The LSRO Report on

DIFFERENTIATING THE HEALTH RISKS OF CATEGORIES OF TOBACCO PRODUCTS

Editor
Kara D. Lewis, Ph.D.
DIFFERENTIATING THE HEALTH RISKS OF CATEGORIES OF TOBACCO PRODUCTS

LSRO

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Editor
FOREWORD

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

This report was developed under a contract between Philip Morris USA, Inc. (Philip Morris) and LSRO. The findings, conclusions, and recommendations contained herein were developed independently of Philip Morris and are not intended to represent the views of Philip Morris or any of its employees.

This report provides the overall conclusions and recommendations of LSRO’s efforts to characterize (1) the relative risks of using smokeless tobacco products compared with cigarette smoking and (2) the relative risks to users of various categories of smokeless tobacco products.

An Expert Panel provided scientific oversight and direction for all aspects of this project. LSRO independently appointed members of the Expert Panel 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. Panel members were selected with the concurrence of the LSRO Board of Directors. An overview of the scientific expertise of Expert Panel members and LSRO staff may be found in Appendix A of this report.

The Expert Panel convened three times between June 2007 and February 2008 to assess available data. Information about the process, including critical literature and presentations on which the Expert Panel based their deliberations, was made publicly available on the LSRO web site at www.lsro.org.

LSRO staff drafted this report on the basis of available information, deliberations of the Expert Panel, and recommendations of the Expert Panel. The draft LSRO report was submitted to experts in relevant disciplines for independent peer review, and their comments were considered for incorporation by LSRO staff, the Expert Panel, and the LSRO Board of Directors. The Expert Panel and LSRO Board of Directors reviewed and approved the final report.
Participation in the preparation of this report or membership on the Expert Panel 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.
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TABLE OF CONTENTS

FOREWORD ................................................................................................................... iii
EXECUTIVE SUMMARY ................................................................................................. 1

1. INTRODUCTION ........................................................................................................ 14
  1.1 PROJECT OBJECTIVES ....................................................................................... 14
  1.2 AREAS OF INITIAL CONSENSUS ...................................................................... 15
  1.3 DIFFERENTIATING TOBACCO RISKS PROJECT ........................................ 15
  1.4 SMOKELESS TOBACCO PRODUCTS ............................................................... 16
    1.4.1 Definitions ..................................................................................................... 16
      1.4.1.1 World Health Organization Scientific Advisory Committee on Tobacco Product Regulation .................................................... 16
      1.4.1.2 Code of Federal Regulations ................................................................ 17
    1.4.2 Selection of Smokeless Tobacco Products ................................................... 17
    1.4.3 Overview of Smokeless Tobacco Product Types and Commercial Brands .......................................................... 17
      1.4.3.1 Powdered dry snuff ................................................................................. 18
      1.4.3.2 Loose-leaf chewing tobacco ................................................................... 18
      1.4.3.3 Moist snuff (including snus) .................................................................... 18
        1.4.3.3.1 Moist snuff, US .................................................................................... 20
        1.4.3.3.2 Moist snuff, Sweden .......................................................................... 20
      1.4.3.4 Hard snuff ............................................................................................... 20
  1.5 REPORT ORGANIZATION ..................................................................................... 20

2. A CONSIDERATION OF RISK ................................................................................. 21
  2.1 INTRODUCTION .................................................................................................... 21
  2.2 RISK ASSESSMENT .............................................................................................. 22
  2.3 COMPARATIVE RISK ASSESSMENT .................................................................. 22
  2.4 HAZARD IDENTIFICATION ................................................................................ 23
  2.5 EXPOSURE ASSESSMENT .................................................................................. 24
  2.6 BIOLOGICAL EFFECTS ASSESSMENT .............................................................. 24
  2.7 HEALTH OUTCOMES ASSESSMENT ................................................................ 25
  2.8 RISK CHARACTERIZATION ............................................................................... 26
  2.9 RISK COMMUNICATION AND RISK MANAGEMENT ...................................... 26
  2.10 POPULATION EFFECTS ................................................................................... 27
  2.11 CONCLUSIONS .................................................................................................. 27

3. MANUFACTURING AND CHEMICAL COMPOSITION ......................................... 28
  3.1 TOBACCO TYPES, CURING METHODS, AND PRODUCTION PROCESSES .......... 28
    3.1.1 Tobacco Types .............................................................................................. 29
    3.1.2 Curing and Production .................................................................................. 29
      3.1.2.1 Loose-leaf tobacco products ................................................................... 30
5.2.1 Routes of Exposure ........................................................... 53
  5.2.1.1 Interactions with mucosal membranes ......................... 53
  5.2.1.2 Role of saliva ............................................................... 54
  5.2.1.3 Ingestion ...................................................................... 54

5.2.2 Tobacco-Derived Chemicals or Metabolites in Biological Fluids ................................................................. 54
  5.2.2.1 Nicotine, minor tobacco alkaloids, and thiocyanate .... 55
  5.2.2.2 Tobacco-specific nitrosamines ..................................... 56
    5.2.2.2.1 Urine ..................................................................... 57
    5.2.2.2.2 Saliva .................................................................... 58
    5.2.2.2.3 Hemoglobin adducts ............................................. 58
  5.2.2.3 Urine mutagenicity ....................................................... 58

5.3 BIOMARKERS OF EFFECT ..................................................... 60
  5.3.1 Cardiovascular Clinical Studies ......................................... 61
    5.3.1.1 Electrical cardiac activity and hemodynamics ............. 61
    5.3.1.2 Endothelial function ..................................................... 62
    5.3.1.3 Atherosclerosis ............................................................ 62
    5.3.1.4 Inflammation ............................................................... 63
    5.3.1.5 Lipid metabolism .......................................................... 63
  5.3.2 Cytological and Cytogenetic Changes ............................... 63
  5.3.3 Reproductive Effects .......................................................... 64
  5.3.4 Summary of Smokeless Tobacco Biological Effects .......... 64

5.4 CONCLUSIONS ....................................................................... 64

6. BEHAVIORAL CONSIDERATIONS ........................................... 66
  6.1 SMOKELESS TOBACCO USE PATTERNS ............................. 66
    6.1.1 Population Use Patterns .................................................... 66
      6.1.1.1 The US ......................................................................... 66
    6.1.1.2 Sweden ........................................................................ 68
    6.1.2 Individual Use Patterns ...................................................... 68
  6.2 COMPARISON OF SMOKELESS TOBACCOS ....................... 70
    6.2.1 Nicotine Pharmacokinetics ................................................ 70
    6.2.2 Initiation ............................................................................. 74
    6.2.3 Gateway to Cigarettes ....................................................... 75
    6.2.4 Psychological Correlates Associated with Use of Smokeless Tobacco ........................................................... 76
    6.2.5 Social Acceptance of Tobacco Product Types ............... 80
    6.2.6 Nasal Use .......................................................................... 81
    6.2.7 Cessation ........................................................................... 81
  6.3 PERCENTAGE OF CONCURRENT USE OF SMOKELESS TOBACCO WITH CIGARETTES ................. 82
  6.4 CONCLUSIONS ....................................................................... 83
7. HEALTH OUTCOMES ........................................................................................................... 84
  7.1 BACKGROUND ........................................................................................................... 84
  7.2 COMPARING SWEDISH SNUS AND US SMOKELESS TOBACCO WITH CIGARETTES .............................................. 86
    7.2.1 Lung Cancer ........................................................................................................ 86
      7.2.1.1 Swedish snus ............................................................................................... 86
      7.2.1.2 US smokeless tobacco products ................................................................. 86
    7.2.2 Chronic Obstructive Pulmonary Disease .......................................................... 88
    7.2.3 Cardiovascular Disease ...................................................................................... 90
      7.2.3.1 Swedish snus ............................................................................................... 91
      7.2.3.2 US smokeless tobacco products ................................................................. 93
      7.2.3.3 Cardiovascular effects of pure nicotine ......................................................... 93
    7.2.4 Oral Cancer ........................................................................................................ 94
      7.2.4.1 Swedish snus ............................................................................................... 95
      7.2.4.2 US smokeless tobacco products ................................................................. 96
    7.2.5 All-Cause Mortality ............................................................................................ 98
      7.2.5.1 Swedish snus ............................................................................................... 99
      7.2.5.2 US smokeless tobacco products ................................................................. 99
    7.2.6 Other Cancers .................................................................................................... 100
      7.2.6.1 Laryngeal cancer ......................................................................................... 100
      7.2.6.2 Esophageal and gastric cancer .................................................................... 100
        7.2.6.2.1 Swedish snus ......................................................................................... 103
        7.2.6.2.2 US smokeless tobacco products ........................................................... 103
      7.2.6.3 Pancreatic cancer ......................................................................................... 104
        7.2.6.3.1 Swedish snus ......................................................................................... 105
        7.2.6.3.2 US smokeless tobacco products ........................................................... 105
      7.2.6.4 Bladder cancer ............................................................................................ 106
        7.2.6.4.1 Swedish snus ......................................................................................... 107
        7.2.6.4.2 Chewing tobacco ............................................................................... 107
      7.2.6.5 Kidney cancer ............................................................................................. 108
    7.2.7 Other Health Effects ............................................................................................ 109
      7.2.7.1 Pregnancy outcomes .................................................................................... 109
        7.2.7.1.1 Swedish snus ......................................................................................... 110
        7.2.7.1.2 Pure nicotine and pregnancy outcomes ................................................. 110
      7.2.7.2 Inflammatory bowel disease ....................................................................... 110
      7.2.7.3 Diabetes .................................................................................................... 111
        7.2.7.3.1 Swedish snus ......................................................................................... 111
      7.2.7.4 Amyotrophic lateral sclerosis ................................................................. 111
    7.3 CONCLUSIONS ....................................................................................................... 112
    7.4 SECONDARY LITERATURE ..................................................................................... 113
8. INTERNATIONAL SMOKELESS TOBACCO PRODUCTS .......... 116
  8.1 ANALYSIS OF INTERNATIONAL SMOKELESS TOBACCO PRODUCTS ................................................................. 116
  8.2 MANUFACTURING, GEOGRAPHIC ORIGIN, AND CHEMICAL COMPOSITION ......................................................... 120
    8.2.1 Tobacco Types, Curing Methods, and Production Processes ................................................................. 120
    8.2.2 Tobacco-Specific Nitrosamines ........................................ 120
  8.3 PRECLINICAL STUDIES ....................................................... 121
    8.3.1 Genotoxicity and Cytotoxicity Assays .............................. 121
      8.3.1.1 Salmonella mutagenicity assay .................................. 121
      8.3.1.2 Mammalian cell mutagenicity ..................................... 123
    8.3.2 Animal Models and Tumor Formation .................................. 124
  8.4 HUMAN EXPOSURE AND BIOLOGICAL EFFECTS ............. 124
  8.5 HEALTH OUTCOMES ............................................................ 125
    8.5.1 Lung Cancer ................................................................. 127
    8.5.2 Cardiovascular Disease ............................................... 127
    8.5.3 Oral Cancer .................................................................... 127
    8.5.4 Esophageal Cancer .......................................................... 131
    8.5.5 All-Cause Mortality ........................................................ 132
    8.5.6 Pregnancy Outcomes ...................................................... 133
  8.6 BEHAVIOR ............................................................................. 134
    8.6.1 Population Use Patterns .................................................. 134
    8.6.2 Nicotine Pharmacokinetics .............................................. 134
    8.6.3 Concomitant Use of Smokeless Tobacco and Cigarettes ................................................................. 135
  8.7 CONCLUSIONS ..................................................................... 135

9. COMPARATIVE RISK ASSESSMENT OF SMOKELESS TOBACCO AND CIGARETTES ................................................................. 137
  9.1 RISK ASSESSMENT OBJECTIVES FOR THE DIFFERENTIATING TOBACCO RISKS PROJECT .......... 137
  9.2 DISEASE RISKS FOR CIGARETTES AND SMOKELESS TOBACCO ................................................................. 138
  9.3 INDIVIDUAL AND POPULATION RISK ASSESSMENT ..... 138
    9.3.1 Risk Reduction ................................................................. 138
    9.3.2 Harm Reduction ............................................................... 139
    9.3.3 Population Risk Assessment .................................................. 139
  9.4 SMOKELESS TOBACCO CATEGORIES .................................. 139
  9.5 SCIENTIFIC UNCERTAINTY .................................................. 140
    9.5.1 Risks to Different Populations .......................................... 140
    9.5.2 Different Smokeless Tobacco Manufacturing Conditions ..................................................................... 140
Differentiating the Health Risks of Categories of Tobacco Products

9.5.3 Information Availability for Smokeless Tobacco ............... 141
9.5.4 Residual Risk for Former Smokers Who Switch to Smokeless Tobacco .......................................................... 141
9.5.5 Switching Between Products ......................................................... 141

9.6 RISK CHARACTERIZATION: CIGARETTES VERSUS SMOKELESS TOBACCO ....................................................... 142
9.6.1 Preclinical Studies ........................................................... 152
9.6.1.1 Product characteristics .............................................. 152
9.6.1.2 Chemistry................................................................... 152
9.6.1.3 Genotoxicity and cytotoxicity assays.......................... 153
9.6.1.4 Animal studies ........................................................... 153
9.6.2 Clinical Studies ................................................................ 154
9.6.2.1 Biomarkers of exposure ............................................. 154
9.6.2.2 Biomarkers of effect ................................................... 154
9.6.3 Health Effects Studies ..................................................... 155
9.6.3.1 Lung cancer ............................................................... 155
9.6.3.2 Chronic obstructive pulmonary disease ................. 156
9.6.3.3 Cardiovascular disease.............................................. 157
9.6.3.4 Oral cancer ............................................................... 158
9.6.3.5 Other adverse health effects ...................................... 159
9.6.3.6 All-cause mortality ..................................................... 160
9.6.4 Behavioral Studies and Surveys ...................................... 161
9.6.4.1 Abuse liability ............................................................. 161
9.6.4.2 Smokeless tobacco as a smoking cessation aid........ 162

9.7 CONCLUSIONS ..................................................................... 162

10. COMPARATIVE RISK ASSESSMENT OF CATEGORIES OF SMOKELESS TOBACCO ............................................................... 164
10.1 RISK CHARACTERIZATION OF CATEGORIES OF SMOKELESS TOBACCO ............................................................... 164
10.1.1 Product Characteristics .................................................... 165
10.1.2 Chemical Composition ..................................................... 166
10.1.3 Genotoxicity and Cytotoxicity Assays .............................. 168
10.1.4 Animal Studies ................................................................. 169
10.1.5 Clinical Studies ................................................................. 169
10.1.5.1 Biomarkers of exposure and effect ............................ 169
10.1.6 Health Outcomes Assessment ........................................ 170
10.1.6.1 Lung cancer and chronic obstructive pulmonary disease ......................................................... 170
10.1.6.2 Cardiovascular disease .............................................. 170
10.1.6.3 Oral cancer ............................................................... 171
10.1.6.4 All-cause mortality ..................................................... 172
10.1.7 Population Risk ............................................................... 172
10.2 PROPOSED FACTORS FOR ASSESSING COMPARATIVE RISK OF SMOKELESS TOBACCO PRODUCTS ............... 173
10.3 STRATIFICATION OF CATEGORIES OF SMOKELESS TOBACCO PRODUCTS ACCORDING TO RISK .......... 174

11. STANDARDIZED METHODS AND RESEARCH NEEDS .............. 177

11.1 STANDARDIZED METHODS .................................................. 177
11.1.1 Preclinical Studies .......................................................... 177

11.2 RESEARCH NEEDS .............................................................. 179
11.2.1 Preclinical Studies .......................................................... 179
11.2.2 Clinical Studies ............................................................. 179
11.2.2.1 Biomarkers of exposure ............................................. 179
11.2.2.2 Biomarkers of effect ................................................... 180
11.2.3 Health Outcomes ........................................................... 180
11.2.4 Behavior ........................................................................ 181

11.3 CONCLUSIONS ................................................................ 182

12. LITERATURE CITATIONS .......................................................... 183

APPENDICES ........................................................................... 233

A. LIFE SCIENCES RESEARCH OFFICE .................................... 233
   Differentiating Tobacco Risks Expert Panel ......................... 233
   Life Sciences Research Office Staff ...................................... 236
   Life Sciences Research Office Board of Directors ................ 240

B. PUBLIC AND INVITED COMMENTS ........................................ 241
   Differentiating the Health Risks of Categories of Tobacco Products Open Meeting Speakers, June 5, 2007 .......... 241
   Differentiating the Health Risks of Categories of Tobacco Products Open Meeting Speakers, October 3, 2007 .......... 241

C. PRECLINICAL STUDIES .......................................................... 242

D. SUMMARY OF HEALTH EFFECTS FOR CIGARETTE SMOKERS AND SMOKELESS TOBACCO USERS .......... 248

E. GLOSSARY, ACRONYMS, AND ABBREVIATIONS ............... 260
   Glossary .............................................................................. 260
   Acronyms and Abbreviations ............................................. 269

INDEX ...................................................................................... 271
## TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>US Smokeless Tobacco Sales (in pounds sold)</td>
<td>17</td>
</tr>
<tr>
<td>1-2</td>
<td>US Smokeless Tobacco Brand Names, Geographic Origins, Constituents, and Methods of Use</td>
<td>19</td>
</tr>
<tr>
<td>3-1</td>
<td>Characteristics of Selected Types of Tobacco</td>
<td>29</td>
</tr>
<tr>
<td>3-2</td>
<td>Manufacturing Processes of US Smokeless Tobacco Products</td>
<td>30</td>
</tr>
<tr>
<td>3-3</td>
<td>Reference Smokeless Tobacco Product Formulations</td>
<td>32</td>
</tr>
<tr>
<td>3-4</td>
<td>Chemical Analysis of Reference Smokeless Tobacco Products</td>
<td>32</td>
</tr>
<tr>
<td>3-5</td>
<td>Chemical Composition of Selected Smokeless Tobacco Products</td>
<td>34</td>
</tr>
<tr>
<td>3-6</td>
<td>Tobacco Specific Nitrosamine Levels in Smokeless Tobacco Products</td>
<td>36</td>
</tr>
<tr>
<td>3-7</td>
<td>GothiaTek® Standard for Maximal Permissible Limits for Undesirable Substances</td>
<td>40</td>
</tr>
<tr>
<td>4-1</td>
<td>Cell Survival as Measured by Lactate Dehydrogenase Release After Treatment with Smokeless Tobacco Extract</td>
<td>44</td>
</tr>
<tr>
<td>5-1</td>
<td>Tobacco-Specific Nitrosamine Levels in Biological Fluids – Urine</td>
<td>59</td>
</tr>
<tr>
<td>6-1</td>
<td>Area Under the Concentration-Time Curve and Maximal Plasma Nicotine Concentrations After Use of Moist Snuff, Modified Smokeless Tobacco Products, or Commit® Lozenge</td>
<td>73</td>
</tr>
<tr>
<td>6-2</td>
<td>Summary of Selected References on a Smokeless Tobacco Gateway to Cigarettes</td>
<td>77</td>
</tr>
<tr>
<td>6-3</td>
<td>Descriptions and Marketing of Selected New Smokeless Tobacco Products</td>
<td>77</td>
</tr>
<tr>
<td>8-1</td>
<td>International Smokeless Tobacco Brand Names, Geographic Origins, Constituents, and Methods of Use</td>
<td>117</td>
</tr>
<tr>
<td>8-2</td>
<td>Manufacturing Processes of International Smokeless Tobacco Products</td>
<td>120</td>
</tr>
<tr>
<td>8-3</td>
<td>Chemical Comparison of the Smokeless Tobacco Product Zarda</td>
<td>121</td>
</tr>
<tr>
<td>8-4</td>
<td>Tobacco-Specific Nitrosamine Levels in International Smokeless Tobacco Products</td>
<td>122</td>
</tr>
<tr>
<td>8-5</td>
<td>Tobacco-Specific Nitrosamine Levels in Biological Fluids – Urine</td>
<td>125</td>
</tr>
<tr>
<td>8-6</td>
<td>Tobacco-Specific Nitrosamine Levels in Biological Fluids – Saliva</td>
<td>126</td>
</tr>
</tbody>
</table>
Table 9-1. Risk Characterization Summary for Lung Cancer .............. 143
Table 9-2. Risk Characterization Summary for Chronic Obstructive Pulmonary Disease ........................................ 146
Table 9-3. Risk Characterization Summary for Cardiovascular Disease ................................................................. 148
Table 9-4. Risk Characterization Summary for Oral Cavity Cancer ............................................................................. 151
Table 9-5. Summary of LSRO’s Confidence in Risk Reduction by Smokeless Tobacco and Estimated Percentage of Smoking-Attributable Mortality ........................................... 160

FIGURES

Figure 3.1 Tobacco-Specific Nitrosamines .................................................. 35
Figure 7.1 Lung Cancer Incidence and Mortality Associated with Cigarette Smoking and Smokeless Tobacco Use .......... 87
Figure 7.2 Chronic Obstructive Pulmonary Disease Mortality Associated with Cigarette Smoking and Smokeless Tobacco Use ........................................................................ 90
Figure 7.3 First Myocardial Infarction Incidence Associated with Cigarette Smoking and Smokeless Tobacco Use .......... 91
Figure 7.4 Oral Cancer Incidence Associated with Cigarette Smoking and Smokeless Tobacco Use ........................................... 95
Figure 7.5 All-Cause Mortality Associated with Cigarette Smoking and Smokeless Tobacco Use ........................................... 99
Figure 7.6 Esophageal Squamous Cell Carcinoma Incidence Associated with Cigarette Smoking and Smokeless Tobacco Use .................................................. 101
Figure 7.7 Gastric Cancer Incidence Associated with Cigarette Smoking and Smokeless Tobacco Use .......... 102
Figure 7.8 Pancreatic Cancer Incidence Associated with Cigarette Smoking and Smokeless Tobacco Use .......... 105
Figure 7.9 Bladder Cancer Incidence Associated with Cigarette Smoking and Smokeless Tobacco Use ...................... 108
Figure 8.1 Oral Cancer Incidence for Tobacco Smokers and Betel Quid Users .................................................. 128
EXECUTIVE SUMMARY

This report presents the findings, conclusions, and recommendations of the Life Sciences Research Office (LSRO) about whether sufficient scientific evidence exists to differentiate the health risks of categories of tobacco products. The report was developed under a contract between Philip Morris USA, Inc. and LSRO. An expert advisory panel, the Differentiating Tobacco Risks (DTR) Committee, guided the development of the report. From this point forward, LSRO, its staff, and the DTR Committee are collectively referred to as LSRO.

CIGARETTE HARM REDUCTION

Each year, more than 400,000 people in the US die prematurely because they smoke cigarettes. Cigarette smokers are advised to quit smoking, and never smokers, not to start smoking. Of the two-thirds of smokers who report that they want to quit smoking each year, more than one-third attempt to quit, but less than 3% of those who attempt to quit are successful. As a result, other approaches to reducing the harms from cigarette smoking have been explored.

Although no tobacco product is safe, accumulating data indicate that various types of tobacco products present different levels of risk to users and individuals in the product use environment. Consequently, it has been proposed that smokers who cannot or will not stop using tobacco can reduce their risk of cigarette smoking-related disease by replacing cigarettes with a tobacco product with a lower risk of adverse health effects. Smokeless tobacco (ST) is one class of potential reduced-risk product. ST is not combusted when used as intended, which eliminates exposure to mainstream smoke and environmental tobacco smoke. However, ST comprises a diverse group of products. Therefore, any risk reduction associated with one category of ST may not translate into risk reduction for other categories. This report focuses on comparing the risks of ST use with those of cigarette smoking and comparing the risks associated with using different types of STs.
DIFFERENTIATING TOBACCO RISKS OBJECTIVES AND APPROACH

The specific objectives of the DTR project were as follows:

1. Develop an independent consensus opinion as to whether ST products meet the criteria for reduced-risk (or reduced-harm) products compared with cigarettes;
2. Identify and characterize the critical characteristics of ST products that contribute to the evaluation of risk; and
3. Develop an independent consensus opinion as to whether sufficient evidence exists to stratify categories of ST products according to risk.

Although LSRO’s review focused on evaluating whether ST reduces the risk of lung cancer (LC), chronic obstructive pulmonary disease (COPD), and cardiovascular disease (CVD), diseases that account for approximately 350,000 of the 400,000 annual deaths from cigarette smoking-related diseases, LSRO also considered the relative risks of oral cancer, other cancers, and all-cause mortality. Comparative risk assessments of tobacco products were conducted by using the evaluative framework described in the LSRO report entitled Scientific Methods to Evaluate Potential Reduced-Risk Tobacco Products. LSRO comprehensively reviewed literature on preclinical studies related to ST and cigarettes (product characteristics, chemical composition, in vitro assays, and animal studies), clinical studies of biomarkers of exposure and biomarkers of effect, health outcome assessments (epidemiological and clinical studies), and behavioral studies. LSRO took a weight of evidence approach to evaluate the relative risks of cigarettes and STs and between different categories of ST products. The order in which the types of studies are mentioned above reflects increasing weight of evidence in the review. LSRO also considered written and oral comments on the topic from the public. LSRO’s findings, conclusions, and recommendations were developed from the literature review, relevant oral and written public comments, and deliberations of the DTR Committee.

