of Respiratory Drug Delivery, and its spin-off, RDD Europe. Dr. Dalby is a Dean’s Distinguished Educator at the University of Maryland School of Pharmacy and a Fellow of the American Association of Pharmaceutical Scientists.

Karl-Olav Fagerström, Ph.D.

Karl-Olav Fagerström graduated from the University of Uppsala as a licensed clinical psychologist in 1975. At that time, he started to operate a smoking cessation clinic. In 1981, he earned his Ph.D.; his dissertation examined nicotine dependence and smoking cessation. In the late 1970s and early 1980s, he served as Editor-in-Chief of the Scandinavian Journal for Behaviour Therapy. From 1983 through 1997, he worked for Pharmacia & Upjohn as Director of Scientific Information for Nicotine Replacement Products. He has contributed to nicotine replacement therapy developments such as nicotine gum, patch, spray, and inhaler. He currently works in private practice.
(Fagerström Consulting and the Smokers Information Center). He is a founding member of the Society for Research on Nicotine and Tobacco (SRNT). He started the European affiliate in 1999 and served as president until 2003. In 2003, he was elected president of SRNT. His main research contributions have been in the fields of behavior medicine, tobacco, and nicotine, with more than 125 peer-reviewed publications (of which he is the first author of 80). His current research interest relates to reducing harm and exposure to tobacco toxins in those who cannot stop smoking. He has developed a scale for nicotine dependence (Fagerström Test for Nicotine Dependence) and was awarded the WHO medal in 1999 for outstanding work in tobacco control.

Jerold Last, Ph.D.

Jerold Last is Professor of Pulmonary/Critical Care Medicine at the University of California, Davis, Medical School and Director of the University of California, Davis, Fogarty International Center, South America. From 1985 to 2004, he was Director of the University of California Systemwide Toxic Substances Research and Teaching Program. His research interests include animal models of asthma and lung fibrosis, inhalation toxicology, and environmental toxicology. Dr. Last has edited 6 books, has served on editorial boards of *Toxicology and Applied Pharmacology* and *Experimental Lung Research*, and is currently Associate Editor of *Toxicology and Applied Pharmacology*. He has provided support and expertise to numerous governmental committees and agencies on issues related to toxic substances in the environment. Dr. Last has published more than 190 articles, including a review of toxic interactions between inorganic gases and particles and studies of effects of exposure to sidestream and environmental tobacco smoke.

Robert Orth, Ph.D.

Robert Orth is a physical chemist at Apis Discoveries, L.L.C. He is also a consultant to the 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. He has conducted research in secondary ion mass spectrometry and taught at the University of Utah and Montana State University. Dr. Orth held positions of increasing responsibility during a 16-year career with the Monsanto Company. His work focused on environmental chemistry and remediation, and in food and agricultural science. His current work at Apis Discoveries includes setting up business units for ultratrace analysis, consulting for companies submitting direct and indirect food
additives to the FDA, and studying analysis and remediation of organic pollutants. He has more than 100 publications and presentations on analytical and physical chemistry and holds two patents.

Glenn Talaska, Ph.D., C.I.H.

Glenn Talaska is Professor of Environmental Health in the Divisions of Industrial and Environmental Hygiene, and Toxicology at the University of Cincinnati and is a Certified Industrial Hygienist. Dr. Talaska received his Ph.D. in genetic toxicology from the University of Texas Medical Branch at Galveston in 1986 and conducted postdoctoral research at the National Center for Toxicological Research in Jefferson, AK from 1986 to 1989. Dr. Talaska has been on the faculty of the University of Cincinnati since 1989. His research is in the field of biological monitoring, with an emphasis on carcinogen biomarkers, and involves metabolite and DNA adduct analysis and cytogenetics. His research on human subjects includes projects investigating the effects of tobacco smoking on levels of DNA adducts in placental tissue, the influence of various diets on DNA damage in exfoliated urothelial cells of smokers, genotoxic exposures in the rubber industry, and the effect of hair dye use on DNA adduct levels in exfoliated urothelial cells. Dr. Talaska also conducts research on DNA damage in human and animal breast tissues caused by polycyclic aromatic hydrocarbons (PAHs) and on the interaction between exposure to arsenic and PAHs. His development and use of an exfoliated urothelial cell assay for DNA adduction and damage is one of the few cases where a representative sample of an important target organ for environmental carcinogens can be obtained by non-invasive means. Dr. Talaska is Vice-Chair of the Biological Exposures Indices Committee of the American Conference of Governmental Hygienists, a member of the American Academy of Industrial Hygiene, and a genetic toxicology consultant to the Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute. He is a member of the editorial board of the Journal of Environmental and Occupational Health and the International Advisory Board of Yonsei Medical Journal and is the associate editor of Polycyclic Aromatic Compounds. Dr. Talaska has published 90 articles and 11 book chapters.

