BIOLOGICAL EFFECTS ASSESSMENT IN THE EVALUATION OF POTENTIAL REDUCED-RISK TOBACCO PRODUCTS

EXECUTIVE SUMMARY

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This is a brief summary of the review by LSRO. It is not a complete document and should be considered within the context of the full report, which can be obtained at WWW.LSRO.ORG

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This report provides findings, conclusions, and recommendations on scientific methods that can be used to assess the biological effects of tobacco products that may pose lower health risks than conventional cigarettes ('potential reduced-risk tobacco products' or PRRTPs). It was developed under a contract between Philip Morris, USA, and the Life Sciences Research Office (LSRO) and is one of three reports associated with the Reduced-Risk Review Project (RRRP). From this point forward, LSRO, its staff, and the expert advisory committees, the Biological Effects Assessment Committee and Core Committee, are referred to collectively as "LSRO".

SMOKING-ASSOCIATED MORBIDITY AND MORTALITY AND TOBACCO HARM REDUCTION

An estimated 400,000 current and former US smokers die each year from smoking-attributable diseases and approximately four to five million deaths worldwide *per* year are caused by tobacco (American Lung Association, 2006; Centers for Disease Control and Prevention, 2002; 2005; Peto & Lopez, 2004). Approximately 350,000 of the 400,000 smoking-attributable deaths that occur each year in the US are due to: lung cancer (LC) (123,800 deaths/year); chronic obstructive pulmonary disease (COPD) (90,600 deaths/year); and cardiovascular disease (CVD) (138,000 deaths/year) (Centers for Disease Control and Prevention, 2005). Epidemiological evidence suggests that COPD, LC, and CVD are smoking-associated comorbidities; LC and CVD are the major causes of death of individuals with mild-to-moderate COPD (Sin *et al.*, 2006).

RRRP OBJECTIVES AND APPROACH

The objectives of the RRRP were to:

- 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.

LSRO's overall findings and recommendations were published in the report *Scientific Methods to Evaluate Potential Reduced-Risk Tobacco Products* (Life Sciences Research Office, 2007). This report provides a detailed state-of-the-science review of various assays, models, and biomarkers of human disease that could be used during premarket testing to draw scientific conclusions regarding the comparative risks of PRRTPs and conventional cigarettes for smokers who cannot or will not quit.

LSRO undertook a comprehensive literature review covering material published through July 2007; conducted in-depth discussions with the Biological Effects Assessment Committee that was composed of scientists and clinicians with relevant expertise; and sought guidance from other individuals who presented or otherwise provided information. LC, COPD, and CVD and certain conditions associated with each disease are the major foci of this report because of their significant contributions to smoking-associated morbidity and mortality.

CONCLUSIONS AND RECOMMENDATIONS

Cigarettes have been evaluated by integrating the information obtained using both analytical and biological methodologies (*i.e.*, physical product and smoke chemistry analyses; *in vitro* genotoxicity and cytotoxicity tests; animal studies; and clinical studies). PRRTPs can be evaluated using similar methodologies.

Preclinical studies

Preclinical studies provide preliminary information that is necessary for decision-making regarding whether a PRRTP should undergo clinical testing (*e.g.,* does a PRRTP reduce toxicological effects in bacteria, cultured cells, and animals relative to conventional cigarettes?)

In vitro testing

In vitro tests (*i.e.*, cytotoxicity and genotoxicity assays) used to test PRRTPs should include:

- Measurements of the combined effects of the vapor and particulate phases (*i.e.*, 'fresh' whole smoke) as well as those of individual components;
- Standardized methods to quantify exposures;
- Measurements of multiple exposure concentrations and durations to obtain accurate dose-response data;
- Control products to standardize data; and
- Generally acceptable laboratory practices.