CONCLUSIONS AND RECOMMENDATIONS

Objective 1: Develop an independent consensus opinion as to whether ST products meet the criteria for reduced-risk (or reduced-harm) products compared with cigarettes

To conduct a comparative risk assessment of ST and cigarettes, LSRO considered the information summarized below.
Preclinical Studies

Product Characteristics

Cigarettes and ST tobacco differ in many ways. For example, moist snuff is made from dark tobacco, whereas cigarettes are made from various types of tobacco, such as flue-cured, Burley, Oriental, cut-roll stem, and reconstituted tobacco. Because cigarettes undergo combustion when used as intended and ST does not, smokers are exposed to combustion products and other substances in the cigarette that transfer directly into cigarette smoke. Cigarettes have diverse formulations and designs, including a wide variety of added ingredients, which may also influence smoke composition. ST products differ in many aspects of the manufacturing, production, and post-production processes. Although ST use eliminates exposure to cigarette smoke, the effects of various STs on individual and population risks of disease may differ.

Chemical Composition

Cigarette smoke contains carcinogens and other toxins. In contrast, because ST does not produce smoke when used as intended, the unburned tobacco leaf contains fewer carcinogens than cigarette smoke. However, ST contains other harmful substances that may contribute to increased risk of certain diseases for ST users compared with non-users of tobacco. Because the causal relationships between constituents of STs and of cigarette smoke and disease development have not been firmly established, it is not known how differences in specific constituents of ST extracts and cigarette smoke affect disease risk. Overall goals for risk reduction of STs should include eliminating or lowering levels of carcinogens and other tobacco product-related toxins as compared to conventional cigarettes' smoke and determining that ST is not a gateway to increased tobacco use.

In vitro Assays

Many studies have shown that cigarette smoke is mutagenic. Some STs also exhibit mutagenicitiy. Because of varied dosing and methodology used in in vitro assays, LSRO found it difficult to draw conclusions from such studies about the risk of STs compared with cigarettes. LSRO recommends that investigators conducting future cigarette and ST studies use a recognized battery of tests for genotoxicity and cytotoxicity and consult the International Organization for Standardization (ISO) guidelines for extracting test substances for such studies. Future research should follow a standardized protocol, such as that proposed by ISO, and should include a range of doses of the test substance, positive controls, reference tobacco products, and products that represent those on the commercial market.
**Animal Studies**

Total particulate matter\(^1\) from cigarette smoke promoted dermal tumor development in animal studies. In addition, inhalation of sidestream and mainstream smoke caused respiratory tract lesions in different rodent models. In contrast, animal studies provide limited evidence of carcinogenicity of ST products, particularly ST products from the US and Sweden. Oral cavity swabbing with ST extract (STE) did not increase tumor formation in rats, possibly because of inadequate STE dosing. Failure to produce tumors following oral swabbing with ST extract may also be due to total dose delivered and duration of exposure. Swabbing with tobacco-specific nitrosamines (TSNAs) increased tumor formation. Placement of moist snuff in a surgically created canal increased the incidence of tumor formation in oral and nasal cavities; however, this exposure method has been criticized because cell proliferation and tumor formation have been associated with mechanical damage and persistent tissue injury. LSRO recommends that future long-term carcinogenicity studies provide a daily dose of the test substances to animals for at least 1 year and that studies adhere to guidelines for carcinogenicity studies. These approaches will result in standard exposures that will permit intra- and inter-study comparisons.

Although some animal studies have investigated the effect of ST on cancer risk, no studies have examined the relationship between ST and CVD and COPD. As is the case with cigarette smoke, STE has developmental effects. Research grade moist STE provided to pregnant mice reduced fetal weight in a dose-dependent manner.

**Clinical Studies**

**Biomarkers of Exposure**

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, cellular or target tissues. Studies have investigated the effect of cigarette smoking and ST use on a limited number of biomarkers. Biomarker of exposure studies showed that compared with smokers, ST users have similar nicotine levels but higher plasma levels of the primary nicotine metabolite cotinine. ST users also have lower levels of serum thiocyanate, a biomarker of exposure for hydrogen cyanide, than cigarette smokers, and urine of ST users is less mutagenic than that of smokers. However, compared with smokers, ST users have higher median levels of the TSNA metabolite

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\(^1\) Particles in smoke, larger than 1 \(\mu\text{m}\) in diameter, that are trapped as the smoke passes through a Cambridge filter; usually obtained from mainstream smoke.
total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) per milliliter of urine and higher levels of the hemoglobin adduct of the TSNA 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB). The saliva of Swedish snuff users contains TSNAs, but questions remain about whether TSNAs are the substances responsible for oral lesions in ST users because the major conversion of TSNAs may occur in the gastrointestinal tract. Excretion of the minor tobacco alkaloids N′-Nitrosoanabasine, N′-Nitrosoanatabine and nornicotine was higher for ST users than for smokers.

**Biomarkers of Effect**

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 and disease. Biomarker of effect studies indicate that, like cigarette smoking, ST use alters electrical cardiac activity, hemodynamics, and endothelial function and causes cytological changes within the oral cavity. Biochemical risk factors for CVD such as plasma fibrinogen, C-reactive protein, and serum lipid and lipoprotein levels are changed toward an increased CVD risk for smokers but not for ST users.

Because biomarkers for inflammation and measures of atherosclerosis, which are more predictive of CVD than some other biomarkers, show more favorable levels for ST users than for smokers, ST may present a lower risk of CVD than cigarette smoking.

**Health Outcomes Assessment**

*(Epidemiological Studies and Clinical Trials)*

**Lung Cancer**

Studies have consistently reported that cigarette smoking significantly increases the risk of LC. Most studies reported that ST users do not have an increased risk of LC compared with non-smokers. Studies with significant methodological issues such as confounding from misclassification of smokers as ST users and exposure of subjects to environmental tobacco smoke reported that ST users have an elevated risk of LC.

**Chronic Obstructive Pulmonary Disease**

Studies have consistently reported that cigarette smoking significantly increases risk of COPD. Few studies have examined the relationship between ST use and the risk of COPD. Some studies with significant confounding indicate an increased risk of COPD for ST users compared with non-tobacco users; however, one study indicated no increased risk of respiratory disease
Differentiating the Health Risks of Categories of Tobacco Products

for ST users in the US. No biologically plausible relationship exists between ST and COPD, but this issue would benefit from additional research.

**Cardiovascular Disease**

Studies have consistently found a significantly increased risk of CVD for smokers compared with non-tobacco users. Two studies showed that Swedish *snus* users have slightly elevated CVD risk compared with non-users, but several studies reported that *snus* does not increase risk. One study determined that US snuff elevates CVD risk; however, another study did not find an increased risk of CVD. A study reported that chewing tobacco used in 52 countries increases CVD risk. In summary, epidemiological studies show that although ST use appears to increase CVD risk above the CVD risk for non-tobacco users, the risk is lower than that associated with cigarette smoking.

**Oral Cavity Cancer**

Many studies have shown that cigarette smoking increases oral cancer risk. Oral cancer risk appears to be different for various ST products. Available evidence suggests that Swedish *snus* either does not increase risk of oral cancer or increases it minimally. Some studies reported that US chewing tobacco and dry snuff increase oral cancer risk, but confounders such as alcohol consumption and smoking were not considered in the interpretation of data. Other studies of US STs reported no increased risk of oral cancer. International STs (*i.e.*, tobacco products other than US STs and Swedish *snus*) may pose a significantly greater risk of oral cancer than do US and Swedish STs. LSRO has a moderate level of confidence that US STs and Swedish *snus* present a lower risk of oral cancer compared with cigarette smoking. LSRO also has a moderate level of confidence that some international ST products present a higher risk of oral cancer compared with cigarettes.

**Other Cancers, All-Cause Mortality, and Pregnancy Outcomes**

Cigarette smoking increases the risk of laryngeal cancer, esophageal squamous cell carcinoma, gastric cancer, pancreatic cancer, and bladder cancer. Studies of the relationship between ST consumption and risk of laryngeal cancer have produced mixed results. Few studies have investigated the relationship between esophageal cancer and ST; however, some studies reported that ST does not increase the risk of esophageal cancer. Most studies did not demonstrate an increased risk of gastric cancer for ST users, but one study reported an increased risk of non-cardiac gastric cancer.
Epidemiological studies have indicated that risk of pancreatic cancer may be elevated for ST users compared with non-tobacco users but lower compared with cigarette smokers. The magnitude of increased risk of bladder cancer for ST users is lower than that for cigarette smokers. Whether cigarette smoking is associated with renal cell carcinoma is controversial. A weak association between renal cell carcinoma and ST use has been reported. The risk of all-cause mortality is significantly lower for users of Swedish snus and American STs than for cigarette smokers. LSRO’s conclusions about the risk of other cancers and all-cause mortality are summarized below. Further research is needed to clarify the relationship between ST use and risk of cancers. Some evidence exists that snuff use is associated with lower birth weight infants, preterm delivery, and/or pre-eclampsia.

**Behavioral Studies**

Cigarette smokers and ST users have similar overall maximal nicotine levels but different nicotine pharmacokinetics. LSRO concluded that ST is not likely to reduce the risk of nicotine addiction compared with cigarettes. There are concerns that ST could be a gateway product for tobacco use in the US; however, some data from Sweden demonstrate its use as a cessation aid and do not support the gateway hypothesis. Few data are available to assess whether easier availability of some types of ST products leads to increased tobacco use.

**Summary of LSRO’s Confidence That Smokeless Tobacco Is a Reduced-Risk Product Compared with Cigarettes**

LSRO used the descriptors low, moderate, and high to summarize its levels of confidence in the potential for risk reduction of ST compared with cigarettes. These definitions for levels of confidence in risk reduction were adapted from the Manual for American College of Cardiology and the American Heart Association Guideline Writing Committees.

**Low confidence in risk reduction:** Evidence is insufficient to assess risk reduction and/or there is general agreement of the DTR Committee that the weight of evidence indicates that ST does not reduce the risk of disease.

**Moderate confidence in risk reduction:** The weight of evidence supports risk reduction, but critical evidence is lacking and/or there is general agreement of the DTR Committee that available data are inconsistent about whether ST reduces the risk of disease compared with cigarettes.
High confidence in risk reduction: Available evidence is sufficient to assess risk reduction and there is general agreement of the DTR Committee that the weight of evidence indicates that ST reduces disease risk compared with cigarettes.

- LSRO’s confidence is high that, compared with cigarettes, ST presents a lower risk of LC and COPD.
- LSRO’s confidence is moderate that ST reduces the risk of CVD and pharyngeal, laryngeal, esophageal, and gastric cancer compared with cigarettes.
- LSRO’s confidence is moderate that US and Swedish ST products present a lower risk of oral cancer than cigarettes.
- LSRO’s confidence is moderate that some international STs present a higher risk of oral cancer than cigarettes.
- LSRO’s confidence is low that, compared with cigarette smoking, ST use reduces the risk of pancreatic and bladder cancer.
- LSRO’s confidence is high that, compared with cigarettes, ST presents a significantly lower risk of all-cause mortality.

Because LSRO has a high level of confidence that compared with smoking, ST reduces risk of LC and COPD (which together account for approximately 49% of smoking-attributable deaths), a moderate level of confidence that ST reduces risk of CVD (which accounts for approximately 32% of smoking-related deaths), and a high level of confidence that ST reduces risk of all-cause mortality, LSRO’s overall confidence that ST is a reduced-risk product compared with cigarettes is high.

Objective 2: Identify and characterize the critical characteristics of ST products that contribute to the evaluation of risk

One component of LSRO’s charge was to identify critical characteristics that contribute to a characterization of risk. In the scientific literature, distinctions have been made among STs on the basis of:

- Geographic origin of tobacco,
- Fermentation of tobacco,
- Heat treatment of tobacco,
- Chemical composition of the ST,
- Manufacturing conditions for the ST,
- Refrigeration of the ST after production,
- Genotoxicity and cytotoxicity assays,
- Animal toxicity studies,
- Biomarker of exposure and biological effect studies,
- Health effects assessment (epidemiological studies and clinical trials), and
- Behavior related to the use of STs.

LSRO concluded that existing information is insufficient to determine the critical factors that influence risk associated with ST products. Consequently, LSRO utilized information from preclinical and clinical studies, health outcomes assessment, and behavioral studies to compare risks of cigarettes and STs. LSRO placed the highest weight of evidence on health outcomes assessment studies and behavioral studies, intermediate weight of evidence on clinical studies, and lowest weight of evidence on preclinical studies. LSRO categorized ST products into Swedish snus, traditional US STs, newer STs, and international STs, because data in the scientific literature were generally organized in this way.

Objective 3: Develop an independent consensus opinion as to whether sufficient evidence exists to stratify categories of ST products according to risk

To determine whether data were adequate to allow stratification of ST products according to risk, LSRO focused on the types of STs that are primarily used in the US and Sweden: moist snuff, loose-leaf chewing tobacco, plug/twist chewing tobacco, dry snuff, and Swedish snus. LSRO emphasized these STs because of their importance (or potential importance) to the US market and because they are associated with the most data with which to evaluate evidence related to health risks.

Preclinical Studies

Product Characteristics

Differences among STs may include the species of tobacco incorporated in the product, aging of the tobacco, fertilization practices during tobacco growth, pesticide use and soil conditions, and climate during tobacco growth. The tobacco cutting size; moisture content; pH; added ingredients; convenience of use; and aspects of the manufacturing process, such as whether tobacco is fermented, are other ways in which STs may differ. For example, tobacco in Swedish snus is heat-treated, whereas tobacco in US moist snuff products is fermented in closed containers under controlled conditions for weeks, which permits survival of bacteria and other microorganisms. Comparing STs may be difficult because manufacturing of some international products is not standardized and is poorly characterized. Post-production handling of STs also differs. Swedish snus is refrigerated until used, whereas other STs are not typically refrigerated after production.
Chemical Composition

The composition of ST products is heterogeneous. As noted previously, some international ST products are manufactured non-commercially, via non-standardized processes, which is likely to increase the variability of their chemical composition.

Levels of some ST constituents (e.g., TSNAs, nicotine, polycyclic aromatic hydrocarbons, heavy metals, and radionuclides) are routinely measured, and STs differ in levels of these substances. As an example, Swedish snus contains lower levels of TSNAs than does US moist snuff, and hard snuff products contain lower TSNA levels than does Swedish snus. However, TSNA levels in US moist snuff products have decreased. International STs generally have higher levels of TSNAs than do Swedish snus and US STs, with Sudanese toombak having up to 100 times the levels in US and Swedish STs. The biological significance of these differences in product constituent levels remains a question because the extent to which TSNAs and other ST constituents alter human health risk has not been determined.

One ST manufacturer, Swedish Match, has set a quality standard for its products called GothiaTek® that defines maximal permissible limits for “suspected harmful elements,” and some other tobacco companies appear to be voluntarily conforming to that standard. In general, there is no rationale for inclusion or exclusion of ST analytes, nor is it known whether use of a product with reduced levels of one constituent would reduce risk compared with another product with or without lower levels of the constituent.

Certain newer ST products—e.g., “hard snuff,” which is compressed, powdered, low-nitrosamine tobacco lozenges designed to dissolve in the mouth without expectoration—are more convenient to use than traditional ST products². This increased convenience has the potential to increase frequency of tobacco consumption by allowing discreet ST use.

The pH of the various ST brands varies, resulting in differences in availability of nicotine for uptake by ST users. The pH of one Swedish snus product was higher than that of 5 traditional US moist snuff products, which indicated a higher proportion of unbound nicotine and suggested more efficient nicotine uptake than that for moist snuff products. Recently, US tobacco companies have developed what they have called snus products, but these products do not have all the attributes of Swedish snus. One recently marketed US snus

² Traditional ST products refers to US ST products other than hard snuff and US snus products that have recently been developed.
product is controversial because it has a lower pH than Swedish snus, which reduces nicotine availability for uptake and potentially limits its ability to satisfy individuals consuming it, in particular, tobacco users who are attempting to switch from cigarettes to ST.

**In vitro Assays**

Limited data suggest that Swedish snus and hard snuff products are not mutagenic in *Salmonella* mutagenicity assays. In contrast, traditional US chewing tobacco, moist snuff, and dry snuff, and many Indian and Saudi Arabian ST products (e.g., betel quid with tobacco, gutkha, shammah, zarda, and mishri) increase the number of revertants in *Salmonella typhimurium*. Some international products also increase the frequency of chromosomal aberrations and number of micronucleated cells (e.g., betel quid with tobacco and lime, mishri, and Indian dry snuff) as well as induce sister chromatid exchange. International products also decrease expression of the DNA repair enzyme methylguanine-DNA methyltransferase. There are currently no published *in vitro* studies on US snus products.

**Animal Studies**

Studies have shown some STs to be carcinogenic, but carcinogenicity studies are not available for all STs. Extracts of the Indian ST betel quid led to formation of murine lung tumors and increased squamous cell carcinoma of the cheek pouch of golden hamsters. At present, there are no published animal studies about US snus products.

**Clinical Studies**

Limited biomarker information is available comparing exposure from and biological effects of the use of different STs. Swedish snus users had higher nicotine levels than did users of some newer STs. US snus products delivered lower amounts of nicotine than cigarettes. Studies have reported differences in TSNA metabolite levels for ST users. Urine and saliva of toombak users had extremely high TSNA metabolite levels. At present, there is limited information about US snus products. No firm conclusions can be reached from biomarker studies about risk for users of different STs.

**Health Outcomes Assessment**

(Epidemiological Studies and Clinical Trials)

More well-conducted studies exist for Swedish snus than for other STs. More data are also available for some international STs, such as betel quid with tobacco, than for others. Epidemiological data about hard snuff and non-
Swedish *snus* products have not been published. The disparity of available data also adds to the complexity of a comparative risk assessment of STs. Because all ST products reduce the risk of LC and COPD compared to cigarettes, the effects of ST on risk of these diseases cannot be used to discriminate between the health risks of different products, and other health outcomes must be utilized for this purpose.

Although some evidence is available that ST users have increased CVD risk compared with non-users, these data do not allow for a distinction to be made among ST products with regard to their risks. Studies from India, Pakistan, and Sudan report a substantially increased risk of oral cancer for users of betel quid with tobacco, chewing tobacco, *toombak*, and *shammah* compared with non-tobacco users. Epidemiological studies indicate that Swedish *snus* has a substantially lower risk of oral cancer than do some international products and that US ST products may confer an intermediate level of risk. Available data do not allow a distinction to be made between STs with regard to risk of all-cause mortality.

**LSRO’s Stratification of Smokeless Tobacco Products According to Risk**

The following reflects LSRO’s stratification of ST products. Epidemiological studies, which are the most heavily weighted of the available studies, suggest that Swedish *snus* may be the least harmful of STs and convey the lowest disease risk of STs. Preclinical studies, such as genotoxicity and cytotoxicity assays and animal studies, which LSRO weighted less heavily than epidemiological studies, also support this idea. LSRO concluded that traditional US STs confer an intermediate risk to ST users. Some data indicate the potential for differences in risk among US STs; this area would benefit from additional research. Preclinical data suggest that some newer STs such as hard snuff products may be less toxic than some moist snuff products; however, no epidemiological studies of these and US *snus* products have been conducted to determine the risk of disease. Swedish *snus*, traditional US STs, and some international products are manufactured commercially and may have a less variable composition than the international STs that are manufactured under non-standardized and poorly-characterized conditions. Less information is available about international products than is available for Swedish *snus*. In general, LSRO considers international ST products to be the most harmful STs on the basis of epidemiological and other studies. LSRO’s stratification of STs is based on limited available information and could change with additional studies.
Future Directions

During the course of the DTR project, LSRO identified several areas in which additional research, application of standardized methods, and use of established guidelines are warranted. LSRO identified the need for rigorous characterization of the chemical composition of ST products using standardized, state-of-the-art analytical methods. Use of available reference ST products and development of a reference ST for Swedish *snus*-like products are recommended. In addition, ISO guidelines for STE preparation and for genotoxicity and cytotoxicity testing according to International Conference on Harmonization and US Food and Drug Administration Guidelines should be applied. In animal studies, daily doses of the test substance should be given for a minimum of 1 year. Development of newer, less invasive, validated animal models of disease for ST studies is also recommended.

LSRO identified a need for additional studies to identify relevant biomarkers of nicotine exposure in ST users. Further investigation of the interaction between constituents of ST and saliva is also needed. Additional studies of the relationship between ST consumption and risk of CVD, oral cancer, and other diseases would allow better characterization of disease risk. Additional, well-designed epidemiological studies of ST products in use today and international products would also contribute to improved understanding of the relationship between ST use and disease. Additional research on health effects of dual use of STs and cigarettes in the US would provide insight into risk associated with ST use. ST is used as a smoking cessation aid, but randomized clinical trials evaluating its efficacy in this capacity are required. Also needed are information about whether ST product design and flavoring affect tobacco initiation rates in youth and studies of the effects of STs on smoking initiation. Population studies detailing patterns of ST use among various demographic groups are also recommended.
1.1 PROJECT OBJECTIVES

The Differentiating Tobacco Risks (DTR) project was a case study that utilized the risk assessment framework developed during the Life Sciences Research Office (LSRO) Reduced-Risk Review Project (RRRP) and published in the report entitled *Scientific Methods to Evaluate Potential Reduced-Risk Tobacco Products* (Life Sciences Research Office, 2007b). The purpose of the RRRP was to develop an approach to scientifically evaluate and assess the risk-reduction characteristics of potential reduced-risk tobacco products. Specific goals included:

- Identification of the types of information needed to assess risk reduction;
- Establishment of criteria to evaluate the scientific information, including identification of comparison products; and
- Development of a process for review of the scientific information.

For the DTR Project, the RRRP framework was used to:

- Develop an independent consensus opinion as to whether smokeless tobacco (ST) products meet the criteria for reduced-risk (or reduced-harm) products compared with cigarettes;
- Identify and characterize the critical characteristics of ST products that contribute to the evaluation of risk; and
- Develop an independent consensus opinion as to whether sufficient evidence exists to stratify categories of ST products according to risk.

The DTR project was developed under a contract between Philip Morris USA, Inc., and LSRO. An expert advisory panel, the DTR Committee, provided oversight and direction for the development of this report. From this point forward, LSRO, its staff, and the DTR Committee are collectively referred to as LSRO.
1.2 AREAS OF INITIAL CONSENSUS

LSRO articulated five areas of initial consensus at the onset of the RRRP that were accepted by the DTR Committee (Life Sciences Research Office, 2007b):

- Smoking is the major cause of preventable death and disease in the US;
- The best approach is to never start smoking;
- The best action for those who smoke is to quit immediately;
- Many smokers will not or cannot quit permanently, and some adolescents will begin to smoke no matter what information is provided or what obstacles are introduced; and
- An ST tobacco product that reduces disease risk for smokers who exclusively switch might benefit some subpopulations of smokers.

1.3 DIFFERENTIATING TOBACCO RISKS PROJECT

Cigarette smoking is the leading preventable cause of death in the US (U.S. Department of Health and Human Services, 2004). Despite the well-known risks of smoking, approximately one-fifth of US adults continue to smoke (Centers for Disease Control and Prevention, 2004). Quitting is difficult; nearly two-thirds of smokers want to stop, and more than one-third will try to quit, but less than 3% of those who attempt to quit will be successful (Centers for Disease Control and Prevention, 2004; Henningfield et al., 1998).

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 lung disease (COPD) (90,600 deaths/year), and cardiovascular disease (CVD) (138,000 deaths/year) (Centers for Disease Control and Prevention, 2005b). Doll and Peto (1981) observed, “No single measure is known that would have as great an impact on the number of deaths attributable to cancer as a reduction in the use of tobacco or a change to the use of tobacco in a less dangerous way.” Exclusive use of ST products instead of cigarettes is one possible change. ST products are used without combustion, which would thus remove both direct respiratory tract exposures to combustion products and environmental tobacco smoke exposures. Exclusive use of ST instead of cigarettes would virtually eliminate two of the major smoking-attributable diseases, LC and COPD, and could cut smoking-attributable deaths by half (Accortt et al., 2002). However, ST-related risk may exist for CVD and other diseases, and it continues to be evaluated by the scientific and medical communities.
Risk reduction for non-users of cigarette tobacco products has already been demonstrated. In Sweden, many smokers replaced cigarettes with Swedish snus, a type of ST. Swedish epidemiological studies indicate that exclusive snus use is associated with reduced mortality and disease risk compared with cigarette smoking. The applicability of these data to widespread use of ST in the US, however, remains uncertain because of several factors:

- **Snus** is not the predominant product used in the US. Instead, individuals choose from a wider array of ST products that differ in tobacco composition, manufacturing process, and use patterns. Therefore, the realized risk reduction may not be the same as that for snus.
- The Swedish population is more homogenous than the US population, and use of snus is more culturally acceptable in Sweden than it is in the US.
- Although it is undeniable that for an individual the replacement of tobacco smoking by ST use would decrease the incidence of smoking-related disease, the population effects of aggressively introducing ST into the US market are uncertain. There are some who maintain that promotion of ST might stimulate individuals, especially young people, to begin the habit of tobacco consumption and maybe even smoking. Some people might become dual users.