William Waddell, M.D.

William Waddell is Emeritus Chairman of Department of Pharmacology and Toxicology at University of Louisville. He served on the faculties of the University of North Carolina and University of Kentucky before his tenure at the University of Louisville, School of Medicine. He is a member of the editorial board of Human and Experimental Toxicology. Dr. Waddell has authored
more than 100 peer-reviewed articles, including studies of localization of nicotine and its metabolites in the mouse and reviews on thresholds of carcinogenicity. He has given more than 100 invited lectures around the world and has served as the chair of national scientific organizations and as a consultant to several drug and chemical companies.

VI.A.2 Reduced-Risk Review Project Core Committee

Alwynelle (Nell) Ahl, Ph.D., D.V.M.

Nell Ahl is Principal Scientist at Highland Rim Research Consulting, Inc. (HRRC). After majoring in biology and mathematics at Centenary College of Louisiana, she obtained her M.S. and Ph.D. in zoology and biochemistry, respectively from the University of Wyoming, and a doctorate in veterinary medicine from Michigan State University where she also served as a Professor in the Department of Natural Science. Before her work at HRRC, Dr. Ahl had a distinguished career at the US Department of Agriculture (USDA) and serving in various capacities, including Deputy Director for Animal Health Training and Chief of Risk Analysis Systems within USDA’s Animal and Plant Inspection Service. She also served in the Senior Executive Service as the first Director of the USDA Office of Risk Assessment and Cost-Benefit Analysis and as a USDA Fellow to the Center for the Integrated Study of Food, Animal and Plant Systems at Tuskegee University in Alabama. She is a fellow of the American Association for the Advancement of Science and has served on several panels at the National Academy of Sciences. Her research interests include public policy for science and veterinary medicine and the use of risk assessment for agricultural issues affecting human health. Dr. Ahl’s presentations and publications total more than 250, and she has edited reports from more than a dozen symposia.

Elizabeth L. Anderson, Ph.D., Fellow A.T.S.

Elizabeth L. Anderson is Group Vice President of the Health Sciences and Food & Chemicals Division of Exponent, Inc. and has more than 20 years of experience, both government and corporate, in health and environmental sciences. She received her B.S. in chemistry from the College of William and Mary, her M.S. in organic chemistry from the University of Virginia, and her Ph.D. from The American University. She formerly served as President and Chief Executive Officer of Sciences International, Inc. and was President, Chief Executive Officer, and Chairman of the Board of Clement International Corporation, where she directed an interdisciplinary group of 200 senior scientists and engineers. She founded and directed the central risk assessment programs at the US Environmental Protection Agency (EPA)
for 10 years. The primary functions of the office were to conduct risk assessments on health effects of a wide variety of toxic chemicals, provide leadership to establish EPA-wide guidelines for risk assessment, and oversee EPA’s risk assessment program. She is the recipient of the EPA Gold Medal for Exceptional Service. Dr. Anderson is an internationally recognized lecturer and consultant and has published numerous journal articles in areas of risk assessment and carcinogenicity. She is in her sixth year as Editor-in-Chief of Risk Analysis: an International Journal.

**Nancy L. Buc, Esq.**

Nancy Buc is a partner at Buc & Beardsley, Washington, DC. She received an A.B. from Brown University, an LL.B. from the University of Virginia, and an LL.D. (Honoris Causa) from Brown University. She has taught food and drug law at Georgetown University Law Center and is a Trustee Emerita of Brown University. She served as Chief Counsel, FDA, from February 1980 to January 1981. She also served as Attorney-Adviser to U.S. Federal Trade Commission (FTC) Chairman Miles W. Kirkpatrick and as Assistant Director of the FTC Bureau of Consumer Protection (1969-1972). Ms. Buc has served as a member on various advisory committees and panels to the National Institutes of Health (NIH), Institute of Medicine, and Office of Technology Assessment.