While a single *in vitro* assay cannot provide toxicity information for all potential genotoxic and cytotoxic characteristics of a PRRTP, increased confidence in *in vitro* analyses is obtained when a battery of complementary tests support proceeding with further testing (*i.e., in vivo* testing) of a PRRTP. Although *in vitro* assays will never completely recapitulate the complex relationships between inhaled cigarette smoke and *in vivo* effects, they contribute to a better understanding of possible mechanisms of effect and to the overall body of scientific evidence.

LSRO recommends a genotoxicity and cytotoxicity test battery based on the merits of individual assays; test battery guidelines of the International Conference on Harmonisation and the US Food and Drug Administration; and recommendations for testing tobacco smoke toxicity by the Centre de Coopération pour les Recherches Scientifiques Relatives au Tabac. The results of the following assays may provide useful information for evaluating PRRTPs:

- The *Salmonella* mutagenicity assay;
- An *in vitro* assay of chromosome damage. Of the three assays considered [chromosomal aberration (ABS), micronuclei frequency (MN), and sister chromatid exchange (SCE)] the MN assay is most useful because it provides a simple, efficient microscopic method to characterize PRRTP-induced chromosomal damage;
- An *in vivo* assay for ABS, MN, or SCE; and
- An *in vitro* assay for cytotoxicity.

Potential epigenetic and apoptotic responses to cigarette smoke and/or PRRTP constituents are not addressed by current genotoxicity and cytotoxicity test batteries. A number of technologies

could be exploited, including stem cell technologies; three-dimensional tissue and organ models; fluorescence-based technologies; and genomic and proteomic approaches. Considerable resources are being directed toward re-evaluating existing toxicological assays and test batteries and validating a number of alternative *in vitro* tests (European Centre for the Validation of Alternative Methods, 2006; Interagency Coordinating Committee on the Validation of Alternative Methods & National Toxicology Program Interagency Center for the Evaluation of Alternative Methods, 2006; National Research Council, 2007). As more sophisticated assays are introduced, the understanding of cellular toxicity mechanisms should improve. If applicable to PRRTP testing, new methods should be used when they are validated.

Animal studies

Animal studies can provide additional preclinical data about the biological effects of PRRTPs. Some animal studies evaluate general toxicity resulting from exposures of various durations (*i.e.,* acute, subchronic, and chronic); others evaluate specific biological processes or health effects caused by an exposure. It is critical to define the actual exposure methods, duration, and concentrations in studies where animals are exposed to smoke or the PRRTP equivalent. The failure to adequately address these exposure issues in many published studies hinders the reproduction of results and creates challenges when attempting to extrapolate the results of animal studies to human exposure conditions. Animal studies that test PRRTPs should include:

- Exposures that simulate potential smoker exposures (*i.e.,* mainstream smoke exposures);
- Appropriate methodology and animal handling for whole-body and nose-only exposures;
- Analytical identification and quantification of the components in the test atmosphere;
- Measurements of biomarkers of exposure to estimate experimental smoke exposures (*e.g.*, carboxyhemoglobin; nicotine or cotinine; urinary mutagens/carcinogens; or deoxyribonucleic acid/protein adducts);
- Comparisons with conventional and reference cigarettes in the context of the same study;
- Filtered-air controls;
- Testing in two animal species, if possible, and of the most sensitive species and gender ideally; and
- International agency standards for the design, conduct, and analysis of animal studies.

Animal toxicity testing is conducted to identify possible adverse effects that result from exposure to an agent and to develop dose-response relationships that permit an evaluation of responses at various exposure levels (National Research Council, 2007). Historically, subchronic (14-, 28-, and 90-day) toxicity studies and carcinogenicity studies using dermal tumor promotion models have been used to evaluate cigarette smoke.

The number and selection of animal studies to be conducted should be based on product characteristics, smoke chemistry, and the results of *in vitro* studies. Animal studies should be able to identify potential adverse health effects. The rationale for the selection of specific methodologies and endpoints should be clearly articulated. As biomarkers of exposure and/or disease are identified and validated, they should be incorporated into rodent toxicity studies to better characterize endpoints of smoking-related diseases or biological processes related to disease etiology.