Education of the public has reduced smoking in the US. This report addresses risk to smokers who switch to ST. Limited evidence suggests the reduction and possible elimination of LC and COPD when ST (Swedish snus or a US ST product) is used exclusively in place of cigarettes. However, switching from cigarettes to ST will not eliminate all risk. Residual risk of LC, COPD, and CVD remains in former smokers, and some new risks associated with ST use are likely unknown. The goal is to reduce the risks of tobacco use in individuals who cannot or will not quit.

### 1.4 SMOKELESS TOBACCO PRODUCTS

#### 1.4.1 Definitions

##### 1.4.1.1 World Health Organization Scientific Advisory Committee on Tobacco Product Regulation

ST products are defined as those that do not require combustion or production of tobacco aerosol (smoke) by other means at the time of use (World Health Organization Scientific Advisory Committee on Tobacco Products Regulation, 2003). The Core Committee report of the RRRP also utilized this definition (Life Sciences Research Office, 2007b).
1.4.1.2 Code of Federal Regulations

The US Code of Federal Regulations (1999) defined ST as any product that consists of cut, ground, powdered, or leaf tobacco that contains nicotine and is intended to be placed in the oral cavity.

1.4.2 Selection of Smokeless Tobacco Products

The DTR Committee chose to emphasize categories of ST that are most common in the US and Sweden: moist snuff, loose-leaf chewing tobacco, plug/twist chewing tobacco, dry snuff, and snus (Swedish moist snuff). The US Federal Trade Commission (FTC) reports domestic sales of four categories of ST products: moist snuff (including snus), dry snuff, loose-leaf chewing tobacco, and plug/twist chewing tobacco. Sales of the last three categories declined between 1986 and 2005 (Table 1-1). In the same period, moist snuff sales rose dramatically and in 2005 accounted for 85.2% of US ST sales (Federal Trade Commission, 2007). Moist snuff is the only ST product that has shown long-term gains in consumption (Capehart, 2005). Use of moist snuff increased 57% between 1980 and 1989 (Connolly et al., 1992).

Table 1-1. US Smokeless Tobacco Sales (in pounds sold)

<table>
<thead>
<tr>
<th>Year</th>
<th>Dry Snuff</th>
<th>Loose-leaf Chewing Tobacco</th>
<th>Plug/twist Chewing Tobacco</th>
<th>Moist Snuff</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>8,110,168</td>
<td>65,697,634</td>
<td>8,835,200</td>
<td>36,135,332</td>
</tr>
<tr>
<td>2005</td>
<td>2,402,904</td>
<td>36,410,287</td>
<td>1,712,921</td>
<td>75,670,894</td>
</tr>
<tr>
<td>% change</td>
<td>–70%</td>
<td>–45%</td>
<td>–81%</td>
<td>+109%</td>
</tr>
</tbody>
</table>


1.4.3 Overview of Smokeless Tobacco Product Types and Commercial Brands

US and Swedish ST products are the primary focus of this report. A discussion of ST products other than those primarily used in the US and Sweden—i.e., international ST products—can be found in Chapter 8. Although fewer types of ST products are used in the US and Sweden, ST is not a homogeneous category (Table 1-2). Three types of ST (powdered dry snuff, loose-leaf chewing tobacco, and moist snuff), which differ with respect to their manufacturing processes and product characteristics, are primarily used in the US.
1.4.3.1 Powdered dry snuff

Dry snuff is made from fermented, fire-cured tobacco that is pulverized into a powder and sold in small metal or glass containers (WHO Study Group on Tobacco Product Regulation (Who Study Group on Tobacco Product Regulation [TobReg]), 2004). Brand names include Al Capone® Powder, Conwood® (Conwood) and Swisher® (Swisher). Nasal inhalation of dry snuff was widely practiced in 17th and 18th century Europe, and manufacturers in Germany and the UK still provide an array of powdered dry snuff (Rodu & Godshall, 2006). However, use of dry snuff in the US, primarily by Southern women (Winn & Pickle, 1986), is declining, with sales falling approximately 70% in the last 15 years (Economic Research Service, 2006).

1.4.3.2 Loose-leaf chewing tobacco

Loose-leaf tobacco (i.e., chewing tobacco) is a special product made for the US market that is usually consumed by men. Large quantities are used, which produces a bulge in the user’s cheek and generates a large amount of saliva that is usually expectorated. Use in the US has dropped by approximately 40% in the last 15 years (Economic Research Service, 2006). Three types of loose-leaf chewing tobacco are produced:

- Loose-leaf tobacco is made from shredded leaf tobacco that is coated with sweet flavoring solutions and packaged in foil-lined pouches. Brand names include Red Man®, Beech-Nut® Wintergreen, and Taylors Pride®.
- Twist tobacco is made from loose-leaf tobacco that is twisted into pliable, rope-like strands that are dried. Typically, no flavoring or sweetener is added. Brand names include Conwood®, R.C. Owen®, and R.J. Reynolds®.
- Plug tobacco is a compressed form of loose-leaf tobacco that is molded and pressed to make a flat bar. Most plug tobacco is flavored and sweetened with licorice. Two forms exist, dry and moist. Brand names of moist plug tobacco include Red Man® Moist Plug, Totems®, RJ Gold®, Levi Garrett® Plus, and Taylors Pride®. Brand names of dry plug tobacco include Days Work®, Conwood®, and Brown & Williamson®.

1.4.3.3 Moist snuff (including snus)

Air-cured and fire-cured dark tobaccos are finely cut or ground to produce moist snuff (Rodu & Godshall, 2006). Some products and brands are flavored. Moist snuff is packaged in round containers, and users compress a pinch or a prepackaged sachet (also known as a portion bag or pouch) between the upper lip and gum. This method produces less salivary gland secretion and no expectoration.
### Table 1-2. US Smokeless Tobacco Brand Names, Geographic Origins, Constituents, and Methods of Use

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Brand Names (Manufacturers)</th>
<th>Geographic Origin</th>
<th>Constituents</th>
<th>Method of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loose-leaf chewing</td>
<td>Red Man®, Red Man® Golden Blend, Red Man® Select, Granger®, Work Horse® (Swedish Match);</td>
<td>US</td>
<td>Leaf tobacco, sweetener, and/or licorice</td>
<td>Chewed or held between cheek and lower lip. Saliva is spit or swallowed. Piece of tobacco is 0.75–1 inch.</td>
</tr>
<tr>
<td>tobacco</td>
<td>Scotten®, Dillon®, Levi Garret®, HB Scott®, Taylors Pride®, Red Fox® (Conwood); Beech-Nut®</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Regular, Beech-Nut® Wintergreen, Beech-Nut® Spearmint (National); Chattanooga Chew® (Swisher)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plug chewing</td>
<td>Red Man® Moist Plug, Totems®, RJ Gold®, Days Work® (Swedish Match); Levi Garrett® Plus,</td>
<td>US</td>
<td>Enriched tobacco leaves, fine tobacco, sweetener,</td>
<td>Chewed or held between cheek and lower lip. Saliva is spit or swallowed.</td>
</tr>
<tr>
<td>tobacco</td>
<td>Taylor’s Pride®, Conwood® (Conwood); Brown &amp; Williamson® (Brown &amp; Williamson)</td>
<td></td>
<td>and/or licorice</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Twist chewing</td>
<td>Conwood® (Conwood), R.C. Owen® (R.C. Owen), R.J. Reynolds® (R.J. Reynolds)</td>
<td>US</td>
<td>Tobacco, tobacco leaf extract</td>
<td>Chewed or held between cheek and lower lip. Saliva is spit or swallowed.</td>
</tr>
<tr>
<td>tobacco</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry snuff</td>
<td>Al Capone® Powder, Conwood® (Conwood); Swisher® (Swisher); US Tobacco® (US Smokeless Tobacco); Brown &amp; Williamson® (Brown &amp; Williamson)</td>
<td>US, UK, India</td>
<td>Tobacco, sodium chloride</td>
<td>Pinch typically held between lip and gum or cheek, or inhaled into nostrils.</td>
</tr>
</tbody>
</table>

1.4.3.3.1 Moist snuff, US

Approximately 3% of Americans, or 7.7 million individuals, were ST users in 2005 (Hatsukami et al., 2007). Different tobacco leaf cutting sizes yield three types of moist snuff that are popular in the US (i.e., fine cut, coarse cut, and long cut). US brand names include Copenhagen®, Skoal® and Skoal® Bandits, Kodiak®, and Hawken®. Moist snuff is now the most popular form of ST in the US; sales of this product doubled in the past 15 years (Economic Research Service, 2006).

1.4.3.3.2 Moist snuff, Sweden

In northern Europe, especially Sweden, the per capita consumption of oral snuff is among the highest in the world. Approximately 18% of Swedish men use oral snuff (Foulds et al., 2003). Prepackaged Swedish snus sachets (i.e., 0.5 or 1 g of tobacco packed in porous papers) are popular and are available in various flavors. Swedish brand names include Catch®, Exalt®, General®, and Timber Wolf®.

1.4.3.4 Hard snuff

New classes of ST products have been introduced that do not fit any traditional ST category. Ariva® and Stonewall® are compressed, powdered low-nitrosamine tobacco lozenges designed to dissolve in the mouth without expectoration (Star Scientific, Inc., 2006; U.S. Food and Drug Administration, 2002).

Because STs differ widely in terms of tobacco type, manufacturing process, and constituents, risk reduction associated with one product may not occur to the same extent with all ST tobacco products.

1.5 REPORT ORGANIZATION

Chapter 2 of this report considers the use of risk assessment in evaluating smoking versus ST use. Chapter 3 details the manufacturing and chemical composition of ST products. Chapters 4 and 5 review preclinical and clinical studies, respectively, performed with ST products or their extracts. Behaviors associated with ST use are discussed in Chapter 6. Chapter 7 examines adverse health effects associated with ST use. International products are reviewed in Chapter 8. Chapter 9 assesses the risks of ST use relative to cigarette smoking. Chapter 10 considers the relative risks associated with use of different categories of ST products. Research needs are described in Chapter 11.
2.1 INTRODUCTION

Cigarette smoking has been demonstrated to cause major harm to human health, including lung cancer, chronic obstructive pulmonary disease, and cardiovascular disease (Centers for Disease Control and Prevention, 2004). In addition, it is a significant cause of premature death of smokers (Centers for Disease Control and Prevention, 2005b). The best way to avoid harm from smoking is to never start, or once started, to quit. However, this method of risk management is neither possible for nor desired by many individuals. Therefore, this report considers an alternative strategy for potential risk mitigation. Evidence exists that use of smokeless tobacco (ST) instead of smoking cigarettes provides a less harmful option for those who cannot or will not quit smoking. However, a large variety of ST products are available worldwide, and not all ST products present the same degree of risk (see Chapter 8) (World Health Organization, 2004). This report considers primarily ST products that are manufactured in a consistent, stable, and repeatable way and that are used primarily in the US or Sweden, for which the most data are available. These products include Swedish snus, moist snuff, dry snuff, loose-leaf chewing tobacco, and plug/twist tobacco. International ST products, defined in this report as those products other than those primarily used in the US and Sweden, are also discussed.

The consideration of risk in this report describes a process of risk assessment to distinguish risks of smoking versus ST use as well as to differentiate risks among ST types. This comparative risk assessment uses a weight of evidence approach to qualitatively integrate information from hazard identification, exposure assessment, biological effects assessment, and health outcomes assessment (epidemiological and clinical studies) into a risk characterization. In addition to risk assessment, risk management and risk communication are briefly discussed. Risk assessment, risk management, and risk communication together form the trilogy often denoted as risk analysis (Byrd & Cothern, 2000). The methods and approaches for this process constitute the heart of this chapter.
2.2 RISK ASSESSMENT

When a health hazard has been well studied and little ambiguity remains, scientists can draw conclusions with some confidence. Such is the case with smoking. However, at times, data are sparse and much is unknown about a hazard, yet decisions must be and are being made. Such is the case with ST. Risk assessment in this situation can be a valuable tool to support decision making (Byrd & Cothern, 2000).

Risk assessment has been described as “…a systematic approach to organizing and analyzing scientific knowledge and information for hazardous activities that might pose risks ….” (National Research Council, 1994). This approach includes the process of identifying potential harm(s) that can come from a particular course of action and estimating the severity of harm on the basis of exposure, knowledge of its biological effects, and human health outcomes. When possible, quantitative risk assessment is preferred. However, when few data exist or they are of poor or variable quality, qualitative risk assessment can be useful. Risk assessment is a process that analyzes hazard identification, exposure, biological effects, health outcomes, and risk characterization (Life Sciences Research Office, 2007b). This study was designed to provide the systematic organization and analysis necessary for differentiating the risks of various tobacco products.

2.3 COMPARATIVE RISK ASSESSMENT

This assessment does not aim to determine whether cigarette smoking or ST use is harmful to human health, for there is ample evidence to demonstrate their harm (Centers for Disease Control and Prevention, 2004; Rodu & Godshall, 2006; Scientific Committee on Emerging and Newly Identified Health Risks, 2007). Rather, this study compares known categories of hazardous products to ascertain whether one is more harmful than another. Within the category of ST, various types—moist snuff, loose-leaf chewing tobacco, plug/twist chewing tobacco, dry snuff, Swedish snus, and international ST products—are compared to determine whether health outcomes associated with use of the various ST products differ. This constitutes comparative risk assessment.

Comparative risk assessment provides a systematic way of looking at hazards that pose different types and degrees of risk. It combines information on inherent hazards of tobacco use, exposure levels, biological effects, health outcomes, and population characteristics to predict health effects (World Bank Group, 1999). Many different types of ST are available; therefore, risks
to be evaluated vary considerably. A consistent approach is necessary for understanding risk. This approach involves putting risks in a rank order, so that risks of one product are compared with those of another similar, but not identical, product.

2.4 HAZARD IDENTIFICATION

Hazards are identified on the basis of product characteristics, their chemical analyses, and a consideration of the way in which the product is used. In this report, the hazard of smoking is undifferentiated by type or kind of cigarette because much of the harm comes from the combustion products. An approach to risk assessment of cigarettes can be found in an earlier Life Sciences Research Office (LSRO) report (2007b).

ST products differ in many ways, and these differences may influence disease risk. The hazards of ST appear to be more related to the type of product used. Each type of ST has its own unique physical characteristics and chemical composition. The chemical constituents are greatly influenced by the variety of tobacco, where it is grown, and the processing after harvest (Idris et al., 1998a). For example, different curing methods (flue, air, and smoke), leaf fermentation, and many other processes change the nature of the final ST product (Davis & Nielsen, 1999). To properly identify the hazards of ST products, a standardized method of extracting chemicals from ST products is required. Without such standardization, chemical analyses of ST products cannot be precise. Use of various extraction methods (e.g., aqueous, alcohol, synthetic saliva) may give a fuller understanding of the compounds to which ST users are exposed.

ST products have different characteristics that may alter user exposures. Certain moist snuff products (e.g., snus) are packaged in sachets that eliminate direct contact between tobacco and the users’ mucous membranes. Conditions of use, such as swallowing rather than spitting, may also expose the digestive system to hazardous materials. Some STs are fermented or partially fermented. Evidence exists that fermentation increases tobacco-specific nitrosamine (TSNA) levels, which may be harmful to human health (Baker, 1999; Brunnemann & Hoffmann, 1991).

Because multiple variables are associated with tobacco growing conditions, processing, production, and use, careful understanding and documentation of these details related to each ST product is essential for clear and unambiguous assessment of risk.
2.5 EXPOSURE ASSESSMENT

Exposure assessment evaluates the actual exposure to the individual using the product by measuring of a chemical constituent (or its metabolites). Thus, exposure assessment follows from a full analysis of chemicals found in ST products. For each ST constituent, it is important to gather information on the nature of toxicity. For example, two types of compounds (TSNAs and polycyclic aromatic hydrocarbons), determined to be carcinogenic by the International Agency for Research on Cancer, occur in many STs (International Agency for Research on Cancer, 2007b).

Cigarette smoking is associated with a change in chemical constituents (e.g., cotinine) in the blood and other body fluids (Galeazzi et al., 1985). Exposure assessment must define the actual exposure to the individual. Whether compounds present in the tobacco actually reach cells of the aerodigestive tract or enter the circulation of the user is important. The quantity or delivery dose is important. For example, some types of snus products are presented to the user in dose-controlled packets. Other ST products are loose in a tin, and the user scoops up a teaspoon or a tablespoon, more or less, for use. Therefore, the actual dose may vary from use to use by the same individual and certainly varies from one person to another. In vivo measurement of tobacco metabolites becomes essential to ensure accurate measurement of exposure.

Even if a chemical is identified in an ST product, it may not be available to the user. For example, salivary pH and composition may or may not influence release of the chemical to the user. Also to be considered are individual variations in physiology that cause the same ST type and quantity to produce different exposures in different people. In addition, evidence suggests that a given user does not respond to the same ST product in the same way each time the product is used (Rodu & Godshall, 2006).

2.6 BIOLOGICAL EFFECTS ASSESSMENT

Cigarette smoking is known to have various effects on users’ physiology. For example, increases in heart rate, systolic blood pressure, and plasma fibrinogen occur (National Institutes of Health, 1997). Values for smokers and ST users or for users of diverse ST products may be similar or may be qualitatively or quantitatively different (Ebbert et al., 2004; Kotlyar et al., 2007). The significance of such differences must be evaluated.
In vitro tests for genotoxicity and cytotoxicity of such compounds must be included in a biological effects assessment. Animal studies that shed light on possible human health effects may also be useful, although results of certain animal studies appear to overestimate the cancer incidence in humans (Grasso & Mann, 1998).

2.7 HEALTH OUTCOMES ASSESSMENT

Tobacco-related health outcomes in individuals or in populations cannot be discerned from short-term studies. Rather, health outcomes become observable after long-term tobacco use (Centers for Disease Control and Prevention, 2005b; U.S. Department of Health and Human Services, 2004). Such evidence originally revealed the health hazards of smoking (Doll & Hill, 1950; Royal College of Physicians, 1962; U.S. Department of Health Education and Welfare, 1964). For that reason, epidemiological studies comparing smokers with ST users are crucial. Epidemiological studies are quantitative and can provide rigorous data for comparisons between and among users of various categories of tobacco. However, such studies can vary enormously in quality, and each study included in the assessment must be carefully analyzed. Each study should be individually evaluated for quality on the basis of sample selection, control for confounders, sample size, and adequate definitions of cases to be included. There are other considerations as well, and a review of any good epidemiology textbook can provide further guidance for such evaluation (Mausner et al., 1985; Rothman, 2002).

Certain unique factors must also be considered when reviewing ST literature. Studies that place all STs in a single category are less useful than those that specify the ST type used by study participants. Manufacturing methods vary and tobacco leaf composition may change from year to year; thus, ST products are not identical over time. It is best when studies specify the composition of the product being evaluated. Some ST users are also smokers, and knowledge of this is important in health outcomes assessment. When cancer in ST users is being investigated, confounders including socioeconomic status, nutritional status, and behaviors such as concurrent alcohol use should always be part of the analysis. When carefully used and properly interpreted, epidemiological data can provide rigor and strength for risk assessment of ST product use.
2.8 RISK CHARACTERIZATION

Risk characterization is the final step in risk assessment. It integrates data and information, both quantitative and qualitative, from exposure assessment, biological effects assessment, and health outcomes assessment (epidemiology). It is developed by using a weight of evidence approach in which the strength of the studies assessed is considered (Atkins et al., 2004; Life Sciences Research Office, 2007b). Weight of evidence requires a clear statement of assumptions, uncertainties, and ways in which the information is weighted. What is known and what is unknown for each comparison must be discussed. The degree of confidence in the conclusions must be specified.

The risk characterization must be delivered in a clear, well-organized format. It should present the best available information for decision making related to managing the risk to human health for those who cannot or will not quit smoking. It should point to the range of risks presented by different categories of ST, with emphasis on those conferring the smallest and the greatest potentials for harm. The nature of the harm must be clearly specified as well. The goal is to clearly present information about the comparative risks associated with smoking versus use of ST products and to outline comparative risks among ST products.

2.9 RISK COMMUNICATION AND RISK MANAGEMENT

The complete risk assessment for each ST type must be communicated for technical and non-technical audiences. The technical audience includes scientists, physicians, and policy makers. These technical readers influence decisions made about future research, health advice for tobacco users, and government policies, respectively. The non-technical audience includes smokers and the users or potential users of ST. Good communication about risk can have an impact on all these audiences.

Risk management may take the form of information dissemination by government or, more forcefully, development of regulations to modify and/or control tobacco use. The actual tobacco user is the ultimate decision maker in health mitigation issues regarding tobacco use. These individuals deserve the best assessments possible to guide them as they make decisions.
2.10 POPULATION EFFECTS

If careful risk assessment demonstrates that certain ST products are safer than smoking, additional considerations of great import arise. Can some perceived “safety” or lessened hazard of STs induce non-tobacco users to become users? Can this information convince smokers who are trying to quit to switch to STs instead of abstaining from tobacco use? Would smokers not quit but instead also start to use ST? If such things were to take place, would the US population as a whole suffer further tobacco-related health deterioration? Careful research is necessary to answer these questions.

2.11 CONCLUSIONS

Studies of STs in the scientific literature are sparse. Epidemiological data on outcome assessments of quality comparable to that available for Swedish snus for each type of ST are unavailable. In such a situation, well-conducted risk assessments can help guide the search for factors that mitigate smoking-related harms as well as guide research toward the development of knowledge related to improving such understanding.
More than 3,000 chemicals have been identified in tobacco leaf, including nicotine, various starches, crude fiber, proteins, reducing sugars, and inorganic compounds (Davis & Nielsen, 1999). Fertilization practices, pesticide usage, and soil and climate conditions where tobacco is grown all influence the final composition of the leaf (Idris et al., 1998a). The chemical composition of the tobacco leaf changes with curing: levels of starch, insoluble proteins, and carotenoids decrease and those of reducing sugars, soluble proteins, ammonia, and amides increase (Davis & Nielsen, 1999). Volatile substances such as fatty acid-derived compounds, terpenoids, and degraded carotenoids impart flavor (Davis & Nielsen, 1999). Maillard products (such as pyrroles and furans), naphthalenes, biphenyls, and phenolics may also be present if the tobacco leaves were cured by using open hardwood fires (Davis & Nielsen, 1999).

Although both cigarettes and smokeless tobacco (ST) share tobacco as a common ingredient, their composition, product characteristics, and chemistry when used differ markedly. Typical US cigarettes are blended from various tobaccos that may include flue-cured, Burley, Oriental, cut-rolled stem, and reconstituted (Fisher, 1999). Moist snuff, the most popular form of ST, is formulated from dark tobacco. Cigarettes are combusted; ST is not. The total number of chemical constituents in cigarette smoke has been estimated to exceed 4,800 (Green & Rodgman, 1996); comparable data for ST are unavailable. Formulation and design characteristics (e.g., sugars, paper porosity, filter design) may also alter the chemical composition of cigarette smoke (Norman, 1999). Although US ST products are generally less complex than cigarettes, different manufacturing methods produce an array of ST products with unique physical and chemical characteristics, which are considered in this chapter.

### 3.1 Tobacco Types, Curing Methods, and Production Processes

Both the type of tobacco and the manufacturing methods used to produce various ST products influence the chemical composition of the final product.
3.1.1 Tobacco Types

Flue-cured Burley and Oriental tobaccos that are used in cigarettes have been subjected to thorough chemical studies. In contrast, less information is available on the chemical composition of dark tobaccos, the primary tobacco type used in ST products. Table 3-1 provides characteristics of various types of tobacco used in ST products.