**Carroll E. Cross, M.D.**

Carroll E. Cross is Professor of Medicine and Physiology at the University of California, Davis, School of Medicine, where he is an attending physician in Pulmonary and Critical Care Medicine. He graduated from Columbia College of Physicians and Surgeons in 1961 and 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. Dr. Cross has published more than 200 papers in such fields as air pollutants, antioxidant micronutrients, inflammatory-immune system oxidants, ozone, nitrogen oxides, 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 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, the NIH, EPA, FDA, and for the Heart, Lung, and Cancer Society Associations.
Louis D. Homer, M.D., Ph.D.

Louis D. Homer is the former Medical Director of Clinical Investigation and Biomedical Research at Legacy Research, Holladay Park Medical Center. He received his Ph.D. in physiology and 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; as Associate Professor at Brown University; and as 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 such as physiology, mathematics, statistics, and computer application. He has also reviewed proposals and has served on site visit teams for the National Institute of Allergy and Infectious Diseases; National Heart, Lung, and Blood Institute; National Science Foundation; and Naval Medical Research and Development Command. He has reviewed articles submitted to the *Journal of Theoretical Biology, Microvascular Research, American Journal of Physiology, and 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-square estimation, and iterative maximum likelihood estimation.

Joseph V. Rodricks, Ph.D., D.A.B.T.

Joseph V. Rodricks is a Founder and Principal of ENVIRON Corporation and an internationally recognized expert in the fields of toxicology and risk analysis. Dr. Rodricks received his B.S. in chemistry from the Massachusetts Institute of Technology and his M.S. in organic chemistry and Ph.D. in chemistry from the University of Maryland. Dr. Rodricks was formerly the Director, Life Sciences Division, Clement Associates (1980–1982); the Deputy Associate Commissioner, Health Affairs; and Toxicologist, FDA (1965–1980); and is a Visiting Professor at the Johns Hopkins University School of Public Health. He has published more than 100 peer-reviewed articles and is the author of *Calculated Risks* (Cambridge University Press), a non-technical introduction to toxicology and risk analysis.

Richard C. Schwing, Ph.D.

Richard C. Schwing is Founder and President of Sustainable Visions, Inc., a firm devoted to the consumer-driven search for more sustainable products and lifestyles. Dr. Schwing received his B.S., M.S., and Ph.D. in chemical engineering from the University of Michigan. Dr. Schwing previously worked at General Motors in research and development. His focus areas included highway safety, environmental pollution, and corporate strategy. As a founding
member of GM’s unique-in-industry Societal Analysis Department, he developed groundbreaking approaches to air pollution health effects, risk analysis, risk trade-offs, human behavior components of risk, and technology forecasting and societal trends. He has authored several technical papers and co-authored the paper Interdisciplinary Vision: The First 25 Years of the Society for Risk Analysis (SRA) 1980-2005.

Emanuel Rubin, M.D.

Emanuel Rubin is Gonzalo E. Aponte Distinguished Professor of Pathology and Chairman Emeritus of the Department of Pathology, Anatomy, and Cell Biology at Jefferson Medical College in Philadelphia. He obtained an M.D. from Harvard Medical School. After completing his 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 in New York. After his fellowship, Dr. Rubin spent the next 14 years at Mount Sinai Hospital's Pathology Service with increasing responsibilities, culminating in Pathologist-in-Chief. Dr. Rubin then became 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 the honors received, the University of Barcelona and the University of Naples named him as Doctor Honoris Causa. He was also given the F.K. Mostofi Distinguished Service Award from the U.S.-Canadian Academy of Pathology, and he received the NIH MERIT Award. He has held many editorial positions and has served as a consultant to many organizations. He has more than 300 publications, including 13 textbooks and a CD-ROM.

Richard Windsor, M.S., Ph.D., M.P.H.