Research into developing animal models for LC, COPD, and CVD is active and promising; however, uncertainties and shortcomings in standardizations of smoke exposure technologies and determinations of delivered dose of cigarette smoke limit the value of many current disease models for evaluating PRRTPs. The most promising models are the:

- $B6C3F_1$ mouse lung cancer model (Hutt *et al.*, 2005);
- A/J mouse model of cigarette-smoke induced emphysema (March *et al.*, 2006); and
- $ApoE^{-/-}$ mouse atherosclerotic plaque formation model in response to cigarette smoke (Gairola *et al.*, 2001).

Each of these models requires verification by independent laboratories before it is accepted as a validated model. Because they are relevant to the effects of smoking, newly validated disease models should be incorporated into the PRRTP evaluation process. Genetically engineered rodents may prove to be the most useful model for testing PRRTPs because a number of risk factors (*e.g.*, hypertension, diabetes, obesity, and hypercholesterolemia) can be superimposed on cigarette smoke exposure.

Preclinical studies and PRRTP assessment

PRRTPs that demonstrate a potential to *reduce* toxicological effects relative to conventional cigarettes in preclinical testing can proceed to clinical studies. PRRTPs that show a potential to *increase* toxicological effects should not be tested in clinical studies; they should either be redesigned and retested or rejected for use as PRRTPs.

Clinical study design

Although analytical, preclinical, and clinical studies will all contribute to the body of scientific evidence for or against the risk reduction potential of a PRRTP, human clinical studies will provide the most relevant information. Generally, the purpose and specific goals of a clinical study should guide its design. Specific study considerations relevant to PRRTPs include: ethics; study population and duration; comparison/control groups; use characterization; and study design and conduct.

Ethics

Participants in clinical studies should be offered assistance in smoking cessation; participants should be limited to current smokers/tobacco users who are of legal age (over 18).

Study Population

A range of different smoker types should be included in PRRTP studies, especially those who match projected characteristics of potential PRRTP users. Sufficient participants should be included to achieve the statistical power necessary to meet study goals. Participant randomization and investigator blinding schemes should be used when possible.

Study Duration

Investigators should allow adequate time to stabilize smoking use/behaviors and to see significant changes in biomarkers of interest. Study duration and stopping rules should be articulated before the study begins.

Comparison/Control Groups

Multiple variables should be used, including the participants' own brand; use of a single control product independent of participants' usual brand; and abstinence with or without nicotine replacement therapy.

Use characterization

Appropriate biomarkers of exposure should be measured. Adequate accounting of the quantity smoked should be provided.

Study design and conduct

Clinical studies should be designed and conducted and the data should be reported according to Good Clinical Practice Guidelines (European Parliament, 2001; International Conference on Harmonisation, 1996, 1997, 2000; U.S. Food and Drug Administration, 1996). Appropriate statistical analyses should be performed (International Conference on Harmonisation, 1998). Crossover study design can be used to minimize inter-individual variation in smoking behavior/topography (Senn, 2002).

Clinical studies of effect

Biomarkers of biological effect can be used to monitor biological changes that occur during clinical studies of PRRTPs. "Biomarkers of effect" are measured effects, including early subclinical biological effects, alterations in morphology, structure or function, or clinical symptoms consistent with the development of health impairment or disease (Committee on Biological Markers of the National Research Council, 1987; Hatsukami *et al.*, 2006; Institute of Medicine, 2001).

Disease pathogenesis is a complex, multifactorial process. Molecular, cellular, tissue, and organ events associated with the development of LC, COPD, and CVD can be used to assess differences in adverse health effects associated with the use of PRRTPs or conventional cigarettes.

Desirable characteristics of biomarkers used to assess biological effects include:

- Biomarker is known to be directly or indirectly affected by smoking;
- Biomarker is known to be associated with pathobiology and clinical events of the disease of interest in humans;
- Biomarker is readily reversible;
- The time frame needed to see a change in the biomarker is appropriate for premarket testing (*i.e.,* months, weeks, days); and
- Use of the biomarker is practical, in terms of cost; intra-individual variability; availability of measurement methodology; analytical reproducibility, sensitivity, specificity, and/or standardization; and acceptability to subjects.