Table 3-1. Characteristics of Selected Types of Tobacco

<table>
<thead>
<tr>
<th>Tobacco Type</th>
<th>Soil</th>
<th>Characteristics</th>
<th>Curing</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bright</td>
<td>Sandy loam</td>
<td>Thinner body, light color</td>
<td>Flue</td>
<td>Cigarettes</td>
</tr>
<tr>
<td>Burley</td>
<td>Silt loam</td>
<td>Mild</td>
<td>Air</td>
<td>Blended cigarettes</td>
</tr>
<tr>
<td>Maryland</td>
<td>Fine sandy loam</td>
<td>Mild</td>
<td>Air</td>
<td>Blended cigarettes</td>
</tr>
<tr>
<td>Oriental</td>
<td></td>
<td>Mild</td>
<td>Sun</td>
<td>Blended cigarettes</td>
</tr>
<tr>
<td>Izmir</td>
<td>Rocky, poor, and</td>
<td>Highly aromatic, oily</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>infertile</td>
<td>Mild, pleasant aroma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basma</td>
<td></td>
<td>Mild</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samsun</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark</td>
<td>Clay/silt loam</td>
<td>Dark color, high chlorophyll</td>
<td>Fire</td>
<td>Dry snuff, moist snuff, cigars</td>
</tr>
<tr>
<td></td>
<td></td>
<td>content</td>
<td>Air/sun</td>
<td>Snus, chewing tobacco, plug chewing tobacco</td>
</tr>
</tbody>
</table>

Adapted from Davis and Nielsen (1999).

aThe species for all types is *Nicotiana tabacum*.

3.1.2 Curing and Production

Although many manufacturers consider their methods to be proprietary, the production of ST products includes certain general steps (Wahlberg & Ringberger, 1999):

- Selection of cured leaf tobaccos suitable for the end product;
- Post-curing processing of the leaf;
- Leaf disintegration (*i.e.*, stripping, threshing, cutting, or grinding);
- Blending of tobaccos;
- Processing of blended tobacco; and
- Finishing of product, with possible inclusion of functional additives such as flavors and stabilizers.

For a brief summary of the manufacturing processes of selected ST products, see Table 3-2.
### Table 3-2. Manufacturing Processes of US Smokeless Tobacco Products

<table>
<thead>
<tr>
<th>Smokeless Tobacco Product</th>
<th>Tobacco Used</th>
<th>Curing</th>
<th>Manufacturing Process</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chewing Tobacco</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loose-leaf</td>
<td>Dark</td>
<td>Air</td>
<td>Steamed, threshed or shredded, and flavored (licorice, sweetener)</td>
</tr>
<tr>
<td>Twisted</td>
<td>Dark</td>
<td>Air</td>
<td>Leaf treated with tobacco extract, twisted into a rope, and dried</td>
</tr>
<tr>
<td>Plug</td>
<td>Dark, Burley, Bright</td>
<td>Air</td>
<td>Leaf and leaf fragments sweated, flavored, and pressed into bars</td>
</tr>
<tr>
<td><strong>Snuff</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US moist snuff</td>
<td>Dark</td>
<td>Fire/air</td>
<td>Aged tobacco cut (fine, coarse, or long cut), moistened, fermented, mixed with flavoring and additives, and packaged loose or in small pouches</td>
</tr>
<tr>
<td><strong>Snus</strong></td>
<td>Dark</td>
<td>Air</td>
<td>Ground, pasteurized, mixed with flavoring and additives, packaged loose or in small pouches, and refrigerated</td>
</tr>
<tr>
<td>Dry snuff</td>
<td>Dark</td>
<td>Fire</td>
<td>Fermented, dried, and ground into powder</td>
</tr>
<tr>
<td>Hard snuff</td>
<td>Dark</td>
<td>Air</td>
<td>Heat-treated, ground and mixed with flavoring, packed into pouches or pressed into lozenges</td>
</tr>
</tbody>
</table>

Adapted from Davis and Nielsen (1999), National Cancer Institute and Centers for Disease Control and Prevention (2002), and Rodu and Godshall (2006).

### 3.1.2.1 Loose-leaf tobacco products

Pennsylvania and Wisconsin air-cured tobacco leaves are often used as the raw material for loose-leaf products: loose-leaf, twist, and plug tobaccos. The cured tobacco is subjected to sweating at slightly elevated temperature and then leaves are threshed into flakes and stems are removed. Loose-leaf chewing tobacco is made from shredded leaf tobacco that is coated with sweet flavoring solutions and packaged in foil-lined pouches. Twist tobacco is made from loose-leaf tobacco that is twisted into pliable, rope-like strands that are dried. Typically, no flavoring or sweetener is added. Plug tobacco is a compressed form of loose-leaf tobacco that is molded and pressed into a flat bar. Most plug tobacco is flavored and sweetened with licorice. Two forms of plug tobacco exist, dry and moist.

### 3.1.2.2 Moist snuff

Moist snuff is primarily made from dark tobacco that is fire- or air-cured. Open hardwood fires are used in the fire-curing process (Wahlberg & Ringberger, 1999). The resultant cured leaves are dark and coated with wood smoke condensate (Burton & Kasperbauer, 1985; Leffingwell, 1999;
Leffingwell & Leffingwell, 1988; Mookherjee & Willson, 1988). In the air-curing process, tobacco is not exposed to wood smoke. Tobacco plants are hung in barns and slowly cured for several weeks (Wahlberg & Ringberger, 1999). Cured tobacco is stored for at least one year before being taken into production (Davis & Nielsen, 1999). After it is cut to the desired size, tobacco is fermented with water and additives. Additional substances are added to the tobacco after fermentation in order to stabilize it and to impart flavor (Davis & Nielsen, 1999).

US moist snuff products tend to be blended with both fire-cured and air- or sun-cured tobacco, whereas Swedish snus manufacturers generally select only tobaccos that are air- or sun-cured (Foulds et al., 2003). Recently, US manufacturers have begun marketing snus-like products that are more similar to Swedish products than US moist snuff with respect to the curing and manufacturing methods used (i.e., air-curing and steam pasteurization).

3.1.2.2.1 Moist snuff, Sweden

After tobacco is cured, it is cut into small strips, dried, ground, and sifted before processing. In Sweden, snus production has included a process in which the tobacco is steam pasteurized for 24–36 hours, with temperatures of approximately 100°C reached (Foulds et al., 2003). Added ingredients include 45–60% water, 1.5–3.5% sodium chloride, 1.5–3.5% humectants, 1.2–3.5% sodium bicarbonate, and less than 1% flavoring. The heating process kills bacteria and produces a product that is then packaged into cans and refrigerated during storage. In Sweden, retailers also keep the product in refrigerators.

3.1.2.2.2 Moist snuff, US

The Swedish manufacturing process contrasts with that traditionally used in the US. Tobacco is fermented in closed vessels under controlled conditions for several weeks rather than being heat-treated, thus allowing survival of bacteria and other microorganisms (Wahlberg & Ringberger, 1999). US products are not usually refrigerated by retailers.

3.1.2.3 Smokeless reference products

Reference products representative of three categories of ST are available from the North Carolina Agricultural Research Service: 2S1 (loose-leaf), 1S2 (dry snuff), and 2S3 (moist snuff) (North Carolina Agricultural Research Service, 2005). Tables 3-3 and 3-4 present the formulations and chemical analyses of these products, respectively. The chemical analyses include the amounts of nicotine and moisture, and the pH. Notably, 2S3 moist snuff is
Table 3-3. Reference Smokeless Tobacco Product Formulations

<table>
<thead>
<tr>
<th>Smokeless Tobacco Reference Product</th>
<th>Nicotine</th>
<th>Moisture</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>2S1 Loose-leaf Chewing Tobacco</td>
<td>0.84 ± 0.06</td>
<td>21.99 ± 1.01</td>
<td>5.81 ± 0.05</td>
</tr>
<tr>
<td>1S2 Dry Snuff</td>
<td>1.32 ± 0.04</td>
<td>11.75 ± 0.41</td>
<td>6.29 ± 0.07</td>
</tr>
<tr>
<td>2S3 Moist Snuff</td>
<td>1.34 ± 0.11</td>
<td>54.46 ± 0.22</td>
<td>7.32 ± 0.20</td>
</tr>
</tbody>
</table>

Adapted from North Carolina Agricultural Research Service (2005).

Table 3-4. Chemical Analysis of Reference Smokeless Tobacco Products

<table>
<thead>
<tr>
<th>Smokeless Tobacco Reference Product</th>
<th>Nicotine</th>
<th>Moisture</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>2S1 Loose-leaf Chewing Tobacco</td>
<td>0.84 ± 0.06</td>
<td>21.99 ± 1.01</td>
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<td>1.34 ± 0.11</td>
<td>54.46 ± 0.22</td>
<td>7.32 ± 0.20</td>
</tr>
</tbody>
</table>

Adapted from North Carolina Agricultural Research Service (2005).

*Reported as percent wet weight ± standard error.
representative of US moist snuff products because it is fermented and contains approximately 25% dark fire-cured tobacco (North Carolina Agricultural Research Service, 2005). Currently, no reference product is available for Swedish snus or snus-like products (i.e., pasteurized reference product produced entirely from air- or sun-cured tobacco).

### 3.2 CHEMICAL COMPOSITION

ST chemistry studies have focused on a relatively small number of tobacco constituents that have been arbitrarily selected; these components include tobacco-specific nitrosamines (TSNAs), nicotine, polycyclic aromatic hydrocarbons (PAHs), heavy metals, and radionuclides (Table 3-5). Substances added during the manufacturing process have also been considered.

#### 3.2.1 Nicotine

Tobacco contains a number of structurally related alkaloids (Saitoh et al., 1985). Nicotine, the major alkaloid, accounts for approximately 95% of the total alkaloid content. Nornicotine and anatabine are the two most abundant minor alkaloids, present in roughly equal amounts at 2–3% each. Anabasine generally makes up about 0.3% of the total alkaloid content.

Cigarettes and other tobacco products have been characterized by the US Surgeon General as addictive (U.S. Department of Health and Human Services, 1988). Nicotine is thought to be one of the primary substances in tobacco that leads to addiction. The total nicotine content of ST products is controlled through selection and blending of tobacco, but the pH of ST products plays an important role in nicotine absorption by the body. The dose of nicotine delivered to the user is not completely characterized. Few nicotine pharmacokinetic studies have been conducted on different categories or among different brands of US STs and Swedish snus. Only nicotine in the free-base form is rapidly absorbed through the mucosal membrane, and the proportion of free-base nicotine is determined by pH (Foulds et al., 2003). Levels of free nicotine are controlled during the manufacturing process by adjusting the pH through fermentation, addition of alkaline buffering agents such as sodium carbonate and ammonium carbonate, or alteration of moisture content. pH levels vary in different brands and types of ST products. A comparison of two commercial oral snuff products found that one product with a pH of 5.84 had 1% of nicotine in free-base form, but another product with a pH of 7.99 had 59% of nicotine in free-base form (Brunnemann et al., 2002).
Swedish snus products typically have pH levels in the range of 7.8–8.5 (Andersson et al., 1994). A leading Swedish snus brand had a higher pH than 5 brands of US moist snuff (Brunnemann et al., 2001), which suggests that Swedish snus products deliver nicotine more efficiently. Marlboro snus, a newly marketed US product, has generated controversy because its low pH will reduce nicotine availability for uptake by users and therefore will diminish the satisfaction of users, especially those attempting to switch from cigarettes to ST (Foulds & Furberg, 2008).

Table 3-5. Chemical Composition of Selected Smokeless Tobacco Products

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Brand</th>
<th>Moisture (% w/w)</th>
<th>pH</th>
<th>Nicotine (mg/g)</th>
<th>TSNAs (µg/g)</th>
<th>B[a]P (ng/g)</th>
<th>Cr (µg/g)</th>
<th>Ni (µg/g)</th>
<th>Pb (µg/g)</th>
<th>As (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moist snuff, Sweden</td>
<td>General®</td>
<td>45.84</td>
<td>7.86</td>
<td>15.2</td>
<td>0.48</td>
<td>1.99</td>
<td>1.54</td>
<td>2.59</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Moist snuff, US</td>
<td>Copenhagen®</td>
<td>48</td>
<td>7.39</td>
<td>25.8</td>
<td>3.5</td>
<td>19.3</td>
<td>1.69</td>
<td>2.64</td>
<td>0.45</td>
<td>0.23</td>
</tr>
<tr>
<td>Hard snuff</td>
<td>Ariva®</td>
<td>2.40</td>
<td>7.57</td>
<td>9.2</td>
<td>NA</td>
<td>0.4</td>
<td>1.4</td>
<td>2.19</td>
<td>0.28</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Adapted from McNeill et al. (2006).

As: arsenic; B[a]P: benzo[a]pyrene; Cr: chromium; NA: not available; Ni: nickel; Pb: lead; TSNAs: tobacco-specific nitrosamines [(N′-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N′-nitrosoanabasine (NAB)].

3.2.2 Tobacco-Specific Nitrosamines

ST has been reported to contain 28 carcinogens (Brunnemann & Hoffmann, 1992); however, the most attention has been given to TSNAs. TSNAs are not present in freshly harvested green tobacco (Baker, 1999; Brunnemann & Hoffman, 1991). TSNAs, including N′-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N′-nitrosoanatabine (NAT), and N′-nitrosoanabasine (NAB), are formed during curing via nitrosation of tobacco alkaloids (Figure 3.1) (Baker, 1999; Brunnemann & Hoffman, 1991). NNN, NAB, and NAT are formed predominantly by N-nitrosation of the corresponding secondary amines that are minor alkaloids (i.e., nornicotine, anabasine, and anatabine). Some NNN is also formed by nitrosation of the tertiary amine nicotine after loss of the methyl group. NNK can be formed only from nicotine by oxidative N-nitrosation after opening of the pyrrolidine ring. The nitrosating agent is nitrite, derived from nitrate, by the action of bacteria and tobacco enzymes during curing (Baker, 1999). Thus, the amount of nitrate in tobacco directly influences the TSNAs levels.

TSNA levels in commercial products also depend on several factors including tobacco variety and agronomic conditions, which affect initial alkaloid and nitrate concentrations; curing conditions, during which nitrosation of alkaloids...
occurs; and storing and processing conditions, during which further nitrosations can occur (Baker, 1999). TSNA levels have been published for numerous ST products (Table 3-6).

**Figure 3.1 Tobacco-Specific Nitrosamines**

![Diagram of Tobacco-Specific Nitrosamines](image_url)

NAB: N′-nitrosoanabasine; NAT: N′-nitrosoanatabine; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: N′-nitrosonornicotine. Adapted from Hoffmann et al., 1988.

Swedish *snus* products typically have lower TSNA levels (2.8–11.2 μg/g) than US moist snuffs (Brunnemann & Hoffmann, 1992). Pasteurization of Swedish *snus* likely prevents microbial reduction of nitrates and activation of nitrites. TSNA levels were examined in *snus* kept at temperatures ranging from -20°C to +23°C for 20 weeks (Österdahl & Slorach, 1983). Exposure to a range of temperatures over time did not significantly increase concentrations of TSNAs, which suggests that *snus* products have decreased bacterial activity. Total TSNA levels in hard snuff products produced from tobacco cured by methods that minimize TSNA formation (i.e., Ariva® and Stonewall®) are even lower than those in *snus* (0.19 and 0.28 μg/g, respectively) (Stepanov et al., 2006).

Although TSNA levels in US ST products are generally higher than levels in *snus*, their range has changed during the last 30 years, with the higher end of the range decreasing. TSNA levels measured in ST products during the 1980s and in 2001 ranged from 1.3 to 289.2 μg/g and 2.8 to 127 μg/g, respectively (European Network for Smoking Prevention, 2005). TSNA levels in 1S3 reference moist snuff more closely approximate levels found in US products than in Swedish products (Table 3-6). Unlike Swedish *snus* products,
### Table 3-6. Tobacco-Specific Nitrosamine Levels in Smokeless Tobacco Products

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Brand</th>
<th>NNN</th>
<th>NAT</th>
<th>NAB</th>
<th>NNK</th>
<th>Total TSNAs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moist snuff, US</td>
<td>Copenhagen® snuff</td>
<td>2.2</td>
<td>1.8</td>
<td>0.12</td>
<td>0.75</td>
<td>4.8</td>
<td>Stepanov et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Copenhagen® Long Cut</td>
<td>3.9</td>
<td>1.9</td>
<td>0.13</td>
<td>1.6</td>
<td>7.5</td>
<td>Stepanov et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Skoal® Long Cut</td>
<td>4.5</td>
<td>4.1</td>
<td>0.22</td>
<td>0.47</td>
<td>9.2</td>
<td>Stepanov et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Skoal Bandits®</td>
<td>0.9</td>
<td>0.24</td>
<td>0.014</td>
<td>0.17</td>
<td>1.3</td>
<td>Stepanov et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Kodiak Ice®</td>
<td>2.0</td>
<td>0.72</td>
<td>0.63</td>
<td>0.29</td>
<td>3.1</td>
<td>Stepanov et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Kodiak® Wintergreen</td>
<td>2.2</td>
<td>1.8</td>
<td>0.15</td>
<td>0.41</td>
<td>4.5</td>
<td>Stepanov et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Revel® Mint</td>
<td>0.62</td>
<td>0.32</td>
<td>0.018</td>
<td>0.033</td>
<td>0.99</td>
<td>Stepanov et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Revel® Wintergreen</td>
<td>0.64</td>
<td>0.31</td>
<td>0.017</td>
<td>0.032</td>
<td>1.0</td>
<td>Stepanov et al., 2006</td>
</tr>
<tr>
<td>Moist snuff, Sweden</td>
<td>General®</td>
<td>0.98</td>
<td>0.79</td>
<td>0.06</td>
<td>0.18</td>
<td>2.0</td>
<td>Stepanov et al., 2006</td>
</tr>
<tr>
<td></td>
<td>General®</td>
<td>NA</td>
<td>NA</td>
<td>0.13</td>
<td>0.27</td>
<td>0.478</td>
<td>McNeill et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Exalt®</td>
<td>2.3</td>
<td>0.98</td>
<td>0.13</td>
<td>0.27</td>
<td>3.7</td>
<td>Stepanov et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Swedish Match products</td>
<td>0.36–0.76</td>
<td>0.27–0.5</td>
<td>0.02–0.04</td>
<td>0.13–0.32</td>
<td>0.82–1.6</td>
<td>Österdahl et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Swedish snus (2001)</td>
<td>2.712.9</td>
<td>0.25</td>
<td>1.1</td>
<td>0.08</td>
<td>0.48</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Chewing tobacco®</td>
<td>1.1</td>
<td>0.94</td>
<td>0.02</td>
<td>0.11</td>
<td>2.2</td>
<td>Österdahl et al., 2004</td>
</tr>
<tr>
<td></td>
<td>US (3) 1983</td>
<td>0.25</td>
<td>0.15</td>
<td>NA</td>
<td>0.08</td>
<td>0.48</td>
<td>Österdahl et al., 2004</td>
</tr>
<tr>
<td></td>
<td>US (2) 1986</td>
<td>0.92</td>
<td>1.1</td>
<td>ND</td>
<td>0.01</td>
<td>2.0</td>
<td>Österdahl et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Sweden (1) 1986</td>
<td>1.7</td>
<td>1.4</td>
<td>NA</td>
<td>0.46</td>
<td>3.6</td>
<td>Österdahl et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Sweden (3) 1992</td>
<td>0.70</td>
<td>2.1</td>
<td>NA</td>
<td>0.09</td>
<td>2.9</td>
<td>Österdahl et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Dry snuff®</td>
<td>0.68</td>
<td>0.31</td>
<td>NA</td>
<td>0.10</td>
<td>1.1</td>
<td>Österdahl et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Germany (2) 1986</td>
<td>1.8</td>
<td>0.82</td>
<td>NA</td>
<td>0.26</td>
<td>2.9</td>
<td>Österdahl et al., 2004</td>
</tr>
<tr>
<td></td>
<td>UK (1) 1986</td>
<td>0.68</td>
<td>0.31</td>
<td>NA</td>
<td>0.10</td>
<td>1.1</td>
<td>Österdahl et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Hard snuff®</td>
<td>0.019</td>
<td>0.12</td>
<td>0.008</td>
<td>0.037</td>
<td>0.19</td>
<td>Stepanov et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Ariva®</td>
<td>0.056</td>
<td>0.17</td>
<td>0.007</td>
<td>0.043</td>
<td>0.28</td>
<td>Stepanov et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Stonewall®</td>
<td>0.019</td>
<td>0.12</td>
<td>0.008</td>
<td>0.037</td>
<td>0.19</td>
<td>Stepanov et al., 2006</td>
</tr>
<tr>
<td>Reference moist snuff</td>
<td>1S3</td>
<td>3.39</td>
<td>3.15</td>
<td>0.25</td>
<td>0.94</td>
<td>NA</td>
<td>Stepanov et al., 2005</td>
</tr>
</tbody>
</table>

---

**Notes:**

- All data reported as µg/g wet weight.
- For chewing tobacco and dry snuff data, numbers in parentheses indicate the number of brands analyzed; detection limit 50 pmol/g tobacco.
- **NA:** not available; **ND:** not detected; **TSNAs:** tobacco-specific nitrosamines \([N'\text{-nitrosonornicotine} \text{ (NNN)}, N'\text{-nitrosoanatabine} \text{ (NAT)}, N'\text{-nitrosoabasine} \text{ (NAB)}, 4\text{-}\text{(methylnitrosamino)}\text{-}1\text{-}(3\text{-}pyridyl)\text{-}1\text{-}butanone \text{ (NNK)}}\).
US STs are not typically stored in refrigerators by retailers or consumers, and nitrite and TSNA levels have been demonstrated to increase in US snuff (10-tin sleeve wrapped in plastic) when stored for 8 weeks in the manufacturer’s packaging at ambient room temperature ($\Delta NNN=2.38 \, \mu g/g$ dry weight; $\Delta NNK=0.71 \, \mu g/g$; $\Delta NAT=1.72 \, \mu g/g$) and at 37°C ($\Delta NNN=9.58 \, \mu g/g$ dry weight; $\Delta NNK=2.91 \, \mu g/g$; $\Delta NAT=5.58 \, \mu g/g$) (Djordjevic et al., 1993). Little to no change in TSNA levels was observed in tins stored at 4°C ($\Delta NNN=0.21 \, \mu g/g$ dry weight; $\Delta NNK=-0.06 \, \mu g/g$; $\Delta NAT=-0.13 \, \mu g/g$) (Djordjevic et al., 1993).

Despite the emphasis on and volume of data measuring TSNA levels in ST products, little is known about the magnitude of risk reduction that results from a reduction in TSNA levels. Given the number of compounds found in tobacco (Hatsukami et al., 2007; International Agency for Research on Cancer, 2007b), it has not been demonstrated that TSNAs alone are responsible for all biological changes caused by ST use.

### 3.2.3 Polycyclic Aromatic Hydrocarbons

Unburned tobacco contains fewer carcinogens than does cigarette smoke because most carcinogens are formed during combustion (Hecht, 2003). Trace amounts of PAHs are typically found in ST products (Table 3-5). In 1985, 5 US snuff brands contained from 0.1 to 63 ng/g dry weight of benzo[a]pyrene (Hoffmann et al., 1987). The levels of PAH and other wood smoke-derived compounds are likely associated with the proportion and origin of fire-cured tobacco used in a product, which helps explain the wide variation in PAH levels among ST products (Davis & Nielsen, 1999).

### 3.2.4 Heavy Metals

Heavy metals including arsenic, cadmium, chromium, lead, and nickel are taken up by plants from the soil and are thus found in tobacco products (Swedish Match, 2007). Metals vary by product as a consequence of the source of the tobacco. Soil contamination results from pollution and the application and content of pesticides and fertilizers in the soil (e.g., cadmium levels in US tobacco are higher than tobacco grown elsewhere because of the fertilizers). The minimization of pesticide application would be a positive step toward reducing the chemical contamination of tobacco leaf.

### 3.2.5 Radionuclides

The $\alpha$- and $\gamma$-emitting $^{226}$Ra (half-life=1,620 years) and to some extent $^{210}$Pb (half-life=22.8 years) are considered to be important radionuclides in moist snuff (Scientific Committee on Emerging and Newly Identified Health Risks,
Differentiating the Health Risks of Categories of Tobacco Products

Moist snuff may also contain $^{210}\text{Po}$, an $\alpha$- and $\gamma$-emitter that decays to stable $^{206}\text{Pb}$ (Gregory, 1965; Harley et al., 1980; Hoffmann et al., 1986). The average total $\alpha$ activity of 5 major brands of US moist snuff was 0.16–1.22 pCi/g (0.006–0.45 Bq/g) (Hoffmann et al., 1986; Martell, 1974). Daily consumption of 20 g of snuff would result in an exposure of 0.12–0.9 Bq, which is negligible compared with background radiation and other sources of ionizing radiation (Chruscielewski & Kaminski, 1999; Scientific Committee on Emerging and Newly Identified Health Risks, 2007). These radionuclides are generally found in products containing tobacco, including cigarettes (Hecht, 1998b).

3.2.6 Additives

All additives used to alter the taste and chemical characteristics of Swedish Match products are approved food additives (Swedish Match, 2007). Pouch materials and additives to two new American snus products are approved by FDA for food use (Fisher, 2007).