Richard Windsor is Professor in the Department of Prevention and Community Health in the School of Public Health and Health Services at the George Washington University (GWU) Washington DC, and is a nationally recognized expert in public health program evaluation. Dr. Windsor obtained a B.S. in community health education from Morgan State College in Baltimore; an M.S. and Ph.D. in public health education and educational/social psychology from the University of Illinois; and an M.P.H. in maternal and child health from The Johns Hopkins University. Prior to joining GWU, he held academic appointments and provided research and management
leadership at a number of institutions, including Ohio State University, University of Alabama Medical Center, and Johns Hopkins University, and has served as Associate Director for Prevention at the National Heart, Lung, and Blood Institute. He served as the Principal Investigator of five and Co-Principal Investigator of three NIH-funded randomized clinical trails to evaluate Smoking Cessation or Reduction in Pregnancy Treatment Methods. Dr. Windsor was recognized by the Agency for Healthcare Research and Quality (2000) Tobacco Treatment Clinical Practice Guidelines and received the C. Everett Koop National Health Award (1997) for his scientific leadership and contributions to the evidence-based treatment for pregnant smokers.

VI.A.3 The Life Sciences Research Office Staff

Michael Falk, Ph.D., is the Director of the Life Sciences Research Office (LSRO). 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, where he supervised as many as 80 senior-level scientists. As a 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 review and subject review panels in infectious diseases, environmental sciences, military medicine, and other health-related fields. He was a peer reviewer for research proposals for the National Science Foundation, Medical Research Council of Canada, and Office of Naval Research. As the Director of the LSRO, Dr. Falk evaluates biomedical information and scientific opinion for regulatory and policy makers in both the public and the private sectors. Among his many accomplishments, he has produced seminal white papers on infant nutrition, food labeling, food safety, and military dental research and has organized two international conferences. Concurrently, he is with MCF Science Consultants and provides analysis and consultation on emerging technologies. Dr. Falk has published more than 60 research articles, abstracts, technical reports, and presentations.

Robin S. Feldman, B.S., M.B.A., is the LSRO Literature Specialist. 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, DC, with a major in zoology, and her M.B.A. from the University of Maryland at College Park, with a concentration in science and technology. She previously 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 (HHMI), she oversaw 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 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. 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.

Karin French, B.S. is a former LSRO Associate Staff Scientist. Ms. French received one B.S. degree in animal science and another in cell and molecular biology and genetics from the University of Maryland at College Park (UMCP). In addition, she earned a College Park “Scholars Certificate in Science, Technology, and Society.” Ms. French worked in the Dairy Nutrition Laboratory at the university where she assisted Maryland dairy farmers in using milk urea nitrogen (MUN) to evaluate herd protein nutrition. She helped design and complete studies to compare and evaluate the MUN analysis techniques used in the National Dairy Herd Improvement Association laboratories. Ms. French is pursing doctoral studies at UMCP.

Rebecca Johnson, Ph.D., is the LSRO Assistant Information Specialist. Dr. Johnson received her B.A. from Wesleyan University and her Ph.D. in anthropology with a concentration in archaeology from the University of Iowa. Her dissertation research examined dietary change between two Native American villages in southeastern Iowa, dated to 1950 and 100 B.P., by looking at fatty acid residues extracted from pottery. Dr. Johnson has performed fieldwork across the Mid-Atlantic and Upper Midwest, as well as in South Carolina, Great Britain, and Poland. Before joining LSRO, Dr. Johnson developed and maintained statewide archaeological databases for Iowa’s Office of the State Archaeologist.
Kara D. Lewis, Ph.D., is a Senior Staff Scientist at LSRO. Dr. Lewis completed postdoctoral research at Yale University, obtained her Ph.D. in biology, with a concentration in neuroscience, from Clark University, and graduated summa cum laude with a B.S. in biology from Spelman College. Dr. Lewis has conducted research on taste and smell of the fruit fly Drosophila melanogaster, and on 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.

Catherine L. St. Hilaire, Ph.D., retired as a LSRO Senior Staff Scientist and Project Leader of the Reduce-Risk Review Project during the course of this project. Dr. St. Hilaire had more than 20 years of experience in environmental and consumer product risk assessment and risk management. She provided leadership in the development of risk assessment policies and procedures, from the original Risk Assessment Guidelines for Carcinogens, and the original guidelines for EPA’s Superfund Risk Assessments, to industry-wide risk assessment approaches for food contaminants. Her contributions to the field were formally recognized through her election as a Fellow of the Society for Risk Analysis and as a recipient of The National Academies’ Certificate for Outstanding Service on the 20th Anniversary of the release of the landmark Red Book. Formally known as Risk Assessment in the Federal Government: Managing the Process, this document became a foundation of theory and practice in the field of risk assessment and provided the framework for public health risk assessments adopted by regulatory agencies worldwide. Dr. St. Hilaire held executive-level posts at Hershey Foods Corporation, International Life Science Institute, ENVIRON Corporation, and Sciences International, Inc. She was the primary author of more than 20 books and publications in the fields of microbiology and toxicology, including carcinogenesis and reproductive and developmental toxicity, risk assessment, and risk management. She was a member of the Society for Risk Analysis and the Society of Toxicology.