Biomarkers used to study effect must be relevant to the biological pathways of the disease being investigated. LSRO considers the following biological processes relevant (but not necessarily directly causal) to the development of LC, COPD, and CVD:

> **LC:** Cytopathological changes, genetic damage, epigenetic alterations, inflammation, oxidative stress, and protein changes;
> **COPD:** Clinical severity and/or airway obstruction, inflammation, protease-antiprotease imbalance, oxidative stress, parenchymal destruction, epithelial injury, and mucus production; and
> **CVD:** Lipid metabolism, inflammation, thrombosis and coagulation, oxidative stress, endothelial function, atherosclerosis, myocardial function, and electrical cardiac activity.

Biomarkers that could be used to study these biological processes were identified and classified as primary, secondary, or tertiary using the following criteria:

> **Primary biomarkers** have been linked to clinical outcomes with strong evidence.
> **Secondary biomarkers** have been linked to clinical outcomes with moderate evidence.
> **Tertiary biomarkers** have been linked to clinical outcomes with preliminary evidence.

LSRO identified a number of biomarkers of effect that could be useful in the assessment of PRRTPs. Chapter III includes a list of these biomarkers and extensive discussions of each.

Biomarkers of biological effect and PRRTP assessment

When biological processes are altered by competing factors such as diet and exercise; occupation; genetics; and co-existing disease, human disease occurs. The major diseases associated with cigarette smoking (*i.e.*, LC, COPD, and CVD) result from numerous deleterious changes that occur and interact over time. The biomarkers for the premarket assessment of PRRTPs should identify readily reversible biological changes that can be linked to both smoking and disease. Few biomarkers of effect possess all characteristics desirable for use in PRRTP testing, but biomarker research is rapidly changing and advancing. For this reason, several overriding principles are provided to direct the selection of biomarkers of effect to assess PRRTPs rather than a list of specific biomarkers:

- The alterations in biological processes caused by cigarette smoking are incompletely understood. Therefore, clinical studies measuring biomarkers of effect in smokers should address as many biological processes that contribute to LC, COPD, and CVD as feasible.
- Because several mechanistic pathways are likely to contribute to specific biological disease processes, multiple biomarkers of effect should be measured. Discrepant results between measurements for the same biological process (*e.g.*, inflammation) should be assessed for potential differences in cellular and molecular pathways to address the apparent inconsistency in results. Attempts should be made to provide theoretical explanations for any discordant findings.
- The best biomarkers available for testing should be used. Priority should be given first to primary, then secondary, and finally tertiary biomarkers when all classes of biomarkers are available for the biological process under investigation.

- The weight of evidence should support the concept that the use of a PRRTP causes biomarker values to move away from values measured in both heavy and light smokers of conventional cigarettes and towards values measured in long-term ex-smokers and never smokers.
- Because a PRRTP may cause beneficial changes in one biomarker, disease process, or disease but may adversely affect others, a PRRTP assessment must consider all evidence and cannot depend on any single biomarker, group of biomarkers, biological process, or disease.

Summary

Generally, preclinical and clinical data should support the reduced toxicity of a PRRTP during pre-market testing. Human biomarker data are the most relevant; unfortunately, the best early predictive markers to measure the development of LC, COPD, and CVD due to cigarette smoking and/or PRRTP use are not clearly established. Instead of being a deterrent, the lack of early predictive markers should be interpreted as a challenge to develop effective measures to evaluate biological alterations in PRRTP users relative to smokers of conventional cigarettes. Better biomarkers of biological effect could be used for other purposes (*e.g.*, to identify subsets of individuals who may be harmed or helped by PRRTPs) or to evaluate therapies for LC, COPD, and CVD. Public databases on smoking and disease could also be built from the clinical data generated from studies comparing cigarette smokers, PRRTP users, and nonsmokers.

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