3.2.6.1 Sodium chloride and ammonium chloride

Sodium chloride is often added to moist snuff and some dry snuffs. Finished products may contain 7–10% (by total weight) sodium chloride, which gives the desired salty taste to the snuff (Davis & Nielsen, 1999). Ammonium chloride is sometimes used instead of, or in combination with, sodium chloride to produce the desired taste. Ammonium chloride is also used to alter pH.

3.2.6.2 Sugars and sweeteners

Sugars and sweeteners such as saccharin, sucralose, and sorbitol are frequently found in ST products. In 1988, some US loose-leaf commercial tobaccos contained as much as 20% sucrose by dry weight (Chamberlain et al., 1988).

3.2.6.3 Conditioners

Benzyl benzoate, a flavor fixative, is sometimes used. Levels in commercial snuff brands have been estimated to range between 30 and 110 $\mu$g/g tobacco (LaVoie et al., 1989). Glycerol, 1,2-propylene glycol, and other conditioners are also found in some ST products (Davis & Nielsen, 1999).

3.2.6.4 Flavorings

Flavorings are commonly added to ST products. Licorice, wintergreen oil, and other essential oils are traditional additives (Davis & Nielsen, 1999). New types of alkali-stable flavorings such as eucalyptus, cassis, and vanilla
coffee are used by Swedish Match in Catch® snus (Swedish Match, 2005). US Smokeless Tobacco Company uses cherry, vanilla, berry, peach, and apple flavorings in Skoal®, the best-selling US flavored moist snuff (U.S. Smokeless Tobacco Company, 2007). Marlboro® snus is flavored by using flavor strip technology. The flavor strip lines the inside of each pouch and is similar to Listerine PocketPaks® Breath Strips, which dissolve as they are used (Fisher, 2007).

3.3 CRITIQUE OF CHEMICAL CONSTITUENT SELECTION

Certain ST constituents are routinely measured despite any real knowledge of whether they increase or decrease human health risks. ST studies have primarily focused on the cancer-causing potential of TSNAs, although other chemical compounds in smokeless products acting independently or synergistically could contribute to adverse human health effects. Swedish Match, the largest manufacturer and distributor of snus in Sweden, has set a quality standard for its products called GothiaTek® that includes maximal permissible limits for “undesirable substances” (Table 3-7) (Swedish Match, 2007).

Because epidemiological evidence supports fewer human health risks associated with the use of Swedish snus than other types of ST, most ST manufacturers appear to be altering the chemical composition of their products to more closely align with the GothiaTek® standard. However, the GothiaTek® standard and others are arbitrary, are without specific merit, and are not enforced by regulatory agencies. Although chemical analyses provide basic information about the composition of ST products, it is unclear how this information might be used in human health assessments.

3.4 CONCLUSIONS

Cigarette and smokeless tobacco differs in terms of composition, product characteristics, and chemistry under conditions of use. In addition ST may include various types of tobacco cured in different ways, include different sizes of tobacco cuttings, contain pasteurized instead of fermented tobacco, contain different amounts of nicotine, and have different pHs that influences availability of nicotine for uptake. Typically a limited number of compounds in ST and cigarette smoke is measured. Analysis of the chemical composition of cigarettes and ST has only a limited role in risk assessment.
Table 3-7. GothiaTek® Standard for Maximal Permissible Limits for Undesirable Substances

<table>
<thead>
<tr>
<th>Component</th>
<th>Limit</th>
<th>Content in 2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite (mg/kg)</td>
<td>3.5</td>
<td>1.0 (&lt;0.5–1.7)</td>
</tr>
<tr>
<td>TSNAs (mg/kg)</td>
<td>5</td>
<td>0.8 (0.5–1.1)</td>
</tr>
<tr>
<td>NDMA (μg/kg)</td>
<td>5</td>
<td>0.5 (&lt;0.5–0.7)</td>
</tr>
<tr>
<td>B[a]P (μg/kg)</td>
<td>10</td>
<td>0.6 (&lt;0.5–1.1)</td>
</tr>
<tr>
<td>Cadmium (mg/kg)</td>
<td>0.5</td>
<td>0.2 (0.1–0.3)</td>
</tr>
<tr>
<td>Lead (mg/kg)</td>
<td>1.0</td>
<td>0.1 (0.03–0.3)</td>
</tr>
<tr>
<td>Arsenic (mg/kg)</td>
<td>0.25</td>
<td>0.06 (&lt;0.03–0.13)</td>
</tr>
<tr>
<td>Nickel (mg/kg)</td>
<td>2.25</td>
<td>0.6 (0.2–0.9)</td>
</tr>
<tr>
<td>Chromium (mg/kg)</td>
<td>1.5</td>
<td>0.4 (0.07–0.7)</td>
</tr>
</tbody>
</table>
| Pesticides      |       | According to Swedish Match pesticide policy

Adapted from Swedish Match (2007).

\*mg/kg = thousandth g/kg product (based on snus with 50% water content); μg/kg = millionth g/kg product (based on snus with 50% water content).

\*All tobacco products manufactured by Swedish Match comply with local pesticide residue requirements in each country in which Swedish Match conducts business. If no pesticide regulations exist in a particular country, internal Swedish Match pesticide residue requirements are enforced.

**IN VITRO TESTS OF BIOLOGICAL ACTIVITY AND ANIMAL STUDIES**

**4.1 INTRODUCTION**

Preclinical studies provide risk assessment data that include potential exposure effects and the concentrations at which effects are observed (National Research Council, 2006). This chapter considers *in vitro* tests of biological activity and *in vivo* animal studies. Smokeless tobacco (ST) products discussed in this chapter are limited to Swedish *snus* and US ST products. International ST products, defined in this report as ST products other than those primarily used in the US and Sweden, are covered in Chapter 8.

**4.2 GENOTOXICITY AND CYTOTOXICITY ASSAYS**

*In vitro* tests (*i.e.*, genotoxicity and cytotoxicity assays) do not provide direct information on human disease potential but are integral components of toxicological assessments and are accepted by regulatory authorities as methods to evaluate biological activity. Numerous criteria exist for the proper conduct of these assays (International Organization for Standardization, 1999; Organization for Economic Cooperation and Development, 2004). *In vitro* test systems can provide insight into mechanisms of toxicity but they do not represent responses of the intact subjects.

**4.2.1 Methodology**

Findings from the ST genotoxicity and cytotoxicity literature are confusing and conflicting because of a failure to adequately address issues related to exposures, controls, and methodology (Organization for Economic Cooperation and Development, 2004; U.S. Food and Drug Administration *et al.*, 2001).

**4.2.1.1 Smokeless tobacco extract**

One critical failing is the lack of standardized methods for producing smokeless tobacco extract (STE). Most *in vivo* and *in vitro* ST studies use STE. In general, ST is mixed with a liquid (*e.g.*, phosphate-buffered saline, alcohol, water, or chloroform), centrifuged, and then filtered or vacuum sterilized (Lindemann & Park, 1988; Oh *et al.*, 1990). Occasionally, the mixture
is dissolved again in media or dimethyl sulfoxide for cell culture experiments. However, no standardized method exists for making STE.

A few ST extraction methods are commonly cited, but the authors do not explain the reasoning behind the choice of method (Lindemann & Park, 1988; Oh et al., 1990). Important variables including STE concentration, acidity, and nicotine content are infrequently determined. Both aqueous and organic solvents are used to resuspend STE, but little is known about the solubility of the chemical components. Sometimes STE is divided into aliquots and frozen for future use. No information is available on differences in the biological activity of fresh versus frozen STE. For these reasons, STE composition is difficult to verify, which complicates the ability of independent laboratories to replicate published results.

Chemicals are routinely tested for mutagenicity with *Salmonella typhimurium*; mutant colonies are commonly referred to as revertants (U.S. Food and Drug Administration et al., 2001). Certain protocols utilize extracts of rat or hamster liver enzymes (RS9) to promote metabolic conversion of a test chemical because bacteria do not have the same metabolic capabilities as mammals (Maron & Ames, 1983). However, human-derived S9 (HS9) gives much more relevant results in the *Salmonella* mutagenicity assay than does rodent-derived S9 (RS9) (Hakura et al., 2005).

The studies evaluated in this chapter use various methods. The International Organization for Standardization (ISO) has well-established and accepted guidelines for extraction methods for preparing test substances for both genotoxicity and cytotoxicity assays (International Organization for Standardization, 2007). The Life Sciences Research Office (LSRO) recommends that investigators consult these guidelines for preparation of STE for *in vitro* studies.

### 4.2.1.2 Control products

Many *in vitro* assays that evaluate ST use untreated cell cultures as controls. For research purposes, reference ST products are available from the North Carolina Agricultural Research Service (North Carolina Agricultural Research Service, 2005). These products include moist snuff (2S3), dry snuff (1S2), and loose-leaf chewing tobacco (2S1). The compositions of these products represent products in the US market and no flavorings are added. (Descriptions of these products and their chemical compositions are presented in Chapter 3 of this report.) No ST reference product is currently available that accurately represents Swedish *snus* or American *snus* products that are pasteurized and do not contain fire-cured tobacco.
4.2.1.3 Sterile smokeless tobacco extract

One important confounding factor when studying ST is potential contamination with lipopolysaccharide (LPS), an immune system stimulant. If ST is not extracted under sterile conditions and kept sterile until use, STE is highly likely to contain LPS. LPS was found in 3 kinds of research grade tobacco: loose-leaf tobacco, dry snuff, and moist snuff (Furie et al., 2000). LPS induces a pro-inflammatory response of the immune system and could confound real pro-inflammatory effects. Researchers should take care to produce STE under sterile conditions and filter-sterilize before use, as well as confirm that the STE contains no LPS.

4.2.1.4 General considerations

In vitro assays used to evaluate ST products should include:

- Positive and negative controls, products representative of the current marketplace, and appropriate reference products;
- Methods used to produce STE and a rationale for selection of a particular method (International Organization for Standardization, 2007);
- Metabolic activation system (e.g., RS9, HS9) in bacterial assays, if appropriate (Mortelmans & Zeiger, 2000);
- Analysis of dose-response relationships when possible (Hayashi et al., 2000; Krishna & Hayashi, 2000);
- Reporting of results in a common metric to allow comparison among tobacco products (Rickert et al., 2007); and
- Analysis of the relationship to expected human exposure under real-life conditions.

4.2.2 Cell Survival

The neutral red assay is a cell survival or viability assay that evaluates the cytotoxic potential of compounds (Andreoli et al., 2003). The assay measures endocytosis at the plasma membrane and lysosomal uptake in cultured cells. Compounds that damage plasma or lysosomal membranes or that interfere with endocytosis impair cellular uptake of neutral red dye that is added to cell culture medium. Endpoints evaluated in the neutral red assay are cell viability and cell number, which can be interpreted as measures of induction of acute toxicity. The assay is rapid, sensitive, quantitative, and reproducible. The Centre de Coopération pour les Recherches Scientifique Relatives au Tabac task force recommended it as a valid assay for cytotoxicity (Doolittle & Massey, 2003). To date, no published studies have investigated ST or STE with this assay. However, some evidence exists that ST manufacturers are using this assay to evaluate their products (Fisher, 2007). Future studies
Differentiating the Health Risks of Categories of Tobacco Products

should utilize this well-established and recommended assay to assess cell survival and viability.

Another measure of cell survival is the lactate dehydrogenase (LDH) assay. This assay measures membrane integrity in cultured cells. The relevance of this assay to specific human disease is questionable. The value of LDH released into culture media compared with total cellular LDH values correlates with the amount of membrane damage and cell death and thus provides an indication of cellular toxicity induced by a test substance. An increase in the percentage of LDH release is interpreted as a cytotoxic response. However, some chemicals can directly inhibit the enzyme, which results in an underestimate of toxicity. As Table 4-1 shows, different ST products produced mixed results with the LDH assay.

The literature lacks consistency across studies of ST effects on cell survival and cytotoxicity. Varied products, dosing, and methodology do not allow definitive conclusions. Future research should follow standardized procedures, such as those proposed by the ISO: include positive controls, reference products, products representative of those on the commercial market, and a range of doses (International Organization for Standardization, 1999).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cell Type</th>
<th>Test Material and Dose</th>
<th>STE</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Müns et al., 1994</td>
<td>Syrian hamster oral epidermoid carcinoma (HCPC-1)</td>
<td>1S3 0.001–5.0% STE</td>
<td>10% STE, aqueous extraction</td>
<td>No effect was found on cell number.</td>
</tr>
<tr>
<td>Johnson et al., 1996</td>
<td>Human gingival keratinocyte culture</td>
<td>1S3 0–10% STE</td>
<td>10% STE, aqueous extraction</td>
<td>Only 10% STE significantly reduced cell viability.</td>
</tr>
<tr>
<td>Mangipudy &amp; Vishwanatha, 1999</td>
<td>HCPC-1</td>
<td>1S3 0.5–2.5%</td>
<td>10% STE, aqueous extraction</td>
<td>No effect was found on cell viability.</td>
</tr>
<tr>
<td>Yildiz et al., 1999</td>
<td>CHO</td>
<td>Research grade ST 0.08–4 mg</td>
<td>% not given; aqueous extraction</td>
<td>STE reduced cell survival and killed all cells at 4 mg.</td>
</tr>
</tbody>
</table>

CHO: Chinese hamster ovary; HCPC-1: hamster cheek pouch carcinoma; STE: smokeless tobacco extract.
4.2.3 Genotoxicity Assays

The International Conference on Harmonisation guidelines for genotoxicity testing state that in vitro tests assessing the ability of a chemical to cause gene mutations, extensive chromosomal damage, chromosomal recombination, and/or alterations in chromosome number may identify potential human carcinogens (International Conference on Harmonisation Steering Committee, 1997b). Commonly used genotoxicity tests are the *Salmonella* mutagenicity assay, chromosomal aberration assay, micronucleus assay, and sister chromatid exchange assay.

4.2.3.1 *Salmonella* mutagenicity assay

The *Salmonella typhimurium* mutagenicity assay allows investigators to determine whether a chemical must be metabolized to express mutagenic activity. Numerous reports of mutagenicity from tobacco smoke condensate that utilize *Salmonella* mutagenicity assays have been published (DeMarini, 2004). Several reliable and reproducible types of *Salmonella* strains should be used (Mortelmans & Zeiger, 2000).

These *Salmonella* strains have specific gene mutations that prevent growth of bacteria in the absence of histidine. The specificity of the test strains can provide beneficial information on types of mutations induced by genotoxic agents. Strains TA100 and TA1535 are sensitive to base-pair substitutions; TA98, TA1538, TA1537, and TA97 are sensitive to frameshifts; and TA102 and TA104 are sensitive to transitions and transversions (Mortelmans & Zeiger, 2000).

Swedish *snus* was not mutagenic when tested with several *S. typhimurium* strains (TA98, TA100, TA1535, and TA1527) and Chinese hamster lung (V79) cells (Jansson *et al.*, 1991). These strains did not detect either frameshift mutations or base-pair substitutions. In these studies, an aqueous extract of Swedish *snus* was made and added to each plate in the presence and in the absence of S9. Although few preclinical studies of Swedish *snus* have appeared, available data suggest that Swedish *snus* is not mutagenic in vitro.

Chewing tobacco produced in the US caused base-pair substitutions and frameshifts in strains (TA98, TA100, and YG1024) of *S. typhimurium*, in the presence and absence of metabolic activation (Guttenplan, 1987; Stamm *et al.*, 1994; Whong *et al.*, 1985). STE from an unspecified type of commercial tobacco caused forward mutations in *S. typhimurium* TM677 with added sodium nitrite (to mimic salivary nitrites) (Shirnamé-Moré, 1991). When these experiments were performed at neutral pH, without added nitrites, they did
Differentiating the Health Risks of Categories of Tobacco Products

not produce mutagenic results. This finding suggests that mutagenicity depends on mouth chemistry in combination with ST. In humans, nitrosation reactions occur under acidic conditions, such as in the mouth (Coulston & Dunne, 1980). The World Health Organization recommended an in vitro nitrosation assay procedure (NAP) to rank compounds for their potential to form N-nitroso derivatives (Coulston & Dunne, 1980). LSRO recommends that the NAP test be applied to in vitro studies of ST to establish the relative nitrosatable potential of ST under conditions that conform closely to human ST use.

Two commercial US ST products (dry snuff and moist snuff) caused base-pair substitutions in strain TA100 with metabolic activation, but no frameshifts in TA98 (Rickert et al., 2007). Products with air-cured (burley, dark air-cured) tobaccos gave a higher number of revertants than did flue-cured products; the authors noted that the higher amount of TSNAs found in air-cured tobacco may be responsible. This study also included a direct comparison of mutagenicity of tobacco smoke condensate and ST (revertants per milligram of nicotine) and found that some ST products were less mutagenic. This finding suggests that the type of tobacco curing (i.e., air-curing) may influence mutagenicity.

Future mutagenicity studies should follow the recommended guidelines for proper conduct of such studies. Several guidelines suggest using a standard set of S. typhimurium strains for mutagenicity testing because one strain is insufficient to confirm a negative result (Mortelmans & Zeiger, 2000; Organization for Economic Cooperation and Development, 1997a). Many studies failed to include a positive control. It is becoming clear that HS9 gives much more relevant results in Salmonella mutagenicity assays than does RS9 (Hakura et al., 2005). Thus, future studies should replace RS9 with HS9.

4.2.3.2 Mammalian cell mutagenicity

Clastogenicity assays screen for possible mammalian mutagens and carcinogens that cause structural chromosomal damage in cultured mammalian cells and provide corroborating evidence of genotoxicity (Organization for Economic Cooperation and Development, 1997b; U.S. Food and Drug Administration et al., 2001). In vitro micronucleus assays do not provide direct evidence of the mutagenic or carcinogenic potency of a substance in humans (Life Sciences Research Office, 2007a).

The micronucleus assay is used to screen for chemicals that are clastogens, agents that cause structural chromosomal alterations and aneugens, agents
that cause the loss or gain of one or more chromosomes (Organization for Economic Cooperation and Development, 1997c). After cell division, lagging whole chromosomes or fragments, because of genetic damage, are incorporated into a micronucleus that can be counted, so that a dose-response curve can be obtained (Andreoli et al., 2003). Swedish snus did not change the frequency of micronucleated cells (Jansson et al., 1991).

The comet assay is a technique for detecting DNA damage in eukaryotic cells. STE made from a US ST commercial product, Copenhagen®, produced DNA single-strand breaks as indicated in the comet assay (Barley et al., 2004).

4.2.4 Cytotoxicity Testing

Cytotoxicity testing is relevant to 4 human situations including irritation and inflammation, cell proliferation and hyperplasia, oxidative stress and damage, and impaired organ function. Several cytotoxicity methods are used to evaluate in vitro biological effects of tobacco products (Andreoli et al., 2003; International Organization for Standardization, 1999). The ISO has well-established standardized methods for quantitating the degree of cytotoxicity (International Organization for Standardization, 1999). Cell-culture systems are often used because they are reliable, reproducible, and relatively inexpensive experimental systems to assess chemical toxicity at the cellular level (National Research Council, 2007).

4.2.5 Inflammatory Responses

ST is an activator of inflammatory responses. STE made from moist snuff, at concentrations of 1.25 mg/plate and below, increased macrophage production of the pro-inflammatory cytokines tumor necrosis factor-α and interleukin (IL)-1 (Seyedroudbari & Khan, 1998). A 10% STE solution also increased the production of pro-inflammatory cytokines IL-1α and IL-1β by cultured human gingival keratinocytes (Johnson et al., 1996). These pro-inflammatory cytokines activate endothelial cells to a pro-inflammatory state and promote extravasation of leukocytes.

STE also increased cyclooxygenase-2 production and expression (Vishwanatha et al., 2003). STE from research grade moist snuff increased IL-2 and interferon-γ (IFN-γ) in T cells from BALB/c mice (Petro & Zhang, 1997). This cytokine response favors the development of inflammation similar to that seen in periodontal disease (Petro & Zhang, 1997). In a later study, the same group found that STE enhanced the promoter activity of IFN-γ and stimulated nuclear localization of nuclear factor-κB (NF-κB) promoters.
Differentiating the Health Risks of Categories of Tobacco Products

(Petro, 2003). NF-κB also promoted hepatocellular and colon carcinoma. These results suggest that STE enhances inflammation through various mechanisms.

The immune system responds to pro-inflammatory cytokines with anti-inflammatory cytokines that serve to dampen the immune response and prevent shock and damage to organs. Annexin-1 (anti-inflammatory) expression increased in HCPC-1 cells treated with 1% STE (Vishwanatha et al., 2003). Production of IL-10, another anti-inflammatory cytokine, decreased in BALB/c splenocytes after exposure to STE made from research grade moist snuff (Petro & Zhang, 1997). A mixed anti-inflammatory response occurs to STE; more research is needed to draw definitive conclusions.

STE from 3 different kinds of research grade ST (loose-leaf chewing tobacco, dry snuff, and moist snuff) increased production of the chemokines IL-8 and monocyte chemoattractant protein-1, which enhance neutrophil migration (Furie et al., 2000). This finding was confirmed by examining neutrophil migration between human umbilical vein endothelial cells monolayers. STE from research grade dry snuff increased concentrations of keratinocyte growth factor, hepatocyte growth factor, and granulocyte-macrophage colony-stimulating factor in a time- and concentration-dependent manner in buccal fibroblasts in vitro (Dabelsteen et al., 2005). Neutrophil accumulation promoted breakdown of the endothelial layers of the oral mucosa and may destroy fragile oral tissue during repeated exposure.

4.2.6 Other Effects

In addition to the ST effects just detailed, ST may affect bone health. STE made from reference moist ST inhibited bone collagen synthesis, decreased oxygen consumption, and increased lactate production in cultured chick embryo tibiae (Lenz et al., 1992). STE made from a commercial ST product, Skoal Bandits®, produced similar findings (Galvin et al., 1988). Galvin et al. (1988) removed nicotine from the STE and continued to find decreased collagen synthesis, which suggests that the bone synthesis inhibitor in STE is not nicotine. Applicability of these results to humans is unknown.

4.3 ANIMAL MODELS

Hoffmann et al. (1992) concluded that animal bioassays support the epidemiological observation that long-term use of ST leads to oral cancer, although this review is limited to pre-1990 studies. However, a later review of the same animal studies reached the opposite conclusion and stated that
current work suggests that snuff is not carcinogenic to hamsters or rats (Grasso & Mann, 1998). Grasso and Mann’s (1998) criticism focused on problematic models that induced mechanical damage (i.e., artificial lip canal in rodents) and subchronic dosing regimens. While there are numerous animal models that simulate certain features of human susceptibility, none fully models the complex combination of genetic, lifestyle, and clinical factors producing the most sensitive human.

LSRO previously compiled a recommended test battery for genotoxicity and cytotoxicity studies (Life Sciences Research Office, 2007b). Also included in this LSRO report was a recommended list of conventional animal toxicity studies. The same recommendations should be applied to evaluation of ST products.

4.3.1 Methodology

Many guidelines for the proper design, conduct, and analysis of animal studies have been published (National Toxicology Program, 2006; Organization for Economic Cooperation and Development, 1981; 1998). There are three in vivo models of ST use: oral cavity swabbing in rodents, surgically created oral test canal in rodents, and cheek pouch assay in hamsters. Oral cavity swabbing is probably the least useful and relevant method as it involves swabbing STE into the oral cavity of the rodent. The material is not kept in the mouth for any length of time, so this method would not predict oral effects of ST use. The other two models involve placing a specific product, such as moist snuff, into the oral cavity. It is then held there for up to 24 hours and removed by the investigator, which more accurately models daily human ST use. The surgically created oral test canal is occasionally criticized because the surgery and healing process may themselves confound the experiment. In vivo assays often use phosphate-buffered saline as a control for ST. Sometimes a cotton pellet is inserted into the oral cavity or air is injected into the cheek pouch as a control measure.

In evaluating ST products, LSRO recommends that for long-term carcinogenicity studies, animals should receive a daily dose of the test substance for a minimum of 1 year (Organization for Economic Cooperation and Development, 1981) and that proper guidelines for attaining standard exposures be followed that will allow intra- and inter-study comparisons.

See Appendix C for a list of preclinical studies consulted for this report. A selected subset is described here.
4.3.2 Tumor Formation

Administration of STE or tobacco-specific nitrosamine (TSNA) promotes tumor formation. Male F344 rats receiving STE via daily oral cavity swabbing did not develop tumors, but a combination of TSNAs, N’-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), increased the incidence of oral tumors (Hecht et al., 1986). In another experiment, moist snuff inserted into an oral test canal increased tumor occurrence in the oronasal cavity. Direct placement of snuff into the oral cavity likely created a different chemical environment in the model, which led to increased tumor incidence. This result was confirmed by other researchers who placed snuff directly into a surgically created oral test canal in Sprague-Dawley rats (Johansson et al., 1989). Johansson et al. (1989) also found that 4-nitroquinoline 1-oxide, a TSNA, produced tumors in rats. Grasso and Mann (1998) raised a number of concerns about the study design and the impact of creating a “lip canal” on carcinogenic outcome, because cell proliferation and tumors have been reported in studies of agents that induce persistent tissue injury. The Grasso and Mann (1998) review also mentioned that the Johansson et al. (1989) study and many other in vivo ST studies failed to use a biologically inert control (such as cotton) to verify the findings.