James Cecil Smith Jr., Ph.D., is a Senior Scientific Consultant at LSRO. Dr. Smith obtained his doctorate in nutritional sciences/biochemistry at the University of Maryland and completed postdoctoral work in the Department of Biological Chemistry at UCLA. He served two years as Health Service Officer at the NIH. Dr. Smith completed 36 years in federal research laboratories with increasing responsibilities in the Veterans Administration medical system and the USDA. Although best known for contributions to trace element nutrition, he is also an authority in the area of vitamin and mineral interactions. Original research directed by him identified a link
between zinc and vitamin A, which was published in *Science*. Other research accomplishments include collaborating on studies that revealed an important interaction between copper and dietary carbohydrates. He directed two large human investigations, funded in part by the National Cancer Institute, which established the USDA Laboratory and Research Center at Beltsville, MD, as original leaders in research to elucidate the role of dietary carotenoids in health and disease. Dr. Smith has authored or co-authored more than 375 publications. He served as an editorial board member, assistant editor, and *ad hoc* reviewer for several national and international journals. He 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 of Bioinorganic Scientists, and he was recently named a Fellow of the American Society for Nutritional Sciences.
VI.A.4 The Life Sciences Research Office Board of Directors

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VI.B GLOSSARY/ACRONYMS AND ABBREVIATIONS

VI.B.1 Glossary

Biological effects assessment
One of two critical evaluations included in a PRRTP risk assessment. Statistically and biologically significant changes indicative of decreased risk in relevant biomarkers of effect in PRRTP users compared to those in conventional cigarette smokers support the likelihood that risk of lung cancer, chronic obstructive pulmonary disease and/or cardiovascular disease will be lower for PRRTP users.

Biologically effective dose
The amount of a constituent or metabolite that is measured interacting with critical subcellular, cellular, and target tissues.

Biological matrix
A discrete material of biological origin (e.g., blood, serum, plasma, urine, or saliva), that can be sampled and processed in a reproducible manner (U.S. Food and Drug Administration, 2001).

Biomarker
A biological response variable that is measured in biological fluids, tissues, cells, and subcellular components and is indicative of exposure and/or effect.

Biomarker of effect
A measurable effect including early subclinical biological effects; alterations in morphology, structure, or function; or clinical symptoms consistent with the development of health impairment and disease.

Biomarker of exposure
A constituent or metabolite that is measured in a biological fluid or tissue and/or is measured after it has interacted with critical subcellular, cellular, or target tissues.

Cancer
A term for diseases in which abnormal cells divide without control.

Cardiovascular disease
Disease of the heart and/or vascular (blood vessel) system including atherosclerosis, coronary artery disease, carotid artery disease, and myocardial infarction (heart attack).
Cessation (smoking)
Abstinence from smoking for at least six months (Centers for Disease Control and Prevention, 2005b).

Chronic obstructive pulmonary disease
A slowly progressive disease of the airways characterized by a gradual loss of lung function. In the US, the term includes chronic bronchitis, chronic obstructive bronchitis, or emphysema or combinations of these conditions.

Cigarette
A rod of tobacco wrapped in paper.

Cigarette smoke condensate
The particulate phase of cigarette smoke that includes liquid and/or solid particles within the smoke aerosol.

Cigarette smoking machine
A machine that generates smoke from a cigarette according to one or more defined protocols.

Cigarette smoking machine regimens
A set of conditions for the production of smoke from cigarettes by using a smoking machine. In general, regimens differ in puffing parameters (e.g., volume, duration, and inter-puff interval) and filter vent-blocking conditions.

Clinical studies
Studies conducted with human subjects.

Conventional cigarettes
Commercial cigarettes that incorporate materials and designs typical of those that have been used in cigarette manufacturing for a number of years (Counts et al., 2006).

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

Crossover study
A study in which each participant is used as his/her own control, removing inter-subject variations in responses.
Dose
The amount of a substance absorbed by an organism.

Dose-response
A relationship between the amount of a substance in environmental media that comes in contact with an organism (humans, animals) and/or the amount of a substance absorbed by an organism or a specified compartment, organ, or tissue, and the biological effects caused by the substance.

Environmental tobacco smoke
Smoke consisting of aged, diluted sidestream smoke and exhaled mainstream smoke.

Epidemiological studies
Epidemiological studies are conducted using human populations to evaluate whether there is a causal relationship between exposure to a substance and adverse health effects. These studies measure the risk of illness or death in an exposed population compared to the risk in an identical population (e.g., same age, sex, race, socio-economic status) unexposed population.

Exposure
The amount of a substance that is absorbed by an organism or specified compartment, organ, or tissue. Also referred to as dose.

Exposure assessment
One of two critical aspects of a risk assessment. The purpose of an exposure assessment is to evaluate all relevant data on an exposure of interest (in this case, chemicals present in PRRTPs and conventional cigarette smoke).

Ex-smokers
Individuals who do not currently use tobacco products and who have managed to abstain from smoking for ≥ 6 months.

FTC method
A cigarette smoking machine procedure adopted by the FTC in 1967 to measure yields of tar and nicotine in mainstream smoke. The method was modified in 1980 to include carbon monoxide.

Filter (cigarette)
A device positioned at the mouth end of a cigarette, which serves as a smoke-permeable mouthpiece; usually composed of cellulose acetate fibers in the US and encased by a wrapper.
Filter vents
One or more rings of small holes or perforations intended to dilute smoke with air, thereby reducing standard yields of tar, nicotine, and carbon monoxide.

Former smoker
A person who reports having smoked ≥100 cigarettes during his/her lifetime but currently does not smoke (Centers for Disease Control and Prevention, 2005c).

Gas phase
The nonliquid (vapor) phase of cigarette smoke, which does not readily condense when it passes through a filter, as compared with the particulate phase.

Harm
An adverse event (e.g., a tobacco-associated disease or health condition).

Harm reduction
A reduction in adverse consequences associated with an activity or exposure to toxic substances. In general, harm reduction operates in an environment in which harm is occurring and cannot be prevented or eliminated.

Lung cancer
Cancer that forms in tissues of the lung, usually in cells lining the air passages. There are four main histological types, squamous cell carcinoma, adenocarcinoma, small cell carcinoma, and large cell carcinoma.

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

Morbidity
A diseased state; prevalence and/or incidence of disease.

Mortality
Death.

N-Nitrosamine
A chemical substance formed by nitrosation of secondary and tertiary amines. Tobacco-specific nitrosamines are formed by nitrosation of the major tobacco alkaloid nicotine (Brunnemann et al., 1996).
Nicotine
A cyclic tertiary amine composed of a pyridine ring and a pyrrolidine ring. It is a colorless to pale yellow, water soluble, fluid alkaloid derived from plants of the genus *Nicotiana*. Nicotine acts as a stimulant in mammals and is one of the main factors responsible for dependence-forming properties of tobacco smoke.

Nicotine replacement therapy
Use of various forms of nicotine delivery methods intended to replace nicotine obtained from smoking or other tobacco use. Examples include nicotine patches, gums, inhalers, nasal sprays, and lozenges.

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

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

Potential reduced-exposure products (PREPs)
Products that could potentially result in reduced exposure to toxicants from a given instance of tobacco use.

Potential reduced-risk tobacco products (PRRTPs)
Tobacco products that may pose lower health risks than conventional cigarettes.

Premarket evaluation
Testing that is conducted before the marketing of a product.

Product characteristics
Composition, design, and engineering of a tobacco product.

Preclinical studies
Studies generally conducted prior to studies in humans. For PRRTPs, preclinical studies include product characterization, chemistry (smoke or product), cytogenicity and genotoxicity assays, and animal studies.

Pyrolysis
Thermal degradation of a chemical substance, generally resulting in smaller chemical fragments.
Reference cigarette
Cigarettes prepared under controlled conditions with uniform, documented source tobaccos producing standardized yields of tar, nicotine, and carbon monoxide.

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).

Risk
The probability that harm (e.g., a smoking-associated disease) will occur.