Swedish snus increases the risk of gastric cancer in mice (Stenström et al., 2007). One study found a positive association (Zendehdel et al., 2008). Commercially available Swedish snus (General®) mixed with rat chow at a concentration of 5–9% was given to wild-type mice and gastrin transgenic mice with or without Helicobacter pylori infection (Stenström et al., 2007). After transgenic mice had 6 months of snus exposure plus H. pylori infection, all developed gastric carcinoma. Without H. pylori infection, 50% of transgenic animals exposed to snus developed gastric carcinoma. Fifty-three percent of wild-type mice that were infected with H. pylori at 6 months of age developed carcinoma after exposure to snus for 6 months.

Gastric cancer is also associated with cigarette smoking and certain studies have reported an association with ST use (Chao et al., 2002; Freedman et al., 2007; Lewin et al., 1998). Several studies reported that Swedish snus was not associated with gastric cancer; however, these studies involved very small numbers of snus users (Boffetta et al., 2005; Lagerrgren et al., 2000; Lewin et al., 1998). Studies suggest that snus exacerbates cancer development in genetically susceptible individuals exposed to H. pylori and that Swedish snus may be co-carcinogenic in a subset of patients. Further research is needed to verify these findings.
Not all in vivo studies reported tumor formation after ST exposure (Barley et al., 2004; Chen, 1989). No tumors were found when STE made from Copenhagen® was placed in the cheek pouch of Syrian golden hamsters (Barley et al., 2004). In this study, an STE was used, and snuff was not placed directly into the oral cavity. The STE was applied only 3 days/week, versus 5 days/week in the studies described above that reported tumor formation. The other study reporting no cancer formation applied ST (0.4 g) only once per week, and this regimen may have been insufficient to produce carcinoma as well (Chen, 1989). This study found that rats treated with ST developed pre-malignant changes such as hyperkeratosis, acanthosis (epithelial hyperplasia), and subepithelial connective tissue hyalinization in the buccal mucosa. These results suggest that daily treatment with STE is necessary for carcinogenicity, in agreement with the Organization for Economic Cooperation and Development guidelines for carcinogenicity studies (Organization for Economic Cooperation and Development, 1981).

### 4.3.3 Immune Response

The immune system plays important roles in periodontal disease and cancer. In addition, an overactive immune system can lead to tissue damage. Snuff placed into an oral test canal in Sprague-Dawley rats reduced natural killer cell activity (Johansson et al., 1991). Syrian hamsters treated with snuff or chewing tobacco in the buccal pouch had fewer peritoneal-derived macrophages, and the cytotoxic capacity of these macrophages significantly declined (Antoniades et al., 1984). At concentrations of 0.05–2.0%, 1S3 research grade snuff increased proliferation of BALB/c spleen cells in vitro, similar to results for controls (Goud et al., 1993). These researchers further found that both T and B cells showed increased proliferation when stimulated with STE. STE also promoted maturation and differentiation of B lymphocytes. IL-1, which is important in maturation and differentiation of B and T cells, increased in a dose-dependent manner in spleen cells of BALB/c mice. No significant differences were found in IL-2 activity or receptor number in stimulated T cells. The authors suggested that long-term ST use may lead to activation of the inflammatory response that contributes to gingivitis.

### 4.3.4 Developmental Effects

Very little research has examined the effects of STE on fetal development in in vivo models. Research grade moist STE given to pregnant Sprague-Dawley dams reduced fetal weight in a dose-dependent manner (Paulson et al., 1994). STE treatment also reduced ossification of many bones including nasal bones, femur, and forelimb. More research is needed to arrive at definitive conclusions, especially in view of possible human implications.
4.4 CONCLUSIONS

Numerous criteria are available for the proper conduct of genotoxicity and cytotoxicity studies (International Conference on Harmonisation Steering Committee, 1995, 1997a; International Organization for Standardization, 1999, 2007; Organization for Economic Cooperation and Development, 2004; U.S. Food and Drug Administration et al., 2001). LSRO previously compiled a recommended test battery for these investigations (Life Sciences Research Office, 2007b). However, much of the research on ST products has not followed such guidelines so that data (e.g., from studies of cell survival after ST exposure) lack consistency, reliability, and generalizability. Varied products, dosing, and methodology do not allow definitive conclusions. Future research should follow a standardized procedure and include positive controls, reference products, and a range of doses.

Several guidelines suggest using a standard set of *S. typhimurium* strains for mutagenicity assays because use of only one strain cannot confirm a negative result (Mortelmans & Zeiger, 2000; Organization for Economic Cooperation and Development, 1997a). However, many studies described here used only one *S. typhimurium* strain. These studies also did not include a positive control for negative findings. Future mutagenicity research should follow the recommended guidelines for the proper conduct of such studies.

STE increases production of pro-inflammatory cytokines and promotes inflammation through various mechanisms *in vitro*. The anti-inflammatory immune response may be suppressed by STE, but current research does not allow definitive conclusions on these effects. STE induces neutrophil accumulation, which promotes breakdown of endothelial layers of the oral mucosa and may destroy fragile oral tissue during repeated exposure. These studies indicate that STE or TSNA administration may promote oral tumors *in vivo*. Daily treatment with ST, for 1 year, is the minimum necessary for evaluating carcinogenicity. Future carcinogenicity studies should also follow proper research guidelines (Organization for Economic Cooperation and Development, 1981).
5.1 CLINICAL STUDIES

Biomarkers of exposure and effect for evaluating physiological changes resulting from cigarette smoking have been described (Life Sciences Research Office, 2007a; 2007b). Similar measures have been used to analyze exposure to and biological effects resulting from smokeless tobacco (ST) use, although significantly fewer clinical studies of ST users have been published. In the published US ST studies, most participants were predominantly young (20–35 years old) white males, which makes extrapolation to other demographic groups difficult at best.

5.2 EXPOSURE

5.2.1 Routes of Exposure

Individuals who use ST are exposed to potential toxicants by absorption through the oral or nasal mucosa and via ingestion of ST constituents (National Toxicology Program, 2002).

5.2.1.1 Interactions with mucosal membranes

Clinical changes observed in the oral cavity consist principally of alterations in non-keratinized mucosa and in gingiva, which correspond to sites of usual ST product placement. The primary mucosal change is a wrinkled mucosa that appears white or yellowish brown because of tobacco stains, in some cases with associated erythema (Scientific Committee on Emerging and Newly Identified Health Risks, 2007). There is evidence that the buccal mucosa barrier is altered during ST use, thus affecting its permeability to nicotine (Andersson et al., 1994; Squier, 1986; Tobey et al., 1988).

Soft tissue changes of the oral mucosa and gingival margin are less pronounced, both clinically and histologically, among portion-bag snuff users than among loose snuff users (Andersson, 1991). The observed difference in tissue response is assumed to be due in part to pH differences of the two products. Both types of snuff are alkaline (pH 7.9–8.6), but the pH of portion-bag snuff is an average 0.5 pH unit lower than that of loose snuff. Nicotine
also appears to have an impact on oral reactions because snuff with a lower nicotine content appears to cause less severe tissue changes (Andersson et al., 1995).

5.2.1.2 Role of saliva

A relative paucity of information exists with regard to biological interactions between saliva and ST constituents. One study of Swedish snus users found elevated salivary pH (7.9–8.5) both during and directly after snus consumption compared with the average pH of resting saliva in a general population (Andersson & Warfvinge, 2003). Saliva pH returned to normal values (~7) after 1 night of abstinence from ST. Despite alkaline salivary pH both during and shortly after snuff use, mucosal changes were recorded only at sites where the pinch of snus was placed.

5.2.1.3 Ingestion

The salivary extract from ST is often swallowed by users (Ebbert et al., 2004). Few studies have specifically quantified absorption of ST constituents from the gastrointestinal tract. Those studies that addressed absorption focused on systemic delivery of nicotine and tobacco-specific nitrosamines (TSNAs) (see Section 5.2.2).

5.2.2 Tobacco-Derived Chemicals or Metabolites in Biological Fluids

Measurement of chemicals in body fluids is one method used to evaluate risk from using tobacco products. 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, cellular or target tissues (Life Sciences Research Office, 2007b). Biomarker of exposure studies suggest:

- Similar nicotine delivery for both smoked tobacco and ST products (Foulds et al., 2003);
- Elevated plasma cotinine levels in ST users compared to levels in cigarette smokers. These levels do not accurately reflect systemic nicotine exposures, however. Serum nicotine concentration may be a more reliable method to evaluate systemic nicotine exposure in ST users (Ebbert et al., 2004);
- Lower serum thiocyanate levels in ST users than in cigarette smokers (Holiday et al., 1995);
- Higher median levels of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) per milliliter of urine than levels found in cigarette smokers (Hecht et al., 2007);
• TSNAs found in saliva of ST users (Hoffmann & Adams, 1981; Nair et al., 1985; Prokopczyk et al., 1992; Wenke et al., 1984); and
• Less urine mutagenicity for ST users than for cigarette smokers (Benowitz et al., 1989; Curvall et al., 1987).

5.2.2.1 Nicotine, minor tobacco alkaloids, and thiocyanate

ST contains and delivers into the systemic circulation quantities of nicotine comparable to those for smoked cigarettes (Foulds et al., 2003). However, the rate of nicotine absorption from ST is slightly slower and lacks the arterial bolus observed with use of smoked products because of nicotine inhalation. A “dose” of ST results in a plasma nicotine content of approximately 15 ng/ml after 30 minutes, and typical steady-state levels of 30 ng/ml are observed with continued ST use (Holm et al., 1992). In comparison, plasma nicotine concentrations peak at 25 ng/ml approximately 5 minutes after smoking a cigarette before declining to 10 ng/ml 30 minutes after smoking the cigarette (Foulds et al., 2003).

Several ST product characteristics affect the rate and extent of nicotine absorption by altering nicotine release from the tobacco matrix (Ciolino et al., 2001). Pouch products slow nicotine release during the first few minutes of contact with saliva and cells in oral mucosal membranes (Nasr et al., 1998). Both the nicotine content of the product and the size of the pinch placed in the mouth will determine the total amount of nicotine available for release. The cut of the tobacco affects the exposed surface area, and resulting in faster release of nicotine for ST products with greater surface areas. Differences in nicotine absorption may also be due to user characteristics including individual differences in saliva pH, rate of salivation and expectoration, and mucosal features (Fant et al., 1999). There is evidence that the buccal mucosa barrier is altered during ST use, which affects its permeability to nicotine (Andersson et al., 1994; Squier, 1986; Tobey et al., 1988).

Plasma and urinary cotinine levels are routinely measured in studies of cigarette smokers to estimate systemic nicotine exposure. Unlike cigarette smokers, ST users may absorb nicotine through the gastrointestinal tract while swallowing tobacco extract. Nearly 80% of the nicotine that is absorbed from the intestine is metabolized to cotinine in the first pass through the liver and never reaches the systemic circulation (Ebbert et al., 2004). Plasma cotinine levels in ST users may overestimate and thus not accurately reflect systemic nicotine exposure. Serum nicotine concentration may be a more reliable method to assess systemic nicotine exposure in ST users (Ebbert et al., 2004).
Gas chromatography-mass spectrometry has been used to determine the quantities of the minor tobacco alkaloids in the urine of cigarette smokers and ST users (Jacob, III et al., 1993). Excretion of nicotine and its metabolite cotinine was similar in all tobacco users, but excretion of minor tobacco alkaloids [N′-Nitrosoanabasine (NAB), N′-Nitrosoanatabine (NAT), and nornicotine] was substantially greater in ST users, presumably because of the absence of pyrolysis of these alkaloids in ST products (Jacob, III et al., 1993). A more recent study also measured higher urinary levels of anabasine and anatabine in ST users than in cigarette smokers (Jacob, III et al., 1999).

Serum thiocyanate levels may be more useful to differentiate cigarette smokers from exclusive ST users (Holiday et al., 1995). Serum thiocyanate values are elevated in smokers because thiocyanate is the chief metabolite of hydrogen cyanide, which is more abundant in tobacco smoke than in ST. Therefore, ST users should have lower thiocyanate levels, although their exposure to environmental tobacco smoke may cause small increases in thiocyanate levels. Serum cotinine levels were similar for smokers (320.9 ± 201.1 ng/ml) and ST users (339.1 ± 327.5 ng/ml). Serum thiocyanate levels were in fact elevated in smokers (145.9 ± 63.7 μmol/L) but were lower for non-users of tobacco (58.2 ± 33.2 μmol/L) and ST users (32.0 ± 16.9 μmol/L).

5.2.2.2 Tobacco-specific nitrosamines

TSNAs including 4-(methylnitrosamo)-1-(3-pyridyl)-1-butanone (NNK), N′-nitrosoanabasine (NNN), and NNAL are the most abundant known carcinogens in ST products (Hecht, 2002). In 1986, Hoffman et al. estimated that levels of total TSNAs in popular US snuff brands (9,600–289,000 ppb) exceeded by at least 2 orders of magnitude the levels of N-nitrosamines in other consumer products. Harris (2001) reported that smoke from 26 brands of cigarettes produced using the Federal Trade Commission Protocol3 for cigarette smoke generation yielded ranges of 99.9–317.3 ng/cig NNN, 55.2–220.7 ng/cig NNK, 95.2–298.6 ng/cig NAT and 14.2–45.5 ng/cig NAB. Tobacco contains secondary and tertiary amines that can be nitrosated in saliva during use of ST when they react with nitrite in the presence of peroxidases and hydrogen peroxide and/or acidic conditions. The N-nitrosoproline test measures the potential for intragastric formation of carcinogenic nitrosamines in humans (Ohshima & Bartsch, 1981).

3 The Federal Trade Commission Protocol is a cigarette smoking machine procedure adopted in 1967 to measure yields of tar and nicotine in mainstream smoke. The method was modified in 1980 to include carbon monoxide.
TSNAs are the only known carcinogens in snuff that have been shown to produce oral tumors in laboratory animals (Hecht et al., 1986). Local application of NNK, NNN, and whole snuff produced oral cancer in rats (Hecht et al., 1986). The formation of TSNA-DNA adducts in the buccal mucosa was hypothesized to lead to DNA replication errors, particularly at sites of snuff application where chronic irritation and activation of inflammatory processes occur (Preston-Martin, 1991). The inflammation then causes cell damage and stimulates cell division, which in turn may enhance the probability that DNA miscoding will occur and that any errors will be reproduced (Preston-Martin, 1991).

NNK is an organoselective carcinogen in rat lung (Hecht, 2008). After intravenous administration to rats, NNK is rapidly reduced to (S)-NNAL, which binds to an unidentified receptor in the lung, whereas (R)-NNAL is rapidly glucuronidated and eliminated (Zimmerman et al., 2004). (S)-NNAL accumulates in the lung, where it is gradually released and reoxidized to NNK, which undergoes $\alpha$-hydroxylation and produces persistent $\sigma^6$-methyl-deoxyguanosine and pyridyloxobutyl-DNA adducts. NNK is readily reduced to (S)-NNAL in humans as well. Although accumulation of (S)-NNAL in human lung has not been reported, this enantiomer is more slowly excreted in human urine than is (R)-NNAL, and the elimination half-life of NNAL (40–45 days) in smokers is long for a water-soluble molecule (Carmella et al., 1999; Hecht et al., 1999).

### 5.2.2.2.1 Urine

The measurement of compounds in urine has certain advantages including availability of the sample in large quantity, reliable analytical methods, and non-invasive sampling. Urinary measurements indicate the relative extent of systemic carcinogen activation and detoxification. Measurement of urinary NNAL and its glucuronide conjugates (NNAL-Gluc) provides an estimate of the systemic delivery of a carcinogen dose in snuff users (Table 5-1). However, results of studies measuring nitrosamine levels in ST users vary, which makes it difficult to draw conclusions.

Correlations have been observed between (1) the number of tins or pouches of ST consumed per week and NNAL plus NNAL-Gluc in the urine of ST users (Hecht et al., 2002); (2) salivary cotinine and NNAL plus NNAL-Gluc in the urine of ST users (Hatsukami et al., 2003); and (3) the frequency and duration of ST use, particularly total dip duration, and NNAL plus NNAL-Gluc (Lemmonds et al., 2005). A trend was observed between the presence of oral leukoplakia and increasing total urinary NNAL levels (Kresty et al., 1996), which suggests that greater exposure to TSNAs from heavy ST product use may be associated with this lesion.
Recently, total NNAL levels were quantified in the urine of cigarette smokers \((n=420)\) and users of US ST products \((n=182; Copenhagen® [31.5\%], Skoal® [12.7\%], Kodiak® [47.0\%], and other brands [8.8\%])\) (Hecht et al., 2007). Multiple regression analysis that adjusted for age and sex showed that ST users had higher median levels of total NNAL per milliliter of urine than did smokers \((3.76 \text{ versus } 2.18 \text{ pmol/ml for males of median age 45, } p<0.0001)\). In a crossover study \((n=14)\), urinary NNAL levels in users of the hard snuff product Ariva® \((0.32; 95\% \text{ CI: } 0.2–0.4)\) were not significantly different from the very low urinary NNAL levels measured in users of the medicinal nicotine product Commit® \((0.34; 95\% \text{ CI: } 0.2–0.8)\) (Mendoza-Baumgart et al., 2007).

The finding of higher NNAL levels in ST users than in cigarette smokers (Hecht et al., 2007) makes it difficult to understand the observed trend of oral lesions occurring with increasing urinary NNAL levels (Kresty et al., 1996). Cigarette smoking was associated with a greater incidence of oral tumors than was ST use. However, NNAL levels were higher in ST users (Mendoza-Baumgart et al., 2007). Major conversion of TSNA may occur in the gastrointestinal tract of ST users, which makes it difficult to conclude that high TSNA levels are primarily responsible for oral lesions in ST users.

### 5.2.2.2.2 Saliva

Volatile nitrosamines and TSNA found in saliva of ST users might be leached out of ST products or formed endogenously from abundant precursors during chewing. Several studies observed TSNA in the saliva of ST users (Hoffmann & Adams, 1981; Nair et al., 1985; Prokopczyk et al., 1992; Wenke et al., 1984). Levels of TSNA in the saliva of habitual Swedish snuff dippers were analyzed every 10 minutes (Österdahl & Slorach, 1988). Detectable levels of at least two TSNA were found in all samples collected between 10 and 30 minutes after snuff had been placed in the mouth. Trace TSNA levels \((2–3 \text{ ng TSNA/g saliva})\) were found in the saliva 20 minutes after the snuff had been removed.

### 5.2.2.2.3 Hemoglobin adducts

Protein adducts have frequently been used as surrogates for DNA adducts because the two parameters tend to be correlated and protein \((i.e., \text{hemoglobin or albumin})\) is easy to obtain in large quantities (Hecht, 2002). Hemoglobin adducts of the TSNA 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) have been measured in blood samples taken from cigarette smokers, ST users, and non-users (Carmella et al., 1990; Schäffler et al., 1993). HPB adduct values were substantially higher in ST users \((517 \pm 538 \text{ fmol HPB/g hemoglobin})\) than in cigarette smokers \((79.6 \pm 189 \text{ fmol HPB/g hemoglobin})\) or non-users \((29.3 \pm 25.9 \text{ fmol HPB/g hemoglobin})\) (Schäffler et al., 1993).
Table 5-1. Tobacco-Specific Nitrosamine Levels in Biological Fluids – Urinea

<table>
<thead>
<tr>
<th>Reference</th>
<th>Product Type (Number of Subjects)</th>
<th>NNAL</th>
<th>NNAL-Gluc</th>
<th>Total NNALb,c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stepanov &amp; Hecht, 2005</td>
<td>Cigarettes (14) Smokeless (11)</td>
<td>NR</td>
<td>NR</td>
<td>1.53 ± 0.7</td>
</tr>
<tr>
<td>Hecht et al., 1999, 2002</td>
<td>Cigarettes Smokeless (13)</td>
<td>0.857 ± 0.514</td>
<td>1.84 ± 0.879</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>Smokeless</td>
<td>0.937 ± 0.813</td>
<td>2.61 ± 2.21</td>
<td>NR</td>
</tr>
<tr>
<td>Hecht et al., 2007</td>
<td>Cigarettes (420) Smokeless (182)</td>
<td>NR</td>
<td>NR</td>
<td>2.33 (2.19–2.47)</td>
</tr>
<tr>
<td>Mendoza-Baumgart et al., 2007</td>
<td>Cigarettes (20; crossover study)</td>
<td>NR</td>
<td>NR</td>
<td>1.58 (1.2–2.2)</td>
</tr>
<tr>
<td>Exalt® (snus)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>0.88 (0.7–1.2)</td>
</tr>
<tr>
<td>Commit® (medicinal nicotine)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>0.36 (0.2–0.7)</td>
</tr>
<tr>
<td>Mendoza-Baumgart et al., 2007</td>
<td>Cigarettes (14; crossover study)</td>
<td>NR</td>
<td>NR</td>
<td>1.02 (0.7–1.6)</td>
</tr>
<tr>
<td>Ariva® (hard snuff)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>0.32 (0.2–0.4)</td>
</tr>
<tr>
<td>Commit® (medicinal nicotine)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>0.34 (0.2–0.8)</td>
</tr>
<tr>
<td>Carmella et al., 2002</td>
<td>Cigarettes (10) Snuff (10)</td>
<td>0.462 ± 0.214</td>
<td>0.322 ± 0.161 (N)</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.48 ± 1.13</td>
<td>0.434 ± 0.343 (O)</td>
<td>NR</td>
</tr>
<tr>
<td>Kresty et al., 1996</td>
<td>Snuff and chewing tobacco (39)</td>
<td>0.92 ± 1.59</td>
<td>3.47 ± 5.86</td>
<td>NR</td>
</tr>
</tbody>
</table>

aNNAL measurements given as pmol/mg creatinine except as noted.
bTotal NNAL = NNAL + NNAL-Gluc.
c95% confidence interval is given in parentheses.

NR: not reported; (N): N-linked glucuronide conjugates; (O): O-linked glucuronide conjugates.
The analytical methods available to measure protein adducts are less reliable than those for urinary metabolites (Hecht, 2002). Numerous not detected values limit the value of published studies in this field.

5.2.2.3 Urine mutagenicity

The *Salmonella* urine mutagenicity assay has been used to screen for exposure to environmental mutagens, including tobacco smoke (Kriebel *et al.*, 1985). The use of US and Swedish ST products considered in this risk assessment is associated with less urinary mutagenic activity than is cigarette smoking. A study with *Salmonella* strain TA98 of the mutagenic activity of urine concentrate from Swedish *snus* users, cigarette smokers, and non-tobacco users found that smokers’ urine had significantly increased mutagenicity but that urine from *snus* users showed no significant difference from non-users’ urine (Curvall *et al.*, 1987). The mean mutagenic activity of smokers’ urine concentrates was $8.6 \times 10^3$ revertants/24 hours (CI: 4.2–17.6 x 10^3), which was significantly higher than corresponding values for snuff users, abstinent snuff users, and non-tobacco users, which were $1.3 \times 10^3$ (CI: 0.3–2.5 x 10^3), $1.3 \times 10^3$ (CI: 0.5–2.4 x 10^3), and $0.9 \times 10^3$ (0.4–2.2 x 10^3), respectively. An important finding was that no significant differences in mutagenic activity were found between urine from snuff users (whether using or abstaining from snuff) and urine from non-tobacco users.

Another study that compared mutagenicity of urine from cigarette smokers, US snuff users (Coppenhagen®, Skoal Bandits® Wintergreen, or Hawken® Wintergreen), loose-leaf tobacco chewers (Redman® loose-leaf tobacco or Redman® plug tobacco), and non-users gave similar results (Benowitz *et al.*, 1989). The urine of cigarette smokers ($76.5 \pm 25.4$ colonies/5 ml urine) was significantly more mutagenic than that of non-users ($15.6 \pm 4.1$ colonies/5 ml urine; $p<0.05$). No significant differences were observed between snuff users ($13.5 \pm 2.8$ colonies/5 ml urine) and non-users ($15.6 \pm 4.1$ colonies/5 ml urine). The value for loose-leaf tobacco users was slightly higher ($25.4 \pm 6.8$ colonies/5 ml urine) but not significantly greater than that of non-users.