Risk assessment
A human health risk assessment is a systematic review and evaluation of data related to risks posed by occupational or environmental chemicals, consumer products, food components and drugs, and other potential health hazards (National Research Council, 1983).

Risk reduction
A decrease in the likelihood that harm will occur.

Shards
A small piece or particle.

Sidestream smoke
The smoke emitted directly into the air from the burning end of the cigarette, largely during the smolder interval between puffs.

Smoke
Fine particles suspended in air that scatter light and are physically visible. Cigarette smoke contains many chemical substances in both gas and liquid state, suspended in a dynamic aerosol created by incomplete combustion which changes both physically and chemically over time.

Smoker
A person who has smoked ≥100 cigarettes and who now smokes every day or some days (Centers for Disease Control and Prevention & National Center for Health Statistics, 2006).

Smoke chemistry studies
Studies that investigate the general composition of smoke or the yield of specific smoke constituents.
Smoking machine
A machine that generates smoke from cigarettes under specific programmed parameters.

Smoking topography
A method to assess how individuals smoke cigarettes or cigarette-like products.

Smoking-related diseases
Diseases that have been reported to be caused by cigarette smoking, such as those listed in the 2004 Surgeon General’s report (U.S. Department of Health and Human Services, 2004).

Standard methods
Testing methods that have undergone appropriate validation (intra- and inter-laboratory evaluation) that are used to compare results.

Structure-activity relationships
Relationships linking chemical structure and toxicological effects.

Surrogate compound
A chemical that is used to represent other chemicals of similar structure or chemical class.

Tar
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).

Tobacco-specific nitrosamines
\( N \)-Nitroso compounds formed by nitrosation of the major tobacco alkaloid nicotine, and a suspected human carcinogen.

Tobacco harm reduction
Reduction of adverse impacts on the health of smokers who will not or cannot abstain from smoking.

Total particulate matter
Particles in smoke, larger than 1 µm in diameter, that are trapped on a Cambridge filter as the smoke passes through the filter; usually obtained from mainstream smoke.
Toxicant
A poisonous substance.

Vapor phase
Material in the gas state; usually material that passes through a filter.

Ventilation
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.

Weight of evidence
A process that assigns different levels of importance (“weights”) to evidence based on a number of factors. The term also refers to conclusions based on the totality of the evidence from all study types.

Yield
The amount of a substance, such as tar, nicotine, or carbon monoxide, as produced in smoke under standard smoking conditions.
### VI.B.2 Acronyms and Abbreviations

<table>
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<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>AAMA</td>
<td>N-Acetyl-S-(2-carbamoylethyl)-L-cysteine</td>
</tr>
<tr>
<td>4-ABP</td>
<td>4-Aminobiphenyl</td>
</tr>
<tr>
<td>4-ABP-Hb</td>
<td>4-Aminobiphenyl-Hemoglobin</td>
</tr>
<tr>
<td>BEA</td>
<td>Biological Effects Assessment</td>
</tr>
<tr>
<td>B[a]P</td>
<td>Benzo[a]pyrene</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BPDE</td>
<td>Benzo[a]pyrene diolepoxide</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CO</td>
<td>Carbon monoxide</td>
</tr>
<tr>
<td>COHb</td>
<td>Carboxyhemoglobin</td>
</tr>
<tr>
<td>CORESTA</td>
<td>Centre de Coopération pour les Recherches Scientifiques Relatives au Tabac</td>
</tr>
<tr>
<td>CreSS</td>
<td>Clinical research support system</td>
</tr>
<tr>
<td>DHBMA or MI</td>
<td>Dihydroxybutyl mercapturic acid</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EA</td>
<td>Exposure assessment</td>
</tr>
<tr>
<td>EBC</td>
<td>Exhaled breath condensate</td>
</tr>
<tr>
<td>EHC</td>
<td>Electrically heated cigarette</td>
</tr>
<tr>
<td>EHCSS</td>
<td>Electrically heated cigarette smoking system</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbtent assay</td>
</tr>
<tr>
<td>EPA</td>
<td>U.S. Environmental Protection Agency</td>
</tr>
<tr>
<td>ETS</td>
<td>Environmental tobacco smoke</td>
</tr>
<tr>
<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
</tr>
<tr>
<td>FTC</td>
<td>U.S. Federal Trade Commission</td>
</tr>
<tr>
<td>GAMA</td>
<td>N-(R,S)-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GC-TEA</td>
<td>Gas chromatography-thermal energy analyzer</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>HCN</td>
<td>Hydrogen cyanide</td>
</tr>
<tr>
<td>1-HOP</td>
<td>1-Hydroxypyrene</td>
</tr>
<tr>
<td>HPB</td>
<td>4-Hydroxy-1-(3-pyridyl)-1-butanone</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>3-HPMA</td>
<td>3-Hydroxypropylmercapturic acid</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Cancer on Research</td>
</tr>
<tr>
<td>IOM</td>
<td>Institute of Medicine</td>
</tr>
<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
</tr>
<tr>
<td>IsoP</td>
<td>Isoprostanes</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<td>--------------</td>
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<tr>
<td>LSRO</td>
<td>Life Sciences Research Office</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid chromatography</td>
</tr>
<tr>
<td>t,t-MA</td>
<td>trans,trans-Muconic acid</td>
</tr>
<tr>
<td>3-Me-Ade</td>
<td>3-Methyladenine</td>
</tr>
<tr>
<td>MHBMA or MII</td>
<td>Monohydroxybutenyl mercapturic acid</td>
</tr>
<tr>
<td>MHBVal</td>
<td>1- and 2-hydroxy-3-butenyl valine</td>
</tr>
<tr>
<td>MS</td>
<td>Mainstream smoke</td>
</tr>
<tr>
<td>NAB</td>
<td>N′-Nitrosoanabasine</td>
</tr>
<tr>
<td>NAT</td>
<td>N′-Nitrosoanatabine</td>
</tr>
<tr>
<td>ND</td>
<td>Not detectable</td>
</tr>
<tr>
<td>NDMA</td>
<td>Nitrosodimethylamine</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>NMTCA</td>
<td>N′-Nitroso-2-methylthiazolidine-4-carboxylic acid</td>
</tr>
<tr>
<td>NNAL</td>
<td>4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol</td>
</tr>
<tr>
<td>NNAL-Gluc</td>
<td>4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol glucuronides</td>
</tr>
<tr>
<td>NNK</td>
<td>4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone</td>
</tr>
<tr>
<td>NNN</td>
<td>N′-Nitrosonornicotine</td>
</tr>
<tr>
<td>NPRO</td>
<td>N-Nitrosoproline</td>
</tr>
<tr>
<td>NSAR</td>
<td>N-Nitrosarcosine</td>
</tr>
<tr>
<td>NTCA</td>
<td>N-Nitrosothiazolidine-4-carboxylic acid</td>
</tr>
<tr>
<td>8-OHdG</td>
<td>8-Hydroxy-2′-deoxyguanosine</td>
</tr>
<tr>
<td>PAH</td>
<td>Polycyclic aromatic hydrocarbons</td>
</tr>
<tr>
<td>PheMA</td>
<td>Phenylhydroxyethylmercapturic acid</td>
</tr>
<tr>
<td>PREP</td>
<td>Potential reduced-exposure product</td>
</tr>
<tr>
<td>PRRTP</td>
<td>Potential reduced-risk tobacco product</td>
</tr>
<tr>
<td>RAL</td>
<td>Relative adduct labeling</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>RRRRP</td>
<td>Reduced-Risk Review Project</td>
</tr>
<tr>
<td>RSP</td>
<td>Respirable suspended particulates</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>S-PMA</td>
<td>S-Phenylmercapturic acid</td>
</tr>
<tr>
<td>SRNT</td>
<td>Society for Research on Nicotine and Tobacco</td>
</tr>
<tr>
<td>SS</td>
<td>Sidestream smoke</td>
</tr>
<tr>
<td>TobReg</td>
<td>Tobacco Regulation</td>
</tr>
<tr>
<td>TOB-HT</td>
<td>Heats but does not burn tobacco</td>
</tr>
<tr>
<td>TPM</td>
<td>Total particulate matter</td>
</tr>
<tr>
<td>TSNAs</td>
<td>Tobacco-specific nitrosamines</td>
</tr>
<tr>
<td>TVOC</td>
<td>Total volatile organic compounds</td>
</tr>
<tr>
<td>USDA</td>
<td>U.S. Department of Agriculture</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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EXPOSURE ASSESSMENT IN THE EVALUATION OF POTENTIAL REDUCED-RISK TOBACCO PRODUCTS

Expert Committee

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