5.3 BIOMARKERS OF EFFECT

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 and disease (Life Sciences Research Office, 2007b).
5.3.1 Cardiovascular Clinical Studies

Cardiovascular changes caused by ST use have been measured by means of various biomarkers of effect. The physiological processes that were examined include electrical cardiac activity and hemodynamics, endothelial function, atherosclerosis, inflammation, and lipid metabolism. The available data for ST use and biomarkers of effect can be summarized as follows (Asplund, 2003):

- Snuff use causes immediate increases in heart rate and blood pressure (Benowitz et al., 1988; Bolinder & de Faire, 1998; Wolk et al., 2005). It is unclear whether these changes have long-term deleterious effects on the heart and vascular tree;
- ST use leads to higher plasma epinephrine levels (Wolk et al., 2005). It is unclear whether ST use causes disturbances of heart rhythm and, if so, whether the risk for sudden death increases;
- Two studies found that snuff users, as opposed to smokers, do not have increased intima-media thickness (IMT) or atherosclerotic lesions when evaluated by ultrasonography (Bolinder et al., 1997; Wallenfeldt et al., 2001); and
- Studies of biochemical risk factors for cardiovascular disease—plasma fibrinogen (Bolinder et al., 1997; Eliasson et al., 1991, 1995), C-reactive protein (CRP) (Wallenfeldt et al., 2001), and serum lipids and lipoproteins (Bolinder et al., 1997; Eliasson et al., 1991; Wallenfeldt et al., 2001)—found significant differences between smokers and snuff users. These factors were significantly altered toward an elevated risk for smokers but not for snuff users. This raises a question as to whether individuals with cardiovascular problems are uniquely sensitive to ST.

5.3.1.1 Electrical cardiac activity and hemodynamics

Heart rate and blood pressure were studied in 10 healthy men, 24–61 years of age who were regular smokers, when they used either one of two brands of US moist snuff (Copenhagen® or Hawken® Wintergreen) or one of three brands of US chewing tobacco (Redman®, Days Work® plug tobacco, or Brown Mule® plug tobacco) (Benowitz et al., 1988). Maximal increases in heart rate and systolic blood pressure responses to ST were greater than those to cigarette smoking.

Hemodynamic and autonomic effects of US moist snuff (Copenhagen®) were investigated in 16 male snuff users (average age, 22 ± 1 years old) by using a randomized, controlled crossover design (Wolk et al., 2005). ST use
increased mean blood pressure by $10 \pm 1$ mmHg and heart rate by $16 \pm 2$ beats/minute during 30 minutes of use. No changes in peripheral vascular resistance, muscle sympathetic nerve activity, or plasma norepinephrine concentration were observed. The plasma epinephrine value increased by approximately 50%. The authors suggested that epinephrine is released from the adrenal gland in response to snuff use, which may have implications for both intravascular thrombosis and cardiac arrhythmias. Epinephrine activates platelets and is prothrombogenic. Epinephrine is also proarrhythmic in animal models and humans.

Short-term hemodynamic effects of Swedish snuff were studied in 9 subjects (8 males, 1 female; mean age, 27 years), 8 of whom were habitual snuff users (Hirsch et al., 1992). After at least 9 hours of abstinence from snuff, measurements were taken at 0, 15, and 30 minutes after snuff intake on 2 different days separated by 2–3 weeks. Snuff use induced a significant increase in heart rate and both systolic and diastolic blood pressures and a decrease in stroke volume during rest. Plasma nicotine and cotinine concentrations did not correlate with the observed hemodynamic changes.

Ambulatory 24-hour blood pressure monitoring was conducted in Swedish male ST users ($n=47$), smokers ($n=29$), and never users ($n=59$) (Bolinder & de Faire, 1998). Daytime ambulatory heart rates were significantly ($p<0.05$) elevated in both ST users (69 ± 14 beats/minute) and smokers (74 ± 13 beats/minute) compared with never users (63 ± 12 beats/minute). A positive relationship was found between cotinine and both systolic ($p<0.001$) and diastolic ($p=0.005$) blood pressures in ST users, whereas an inverse relationship was found in smokers.

### 5.3.1.2 Endothelial function

Endothelial function was analyzed in 20 Swedish snuff users (mean age, 34 ± 6 years old) by using ultrasound measurements of endothelial-dependent flow-mediated dilatation of the brachial artery (Rohani & Agewall, 2004). Brachial artery responsivity declined significantly (from 3.4 ± 2.0% to 2.3 ± 1.3%, $p<0.05$) 35 minutes after administration of 1 g of oral moist snuff. Heart rate and systolic and diastolic blood pressures increased significantly ($p<0.05$) after snuff administration.

### 5.3.1.3 Atherosclerosis

Atherosclerotic changes in Swedish moist snuff users were studied via ultrasonography to measure carotid and femoral IMT (Bolinder et al., 1997; Wallenfeldt et al., 2001). In the first study, long-term snuff users ($n=28$) did
not differ significantly from never users (n=40) with regard to carotid bulb IMT (0.80 ± 0.13 mm versus 0.78 ± 0.12 mm) or common carotid IMT (0.67 ± 0.11 mm versus 0.68 ± 0.11 mm), whereas smokers (n=29) had significantly increased wall measurements (carotid bulb 0.87 ± 0.19 mm, p=0.002; common carotid 0.74 ± 0.13 mm, p=0.03) compared with never users (Bolinder et al., 1997). A later study obtained similar results (Wallenfeldt et al., 2001). Current snuff users (n=48) did not differ significantly from never users (n=310) in carotid bulb IMT (1.04 ± 0.26 mm versus 0.99 ± 0.26 mm), common carotid IMT (0.82 ± 0.12 mm versus 0.80 ± 0.13 mm), or femoral artery IMT (1.12 ± 0.43 mm versus 1.05 ± 0.49 mm). Compared with never smokers, current smokers (n=96) had significantly increased carotid bulb wall measurements (0.95 ± 0.22 mm versus 1.05 ± 0.35 mm, p<0.05) and femoral artery wall measurements (0.87 ± 0.28 mm versus 1.31 ± 0.62 mm, p<0.001).

### 5.3.1.4 Inflammation

Three Swedish studies measured plasma fibrinogen levels to evaluate inflammation in snuff users (Bolinder et al., 1997; Eliasson et al., 1991; 1995). Unlike cigarette smokers, snuff users did not have significantly altered fibrinogen levels compared with never users. A more recent study also found that although cigarette smoking was associated with elevated CRP levels, Swedish moist snuff use was not (Wallenfeldt et al., 2001).

### 5.3.1.5 Lipid metabolism

Three studies considered changes in lipid metabolism related to Swedish moist snuff use (Bolinder et al., 1997; Eliasson et al., 1991; Hirsch et al., 1992; Wallenfeldt et al., 2001). No significant differences in total serum cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, or apolipoprotein A-I levels were observed in snuff users versus never users. One study found an association between snuff use and elevated triglyceride levels (Wallenfeldt et al., 2001).

### 5.3.2 Cytological and Cytogenetic Changes

ST use causes cytological changes within the oral cavity. The prevalence of somatic cell genetic damage in the oral cavity of never users (n=24) and users (n=24) of ST (US smokeless products, not otherwise specified) was evaluated (Livingston et al.1990). The frequency of micronucleated cells was significantly (p<0.01) higher in the labial mucosa of exposed (2.22%) compared with unexposed (0.27%) individuals. The frequency of micronuclei varied widely between exposed subjects but was higher in heavily exposed (2.48%) than in lightly exposed (1.29%) individuals as measured by saliva
cotinine levels. Morphological classification of epithelial cell nuclei showed that the occurrence of cells with normal nuclear structure was significantly reduced ($p<0.01$) in exposed individuals. Unlike the case with cigarette smoking, exposure to ST did not affect peripheral lymphocyte sister chromatid exchange frequency (Livingston et al., 1990).

### 5.3.3 Reproductive Effects

Adverse effects possibly associated with snuff use during pregnancy include lower birth weight, preterm delivery, and pre-eclampsia. Reproductive effects were examined in female Swedish snuff users ($n=789$), cigarette smokers ($n=11,240$), and non-tobacco users ($n=11,495$) (England et al., 2003). Compared with non-tobacco users, adjusted mean birth weight was reduced for snuff users by 39 g (95% CI: 6–72 g) and for smokers by 190 g (95% CI: 178–202 g). The occurrence of preterm delivery increased for snuff users and smokers (adjusted ORs, 1.98 [95% CI: 1.46–2.68] and 1.57 [95% CI: 1.38–1.80], respectively). Pre-eclampsia frequency was reduced in smokers (adjusted OR, 0.63; 95% CI: 0.53–0.75) but increased in snuff users (adjusted OR, 1.58; 95% CI: 1.09–2.27). These results suggest that snuff use during pregnancy is not without risk and may result in lower birth weight, preterm delivery, or pre-eclampsia.

### 5.3.4 Summary of Smokeless Tobacco Biological Effects

Available data from the few studies measuring biomarkers of effect in small numbers of subjects suggest the following:

- ST use causes certain changes in electrical cardiac activity, hemodynamics, and endothelial function. In contrast, measures of atherosclerosis, inflammation, and lipid metabolism in ST users are not significantly different from those in non-users;
- ST use leads to cytological changes in the oral cavity; and
- Snuff use during pregnancy is associated with possible adverse effects, including lower birth weight, preterm delivery, and pre-eclampsia.

### 5.4 CONCLUSIONS

Biomarker of exposure studies suggest that ST products and cigarettes have similar nicotine delivery but that ST users have higher plasma cotinine levels than do cigarette smokers. ST users also have higher levels of NNAL per milliliter of urine than cigarette smokers. ST users have less urine mutagenicity and lower serum thiocyanate levels than cigarette smokers. ST use and cigarette smoking result in similar maximal increases in heart
rate, however ST alters integrated heart rate and systolic blood pressure to a greater extent than does cigarette smoking.

Biomarker of effect studies indicate that in contrast to cigarette smokers, snuff users do not have increased IMT or atherosclerotic lesions. Whereas smokers’ plasma fibrinogen, C-reactive protein and serum lipids and lipoproteins are significantly altered toward an increased risk for cigarettes smokers, there are no such alterations for ST users. ST use increased frequency of micronucleated cells. Unlike cigarette smoking, ST use did not increase peripheral lymphocyte sister chromatid exchange frequency. Snuff may also affect pregnancy outcomes by increasing the risk of preterm delivery, preeclampsia, and low birthweight.
6.1 SMOKELESS TOBACCO USE PATTERNS

Concerns have been raised that smokeless tobacco (ST) use could increase the occurrence of tobacco consumption if people who did not previously use tobacco start to use ST (Haddock et al., 2001; Tomar, 2003a, 2003b) or if current cigarette smokers take up ST use in addition to smoking (Tomar et al., 2003). Tobacco use characteristics such as frequency and duration of use can also affect individual exposure to ST toxins and influence the risk of adverse health effects. This chapter attempts to identify differences in population and individual behaviors related to ST and cigarette use that may help distinguish between cigarettes and ST products and among different ST products.

6.1.1 Population Use Patterns

6.1.1.1 The US

The predominant form of tobacco used before 1900 was ST (Christen et al., 1982; Fisher et al., 2005). The invention of the cigarette rolling machine in 1880 resulted in mass production of cigarettes (Rouse, 1989). This, combined with the fear that tuberculosis and other airborne diseases could be spread by spitting tobacco juices (Rouse, 1989) and the issuing of cigarettes as a part of soldiers’ rations during World War I, led to the later decline in ST use, during the 1950s and 1960s (Capehart, 2005; Christen et al., 1982; Fisher et al., 2005).

The resurgence in popularity of ST during the 1970s and 1980s is largely attributed to innovative, wide-scale advertising and marketing campaigns and the introduction of new brands by the ST industry (Capehart, 2005; Connolly, 1995; Giovino et al., 1994; Nelson et al., 2006). However, reports from the Consensus Conference (1986) and the Advisory Committee to the Surgeon General on ST (1986a) raised public awareness that ST was a major public health problem (Fisher et al., 2005; Grunbaum et al., 2002; National Cancer Institute & Smoking and Tobacco Control Program, 1992; Nelson et al., 2006; U.S. Department of Health and Human Services, 1994a). ST use thereafter declined. In 1986, the US Congress passed the Comprehensive Smokeless Tobacco Health Education Act, which required
ST products to carry warning labels and banned ST advertising “… on any medium of electronic communications …” (U.S. Congress, 1986). However, this decline was short-lived; by 1988, sales and use of ST products had once again increased (Glover & Glover, 1992).

A study of tobacco use prevalence by the 2005 National Health Interview Survey (NHIS) defined a current ST user as an individual who reported using chewing tobacco or snuff at least 20 times during his or her lifetime and who used chewing tobacco or snuff every day or some days (Centers for Disease Control and Prevention, 2006). An analysis of NHIS data by the Centers for Disease Control and Prevention (CDC) revealed that, in 2005, 2.3% (95% CI: ± 0.3) of the US population were current ST users. ST use prevalence was higher for men (4.5%) than for women (0.2%). In comparison, in 2005, 20.9% of US adults were current cigarette smokers (defined as having smoked at least 100 cigarettes in their lifetimes and currently smoking every day or some days) (Centers for Disease Control and Prevention, 2006).


In 2005, an estimated 7.7 million Americans 12 years of age or older (3.2% of the population in that age range) had used ST in the previous month (Substance Abuse and Mental Health Services Administration, 2006). However, ST use clusters within specific demographic groups. In the US, ST use is primarily a behavior of men, young adults, whites, Native Americans/Alaska Natives, people who live in the South or West, people who live in rural communities, and individuals with lower education levels (Centers for Disease Control and Prevention, 1993; Howard-Pitney & Winkleby, 2002; Marcus et al., 1989; Nelson et al., 1996; Tomar, 2002). Professional athletes, particularly professional baseball players, have historically had high ST use (Connolly et al., 1988; Ernster, 1989; Ernster et al., 1990).

The greatest increase in ST use during the last 20+ years has been seen in adolescents and young adults 18–24 years old and individuals 75 years of
age and older (Spangler & Salisbury, III, 1995). This increase has coincided with aggressive advertising of new ST products that appeal to young males (Centers for Disease Control and Prevention, 1993). Among male adolescents who use ST, whites and Hispanics are more likely to use ST than are blacks and Asians, with the percentage of users highest for Native Americans who begin the habit at an early age (Johnson & Squier, 1993; Wang et al., 1994). ST use is highest in the southern region of the US for adolescent boys and girls regardless of economic status (Wang et al., 1994).

The pattern of use of different types of ST products in the US has changed. In 1970, chewing tobacco was the most common form of tobacco used. However, chewing tobacco and dry snuff sales have decreased (Tomar, 2003a). Between 1986 and 2003, sales of chewing tobacco decreased by 49% and sales of dry snuff decreased by 67% (Nelson et al., 2006). Moist snuff is the sole tobacco product for which sales have increased yearly since the mid-1980s (Tomar, 2003c). Between 1986 and 2003, sales of moist snuff products increased by 87% (Nelson et al., 2006).

6.1.1.2 Sweden

Although chewing tobacco is also sold in Sweden, sales of it constitute only 1% of snus sales (Foulds et al., 2003). The sale of some types of ST, including snus, is banned in other parts of the European Union. In Sweden since the mid-1970s, snus consumption has increased significantly but cigarette smoking has decreased significantly. In 1976, 40% of Swedish men were daily smokers, compared with 15% in 2002. During the same time period, daily snus use by men increased from 10% to 23%. A cross-sectional study of 31,213 Swedish male and female twins conducted between 1998 and 2002 reported that 63% of participants were ever users of cigarettes, 39.3% were former smokers, and 23.7% were current smokers (Furberg et al., 2006). Of the men, 30.4% were ever users of snus; only 2.5% of women were ever users of snus. Snus has been suggested as having played a major role in the decline in smoking in Sweden (Bates et al., 2003; Foulds et al., 2003). However, other contributing factors may include the Swedish government’s campaigns against smoking that started in the 1970s and continue to date, as well as additional social and cultural factors (Henningfield & Fagerström, 2001). Snus use is more common among higher socioeconomic groups than is cigarette smoking (VECA Research and Consulting., 2001).

6.1.2 Individual Use Patterns

The 1986 Report of the Advisory Committee to the Surgeon General concluded that ST is an addicting drug (U.S. Department of Health and
Behavioral Considerations

The definition of addiction varies. Another 1986 US Surgeon General’s report noted that ST shares characteristics with other addictive drugs such as cocaine and heroin (U.S. Department of Health and Human Services, 1986b). ST provides users with psychoactive, dependence-producing levels of nicotine to which they develop tolerance over time (Tomar et al., 1995) and therefore seek higher and more frequent doses. Users experience withdrawal symptoms when they stop using ST (Hatsukami et al., 1987).

Use behaviors of ST products include the frequency of consumption, the amount of product used, and the length of time the product is kept in the mouth. Examples of measures of ST use are the number of tins of product used per week, number of dips used per day, dip duration, and total dipping time (amount of time from the first daily dip of tobacco to the last dip) (Lemmonds et al., 2005).

Some measures of use behavior are better indicators of exposure than others. Nicotine exposure was better reflected by the total dip duration per day and total number of dips than by the number of days a container of ST lasted and the number of pouches of ST used per day (Hatsukami et al., 1987, 1988, 1991b; Lemmonds et al., 2005). Another smaller study showed that saliva cotinine concentration correlated with the total number of dips per day, minutes per dip, days per can, and weight per dip (Severson et al., 1990).

Age may affect ST use behavior and length of use. Lemmonds et al. (2005) reported that the mean daily duration of ST use was 7.2 hours for subjects who were 21–65 years old. Other studies have reported daily ST use durations of 4.2 hours for subjects 18–30 years old (Hatsukami et al., 1988) and 4.7 hours for a population of ST users with a mean age of 20.5 years (SD=2.3) (Hatsukami et al., 1991b).

US male adolescent snuff users reported consuming one tin or can of ST on average every 5.1 days. They reported taking a mean of 5.3 dips/day, with an average dip size of 1.3 g, and held each dip in the mouth for 10–20 minutes (Ary et al., 1987). Another study of young adult females reported

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4 As defined by the National Institute on Drug Abuse, addiction is a chronic, relapsing disease characterized by compulsive drug seeking and abuse and by long-lasting neurochemical and molecular changes in the brain (National Institute on Drug Abuse, 2006).

5 A dip is a pinch of ST that is placed between the cheek or lip and the gum (Brunnemann & Hoffmann, 1992).
Differentiating the Health Risks of Categories of Tobacco Products

that daily ST users \((n=18)\) took a mean of 3.6 dips/day, with a mean mass of 1.64 g \((\text{range}=0.2–4.6\) g), and held each dip in the mouth for a mean duration of 22 minutes \((\text{SD}=9.6)\). Each can lasted an average of 6 days \((\text{range}=2\) days–3 months) (Boyle et al., 1998).

On their first use of ST, 74% of adolescent boys reported general negative physical effects: 32.1% felt sick and 30.3% felt dizzy. After their second use, 53% reported general negative physical effects from ST (Ary et al., 1989).

6.2 COMPARISON OF SMOKELESS TOBACCOS

6.2.1 Nicotine Pharmacokinetics

Nicotine is a primary reason for maintenance of a tobacco use habit. The nicotine dose is determined by the following:

- Amount of tobacco used;
- Length of time it remains in the mouth;
- pH of the product (determines the ratio of unprotonated nicotine to free nicotine);
- pH of the saliva;
- Position of tobacco in the mouth;
- How much the product is moved around in the mouth;
- Presence of a membrane between the product and the mucosa (Fant et al., 1999); and
- Nicotine content of the product and other product constituencies.

Because non-ionized nicotine crosses membranes more readily than does ionized nicotine, products with higher pH (which contain more non-ionized nicotine) have better nicotine delivery efficiency than products with lower pH. The pH of ST products has been determined by placing a sample of ST in distilled water, weighing the mixture, and measuring the pH of the mixture with a pH meter (Richter & Spierto, 2003). ST product manufacturers can alter the pH of the product by fermenting the tobacco, adding alkali buffer, or changing the product’s moisture content (Connolly, 1995).

ST products have a range of pH values. A surveillance study conducted by the CDC revealed a mean pH of 7.26 for 8 of the best selling moist snuff tobacco products in the US (Richter & Spierto, 2003). The pH of snus is 7.5 to 8.5 (Andersson, 1991; Hirsch & Thilander, 1981). A survey of 8 STs in the loose moist snuff and tobacco pouch category reported a lowest mean pH \((\pm \text{SD})\) for Hawken® Wintergreen moist snuff of 5.35 \((0.04)\) and the highest mean pH \((\pm \text{SD})\) of 8.28 \((0.03)\) for Kodiak® Wintergreen (Richter & Spierto,
For the 10 loose-leaf tobacco (chewing) products included in the study, the lowest mean pH (± SD) was 5.33 (0.05) for Chattanooga Chew® Premier Flavor and the highest mean pH (± SD) was 6.41 (0.02) for Levi Garrett®. The brands of moist snuff and the tobacco pouch category studied accounted for 91% of the market share for moist snuff; the loose-leaf tobacco products studied represented 74% of the market share for loose-leaf tobacco products in the US.

Other product characteristics such as the size of tobacco cuttings and inclusion of binding ingredients also influence the efficiency of nicotine dose delivery (Connolly, 1995; Henningfield et al., 1995). According to Hatsukami and Severson (1999), long cut ST products incorporate bigger pieces of tobacco and include a binder that allows tight packing of the tobacco, which results in slower nicotine release compared with short cut ST products. Packaging of ST products in teabag-like containers (e.g., sachets or pouches) limits nicotine absorption (Connolly, 1995; Hatsukami & Severson, 1999). Chewing tobaccos tend to be more coarsely cut and acidic and have a lower moisture content than moist snuff products. Exposure to saliva results in moistening of the chewing tobacco, an increase in pH, and an increase in the speed of nicotine delivery (Henningfield et al., 1997).

The abuse liability of different ST products may also provide insight into their potential population effects. Abuse liability refers to the potential for a drug to be abused. The major determinants of abuse liability of a drug are the speed at which it reaches the brain and the magnitude of its effect (Hatsukami & Severson, 1999). Drugs that reach the brain faster and have a higher increase in nicotine levels have greater abuse liabilities. Nicotine is a psychoactive drug and as such its abuse liability is partly determined by its bioavailability and pharmacokinetics (Henningfield & Keenan, 1993). Henningfield and Keenan (1993) determined that slower nicotine delivery systems seem to have significantly lower abuse liability than faster delivery systems. The patterns of nicotine absorption are different for cigarette smoking, ST consumption, and nicotine gum use. During cigarette smoking, blood nicotine levels peak within 5 minutes and fall rapidly when the cigarette has been consumed because nicotine is distributed from the pulmonary vasculature to the tissues (Benowitz et al., 1988). Nicotine levels then fall more gradually after the initial rapid decline, which reflects equilibrium kinetics among different tissue compartments. Studies examining nicotine pharmacokinetics for moist snuff products are found below.

Fant et al. (1999) studied nicotine levels before and after consumption of 4 brands of moist snuff products (Skoal® Bandit pouch, Copenhagen®, Skoal®
Long Cut Cherry, and Skoal® Original Wintergreen) and 1 of 2 non-tobacco snuff products. The moist snuff products had the same amount of nicotine but different pH values. Subjects avoided tobacco use for 3 hours before the beginning of each of the 5 sessions. Subjects then used 1 Skoal® Bandit pouch containing approximately 0.5 g tobacco (pH 6.9) or 2 g of Copenhagen® (pH 8.6), Skoal® Long Cut Cherry (pH 7.5), or Skoal® Original Wintergreen (pH 7.6), or either non-tobacco Smokey Mountain® Snuff or Oregon® Mint Snuff. The product was placed between the cheek and gum and held there for 30 minutes, with subjects expectorating as desired. Subjects then removed the tobacco and rinsed their mouths with water.

The time to reach a nicotine concentration of 10 ng/ml was faster for Copenhagen® (4 minutes) than for Skoal® Original Wintergreen (10 minutes) and Skoal® Long Cut Cherry (15 minutes). The mean nicotine level before ST use was 5.3 ng/ml. Copenhagen® produced the highest mean increase in nicotine levels (19.5 ng/ml; SEM=4.1), whereas the mean nicotine boosts for the Skoal® Bandit pouch, Skoal® Long Cut Cherry, Skoal® Original Wintergreen, and Mint Snuff were 4.2 ng/ml (SEM=1.4), 14.9 ng/ml (SEM=3.0), 14.9 ng/ml (SEM=2.4), and 0.9 ng/ml (SEM=0.4), respectively. Significant inter-individual variation in nicotine absorption occurred, even though the same nicotine dose was placed in the mouth. The authors proposed that this variation may have been caused by differences in saliva pH, salivation and expectoration rates, and mucosa absorption kinetics of different individuals (Fant et al., 1999). Absorption continued after removal of snuff from the mouth, possibly because of the slow release of nicotine from the mouth mucosa or uptake of swallowed nicotine from the gut.

Nicotine absorption is slower during ST use than during smoking; however, the mean maximal venous blood nicotine concentrations were reportedly similar (14.3 ng/ml) (Benowitz et al., 1988). During ST use, nicotine levels also peaked within 5 minutes of the start of use, but absorption continued for 30 minutes after oral snuff or chewing tobacco was removed, with plasma levels declining to baseline by 90 minutes. The mean nicotine concentration before tobacco consumption for 10 regular smokers after abstaining from tobacco use and food consumption overnight was 2.7 ng/ml (Benowitz et al., 1988). Subjects absorbed an estimated nicotine dose of 1.8 mg after smoking 1 cigarette, 3.6 mg after using 2.5 g of oral snuff, 4.5 mg after chewing tobacco (mean mass=7.9 g [range=0.9–17.8 g]), and 1.9 mg after chewing nicotine gum. Worldwide, smokers inhale approximately 1–1.4 mg nicotine/cigarette (Fagerström, 2005).
Holm et al. (1992) reported that placement of a 2-g pinch of ST in the mouth of 10 ST users resulted in a mean venous blood nicotine level of 9.9 ng/ml after 10 minutes and a maximal nicotine level of 14.5 ng/ml. Twenty-seven regular snuff users had a maximal blood nicotine concentration of 36.6 ng/ml (SD=14.4), and 35 cigarette smokers had similar a maximal blood nicotine concentration of 36.7 ng/ml (SD=16.1) (Holm et al., 1992).

Few studies have compared nicotine pharmacokinetics of different types of ST products. Kotlyar et al. (2007) compared nicotine pharmacokinetics and subjective responses to 3 types of modified ST products (Ariva®, Stonewall®, and Revel®), moist snuff (Copenhagen®), and the Commit® nicotine lozenge (Table 6-1). The 10 study participants who completed the analysis had been regular Copenhagen® moist snuff users for more than 1 year. Subjects participated in 5 sessions and were assigned to use 1 type of modified ST, the moist snuff, and the nicotine lozenge in random order. Subjects abstained from using tobacco 12 hours before the start of the study. During the study, they used the product for 30 minutes. Nicotine exposure was significantly higher ($p<0.001$) after subjects used Copenhagen® [area under the concentration-time curve (ng x min/ml)=1,038 (range=806–1,336)] than after they used the other products. Areas under the curve for the 3 modified ST products were not significantly different. Craving for nicotine during use of Copenhagen® was significantly lower than that during use of the other products. Subjects also reported liking Copenhagen® more, with more positive effects during its use.

Table 6-1. Area Under the Concentration-Time Curve and Maximal Plasma Nicotine Concentrations After Use of Moist Snuff, Modified Smokeless Tobacco Products, or Commit® Lozenge

<table>
<thead>
<tr>
<th>Product</th>
<th>Area Under Curve* (AUC) 0–90 min, ng x min/ml</th>
<th>$C_{\text{max}}^*$, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Revel®</td>
<td>189 (146–244)</td>
<td>2.6 (2.0–3.5)</td>
</tr>
<tr>
<td>Ariva®</td>
<td>192 (149–248)</td>
<td>2.7 (2.0–3.6)</td>
</tr>
<tr>
<td>Stonewall®</td>
<td>292 (226–376)</td>
<td>4.1 (3.1–5.4)</td>
</tr>
<tr>
<td>Commit®</td>
<td>467 (361–604)</td>
<td>7.3 (5.5–9.8)</td>
</tr>
<tr>
<td>Copenhagen®</td>
<td>1,038 (806–1,336)</td>
<td>16.1 (12.1–21.5)</td>
</tr>
</tbody>
</table>

Adapted from Kotlyar et al. (2007).

*Range in parenthesis

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6 Ariva® and Stonewall® are hard snuff products.

7 Revel® is a low-nitrosamine moist ST.
**Snus** is packed in pouches or sachets. The pH of Swedish *snus* is typically more alkaline (range = 7.8–8.5) (Andersson et al., 1994) than that of US snuff (range = 5.6–7.3) (Brunnemann & Hoffmann, 1992). Lunell and Lunell (2005) compared nicotine pharmacokinetics for 1 g of General® *snus* (pH 8.4), 1 g of Catch® Licorice (pH 8.5), 0.5 g of Catch® Mini (pH 8.4), 0.3 g of Catch® Dry Mini (pH 7.3) and 2 mg of Nicorette® gum. Each of the 11 non-smoking male subjects participated in five 12-hour sessions, during which they were provided with multiple doses of 1 product. A different product was tested each session. Subjects positioned and kept the *snus* between the gum and the upper lip for 30 minutes. The mean nicotine extracted\(^8\) for General® *snus*, Catch® Licorice, Catch® Mini, Catch® Dry Mini, and Nicorette® gum were 31%, 22%, 44%, 22%, and 44% of the dose, respectively (Lunell & Lunell, 2005). The maximal blood nicotine levels after use of General® *snus* were significantly \((p=0.002)\) higher than levels after use of the other products. Plasma nicotine levels for Catch® Dry Mini were similar to levels after smoking 7–10 cigarettes/day, and hourly doses of Catch® Mini and Catch® Licorice produced nicotine levels similar to levels after smoking 15–20 cigarettes/day. General® *snus* produced plasma nicotine levels similar to those produced by smoking 25–40 cigarettes/day.

Within 10 minutes of nasal snuff consumption, plasma nicotine levels were similar to levels after smoking a single cigarette (Russell et al., 1981). The ST use experience of the nasal snuff user is thought to influence nicotine uptake, because daily users had higher nicotine levels (12.6 ng/ml) than occasional users (individuals who typically used nasal snuff less than once a week) (2.0 ng/ml) and new users. Some snuff users were also cigarette smokers.

### 6.2.2 Initiation

Approximately 75% of adult users of cigarettes, cigars, and ST report that they first used tobacco between 11 and 17 years of age (Riley et al., 1996a, 1996b; U.S. Department of Health and Human Services, 1994a, 1994b). Tenth grade students stated that curiosity, social group activity, peer pressure, and availability of ST were the most common reasons for trying ST. A study of male adolescents reported that the average age at first use of ST was 11.2 years (Ary et al., 1987) and that 68.8% of the boys acquired their first ST product from a peer. Most adolescents receive their cigarettes through a social source (White et al., 2005).

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\(^8\) The mean nicotine extracted was the difference between the amount of nicotine in unused *snus* and the amount left in *snus* that had been used.
Some tobacco companies have developed what critics have called starter brands of ST, such as Skoal® Bandits and Skoal® Long Cut, which appear to some to be especially marketed to youth (Connolly, 1995). These ST products have lower levels of nicotine, which reduces the likelihood of negative effects, such as nausea and dizziness, during the initial stages of ST use. These products are also highly flavored, which dampens the tobacco taste, and the tobacco has a longer cut, which may reduce swallowing of the product and floating of tobacco around the mouth (Henningfield et al., 1997). Concerns have been expressed that these products could increase tobacco product use by youth. Foulds and Furberg (2008) recently reported that a new type of US snus, Marlboro® snus, which has a lower nicotine concentration than Swedish snus products, may be a graduation product (i.e., one from which novice users move to STs with higher amounts of nicotine.)

A study of Swedish tobacco product users reported that 91% of smokers initiated daily smoking before age 23, whereas 33% of daily snus users started using snus after age 22 (Ramström & Foulds, 2006). The pattern of tobacco product initiation is changing in Sweden. The younger cohort more commonly starts with snus. In contrast, the older cohort more often started with cigarettes. Riley et al. (1996b) reported that individuals who initiated tobacco use with chewing tobacco were more likely to shift to using moist ST than vice versa.

6.2.3 Gateway to Cigarettes

Concerns have been expressed that individuals who had never been smokers who start to use ST and become addicted to nicotine have an increased likelihood of becoming cigarette smokers than those who had not used ST (Haddock et al., 2001; Institute of Medicine, 2001; National Cancer Institute & Smoking and Tobacco Control Program, 1992; Tomar, 2002, 2003a). If this were the case, ST use would be a causal gateway to cigarette smoking. Table 6-2 presents findings from studies that support this theory and those that do not support it. Some of these are discussed below.

Most studies support the idea that ST is not a gateway to cigarette smoking. Tomar (Tomar, 2003a) analyzed data from the 1989 Teenage Attitudes and Practices Survey (TAPS-I) and the 1993 follow-up study (TAPS-II). Subjects were questioned about whether they chewed tobacco or used snuff and whether they currently used any of these products. Questions about chewing tobacco and snuff were not asked separately.
Tomar (2002) also analyzed data from the 1998 NHIS. Data were collected during personal interviews of 13,865 men about their cigarette and ST use. Former snuff users were more likely to become smokers (39.4%) than men who used snuff on some days (38.9%) or every day (19.2%) or who had never used snuff (25.4%).

Ramström and Foulds (2006) studied trends in smoking and *snus* use in Sweden to determine whether *snus* use leads to smoking. They analyzed data from a cross-sectional survey of 3,238 men and 3,514 women conducted from 2001 to 2002. The likelihood of starting daily smoking was lower for tobacco users who had initiated tobacco use with *snus* than for those who had started with cigarettes.

Furberg *et al.* (2005) analyzed data from the screening across lifespan twin study of 14,932 men in the Swedish Twin Registry to assess whether *snus* use was associated with smoking initiation or smoking cessation. The authors developed categories of *snus* users. Men who currently or before the study “smoke(d) at parties” ($n=602$), “smoke(d) now and then” ($n=669$), or “smoke(d) regularly” ($n=7,880$) were “ever smokers”. “Never smokers” were participants who “only tried cigarettes” or “never smoked.” Study participants were “ever *snus* users” if they used or formerly used *snus* “now and then” or “regularly.” The questionnaire did not define “regular” or “now and then”; participants categorized themselves. Regular *snus* users were less likely to start smoking (4.1% became smokers) than were men who had not used ST before smoking (18.5%) (Furberg *et al.*, 2005). Of those participants who were ever smokers, 0.5% had used *snus*; of those who were never smokers, 1.1% had used *snus*.

### 6.2.4 Psychological Correlates Associated with Use of Smokeless Tobacco

According to 10th grade students, curiosity, social group activity, peer pressure, and product availability were the most common reasons for trying ST. A risk-taking attitude was linked to experimenting with ST but not with maintained use of ST (Riley *et al.*, 1991). One US study reported that this was also the case for cigarette smoking. Eighty-three percent of boys who used ST daily also used alcohol, marijuana, or cigarettes (Ary *et al.*, 1987).

Perceptions about ST may also affect young people’s behavior. Parents are more likely to provide ST and have a more favorable view of ST than of cigarettes (Henningfield, 1995). A study of US teenage boys who were non-users of tobacco reported that although their parents and friends would
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Participants</th>
<th>Study Design and Findings</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haddock et al., 2001</td>
<td>Males, average age 20, (n = 7,865) Individuals entering US Air Force Basic Training (n = 14,340) never smokers and 21,690 initial participants</td>
<td>Men were asked about tobacco use during basic training and after 1 year. ST users were 2.27 times more likely than those who had never used ST to start smoking. Former ST users were 2.33 times more likely to start smoking.</td>
<td>ST use was a significant gateway to smoking in this population. Smoking initiation was greater than that in civilian population. Note: Duty assignment location does not appear to be a factor in the analysis nor have a role in availability of tobacco and social norms of tobacco use in areas where men were posted overseas.</td>
</tr>
<tr>
<td>Tomar, 2003a</td>
<td>Males, ages 12–18, (n = 3,996) TAPS-I and -II (1989 and 1993) (CDC, US)</td>
<td>After 4 years the risk of regular ST users to start smoking was 3.45. After 4 years the risk of former ST users to start smoking was 2.01.</td>
<td>ST use was a significant gateway to smoking in this population. Note: This population was young, and the follow-up period was only 4 years.</td>
</tr>
<tr>
<td>O'Connor et al., 2003</td>
<td>Males, ages 12–18, (n = 3,996) TAPS-I and -II (1989 and 1993) (CDC, US)</td>
<td>Reanalysis of data published by Tomar (2003a). Analysis included known factors in a multivariate model for predicting smoking in 4 years smoking. Regular ST use had OR = 1.68 Non-regular ST use had OR = 1.41</td>
<td>The authors concluded that complex behaviors required multivariate models and that the simplistic approach of Tomar was not reliable. ST was not a predictor of subsequent smoking. Note: Tomar had concluded that ST users were 3.45 times more likely to start smoking than non-ST users.</td>
</tr>
<tr>
<td>Reference</td>
<td>Study Participants</td>
<td>Study Design and Findings</td>
<td>Conclusion</td>
</tr>
<tr>
<td>-------------------</td>
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<td>------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>O’Connor et al., 2005</td>
<td>Males, ages 22–34 n = 7,956</td>
<td>Excluded prior smoking before considering ST as a gateway.</td>
<td>Little evidence that causal effects of ST use to initiate smoking has changed since 1987.</td>
</tr>
<tr>
<td></td>
<td>NHSDA 2000 (US)</td>
<td>ST risk for smoking initiation was 1.39.</td>
<td>Significant overlap between chewing tobacco and snuff suggests experimentation. It may also suggest that concurrent smoking is coincidental rather than causal.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Snuff risk for smoking initiation was 1.09.</td>
<td>ST is a weak predictor of smoking.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chewing tobacco risk for smoking initiation was 1.69.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Snuff was a non-significant causal gateway to smoking; chewing tobacco and combined ST had minor effects.</td>
<td></td>
</tr>
<tr>
<td>Rodu et al., 2002</td>
<td>Males, mean age 45.4 Females, mean age 45</td>
<td>Studied trends in smoking and ST use.</td>
<td>Tobacco use remained stable in northern Sweden, but ST use increased and smoking decreased in males.</td>
</tr>
<tr>
<td></td>
<td>males n = 2,998 Females n = 3,092</td>
<td>Use of ST increased in males from 22% to 30%, whereas smoking decreased from 23% to 14%.</td>
<td>A significant number of smokers switched to ST use during that time, but fewer ST users started smoking.</td>
</tr>
<tr>
<td></td>
<td>Cohort study in northern Sweden: 1988, 1990, 1884, and 1999</td>
<td>Smoking decreased moderately in females from 27% to 22%, and ST use remained stable at 6%.</td>
<td>ST use appears to have contributed to the lower rate of smoking in males in northern Sweden.</td>
</tr>
</tbody>
</table>
Table 6-2. Summary of Selected References on a Smokeless Tobacco Gateway to Cigarettes (continued)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Participants</th>
<th>Study Design and Findings</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furberg et al.,</td>
<td>Males, born before 1959</td>
<td>ST use was inversely related to smoking initiation (OR=0.2).</td>
<td>ST use did not lead to smoking in this survey. ST use was associated with smoking cessation but not smoking initiation.</td>
</tr>
<tr>
<td>2005</td>
<td>(n = 14,424)</td>
<td>Regular ST use was more prevalent in men who never smoked compared with men who had smoked.</td>
<td>Note: Authors stated that cross-sectional data correlations do not necessarily indicate causal relationships.</td>
</tr>
<tr>
<td></td>
<td>SALT (cross-sectional twin survey, Sweden)</td>
<td>ST users were 3.7 times more likely to be former smokers than current smokers.</td>
<td></td>
</tr>
<tr>
<td>Ramström &amp; Foulds,</td>
<td>Males, ages 16–79</td>
<td>Male snus users were less likely to start smoking than non-snus users (OR=0.28).</td>
<td>ST (snus) reduced the risk of smoking initiation in this Swedish male population and improved the odds of smoking cessation.</td>
</tr>
<tr>
<td>2006</td>
<td>Females, ages 16–79</td>
<td>Male smokers were more likely to quit smoking when switching to snus than those who did not switch to snus (OR=5.7).</td>
<td>Note: There are questions about comparisons in terms of OR, but trends appear valid and the high incidence of ST use in Sweden and wide range in age may give a better indication of what happens as users age.</td>
</tr>
<tr>
<td></td>
<td>(n = 6,752)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>YCYL (cross-sectional survey of adults,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sweden)</td>
<td></td>
<td></td>
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</tbody>
</table>

**NHSDA**: National Household Survey on Drug Abuse; **OR**: odds ratio; **SALT**: Screening Across Lifespan Twin Study; **ST**: smokeless tobacco; **TAPS-1**: Teenage Attitudes and Practice Survey; **YCYL**: Your Country and Your Life.
have a negative reaction to their use of any tobacco product, they would feel less negatively about chewing tobacco than about cigarette smoking (Chassin et al., 1985). Approximately 41% of male teenage smokers reported that their parents were aware of their smoking habit in contrast to approximately 71% of chewers whose parents were aware that they chewed tobacco. Impressions about those who chewed tobacco were better than those about smokers; they were perceived as more confident, braver, having better grades, more popular, more industrious, healthier, and more athletic than smokers (Chassin et al., 1985).

Involvement in organized sports was associated with a 33% higher risk of ever trying and currently using chewing tobacco (OR=1.33; 95% CI: 1.12–1.58) (Castrucci et al., 2004). Other studies also described a higher risk of using chewing tobacco for teenagers who participated in organized sports (Baumert, Jr. et al., 1998; Davis et al., 2007; Rainey et al., 1996).

6.2.5 Social Acceptance of Tobacco Product Types

Because ST products do not generate smoke during use, ST users can use some products without anyone being aware that they do so, which provides more opportunities for use of the product than for use of cigarettes. Ohsfeldt et al. (1997) reported that in the US, clean air laws had more of an impact on cigarette smoking than on ST use. ST users can consume the product throughout the day and while engaging in other activities. Some ST users reported using the product while sleeping (Severson, 2003).

During snuff dipping and tobacco chewing, tobacco is sucked on and saliva and tobacco juices are expectorated as desired (Office of Inspector General, 1992). People can hide their ST use by swallowing the juice. Some companies have marketed ST products with a view to improvements in acceptability.

Changes in product packaging and formulation may influence frequency of ST product use. ST use is not as obvious as smoking and can occur surreptitiously in indoor environments (i.e., classroom or workplace) where cigarettes are not permitted (Johnson & Squier, 1993). Table 6-3 lists certain modified tobacco products that do not require spitting and provides marketing information about the product.
Table 6-3. Descriptions and Marketing of Selected New Smokeless Tobacco Products

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Marketing</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ariva®</td>
<td>Compressed powdered tobacco lozenge with low TSNA levels</td>
<td>To be used by smokers when they cannot or choose not to smoke</td>
<td>Kotlyar et al., 2007</td>
</tr>
<tr>
<td>Stonewall®</td>
<td>Compressed powdered tobacco lozenge with low TSNA levels</td>
<td>Spit-free alternative for regular users of moist snuff</td>
<td>Kotlyar et al., 2007</td>
</tr>
<tr>
<td>Revel®</td>
<td>“Spit-free smokeless tobacco packet”</td>
<td>For smokers seeking a discreet alternative to smoking</td>
<td>Kotlyar et al., 2007</td>
</tr>
<tr>
<td>Taboka®</td>
<td>“Spit-free, smoke-free tobacco pouches”</td>
<td>To be used by adult smokers interested in ST alternatives to smoking</td>
<td>Philip Morris USA, 2006</td>
</tr>
</tbody>
</table>

ST: smokeless tobacco; TSNA: tobacco-specific nitrosamine.

6.2.6 Nasal Use

Nasal snuff was the primary type of tobacco product used until the late 19th century. After automation of methods for wrapping tobacco into a cylinder, sales of nasal snuff were eclipsed by sales of cigarettes (Sapundzhiev & Werner, 2003). Nasal snuff use is not as common as oral tobacco use in the US and Sweden, but it is common in all parts of India.

6.2.7 Cessation

According to the NHIS, 19.2 million US smokers (i.e., 42.5% [95% CI: ±1.7] of US smokers) reported stopping smoking for at least 1 day within the last 12 months in an attempt to quit smoking (Centers for Disease Control and Prevention, 2006). ST, snus in particular, has been reportedly used as a smoking cessation aid (Furberg et al., 2005; Ramström & Foulds, 2006).

In a study of 6,752 Swedish tobacco users, 88% of former cigarette smokers who switched to using snus successfully quit smoking (Ramström & Foulds, 2006). In contrast, 56% of daily smokers who had not switched to using snus quit smoking. Ramström and Foulds (2006) noted that snus might be useful for smoking cessation because of its ability to increase venous nicotine to levels achieved from cigarette smoking. A smoker can attain plasma nicotine levels that peak at 25 ng/ml within 5 minutes of smoking one cigarette, then declines to 10 ng/ml 30 minutes after the last cigarette (Foulds
Differentiating the Health Risks of Categories of Tobacco Products

Ten Swedish *snus* users who abstained from tobacco use overnight achieved a mean + SD plasma nicotine level of approximately 9.9 + 6.5 ng/ml after 10 minutes and 14.5 + 4.6 ng/ml following consumption of a 2g dose of *snus* for 30 minutes (Holm *et al.*, 1992) and *snus* users can have levels of 30 ng/ml or more with multiple uses. Furthermore, *snus* users tend to use the product to help achieve cessation for a longer time than if they use nicotine replacement therapy. The authors reported smoking cessation rates of 72% (95% CI: 68–76%) for men with a history of *snus* use and 51% (95% CI: 48–54%) for men without a history of *snus* use. Corresponding rates for women were 71% (95% CI: 61–81%) and 48% (95% CI: 45–51%).

Furberg *et al.* (2005) reported an association between regular *snus* use and smoking cessation for regular users and now and then users in Sweden. They compared men who were *snus* users before smoking to never cigarette smokers. Of former smokers, 34.6% were current, regular users of *snus* in contrast to 13.7% of current smokers who reported using *snus* regularly.

6.3 PERCENTAGE OF CONCURRENT USE OF SMOKELESS TOBACCO WITH CIGARETTES

Concurrent use refers to regular use of both cigarettes and ST. A 1991 NHIS study reported that 23% of ST users smoked cigarettes and 2.6% of current smokers also used ST (Centers for Disease Control and Prevention, 1993; Tomar *et al.*, 2003). Lando *et al.* (1999) reported that 29% of current smokers used ST and 4.9% of ST users smoked.

Mumford *et al.* (2006) analyzed data from the CPS-TUS study and reported an overall decrease in concurrent use of ST and tobacco in the population related to reductions in cigarette and ST use. The concurrent use prevalence of chewed and smoked tobacco in the US decreased from 1% (95% CI: 0.94–0.11%) in 1992 to 0.56% (95% CI: 0.50–0.62%) in 2001/2002. Because of low ST use by females, any estimate of change in prevalence was unreliable.

Wetter *et al.* (2002) conducted a study of participants in the Working Well Cancer Prevention Trial. Study subjects were employed adults living in the southeastern US. Five percent of males used both ST and cigarettes. They were more likely to be single and have higher estimated cotinine levels than other tobacco users. Concurrent users of cigarettes and ST were less likely to quit tobacco use during 4 years than were individuals who used either cigarettes or ST (Wetter *et al.*, 2002). A study of Lumbee Indian women reported dual cigarette and ST use for 18.3% of smokers (Spangler *et al.*, 1997).
A study of Swedish tobacco users reported that 2% of men were likely to be dual users of cigarettes and snus, whereas 0% of women were likely to be dual users (Ramström & Foulds, 2006). In a study of Northern Swedes, 2% of men and 2% of women were likely to be dual users of cigarettes and snus (Stegmayr et al., 2005). The incidence of dual tobacco use decreased from 4% (CI: 3–7%) in the 1986, 1990, and 1994 surveys to 2.4% (CI: 1.5–3.8%) in 2004. Smoking prevalence for men also decreased from 19% (CI: 16–55%) in 1986 to 9% (CI: 7–11%) in 2004 (Stegmayr et al., 2005).

6.4 CONCLUSIONS

The heterogeneity of ST products leads to differences in use, with some ST products possibly more addictive than other products. Cigarettes and ST have different nicotine pharmacokinetics, as do various types of ST products. Environment and culture appear to have a strong impact on ST use behavior, and ST products may have a more positive image than cigarettes. Moist snuff is the most popular form of ST used in the US. Differential effects of ST on subgroups in the population, for example, young men, may exist. Evidence that snus is not a gateway product is available for Sweden, but in the US conflicting results about its being a gateway product have been published. Furthermore, some data support the use of snus as a cessation aid product, but additional work should be done to determine its effectiveness in this capacity.
7 HEALTH OUTCOMES

7.1 BACKGROUND

Compared with cigarette smoking, smokeless tobacco (ST) use is generally associated with far fewer and considerably less serious health consequences. Approximately 44.5 million Americans (21% of the US population) currently smoke cigarettes, and about 400,000 die each year because of past or current smoking (Centers for Disease Control and Prevention, 2005a). The most common causes of death for cigarette smokers are lung cancer (LC), chronic obstructive pulmonary disease (COPD), and cardiovascular disease (CVD) (Centers for Disease Control and Prevention, 2005b). The primary diseases associated with ST use are oral pathologies, including oral cancer, and CVD (International Agency for Research on Cancer, 1985; U.S. Department of Health and Human Services, 1986a). This chapter compares health outcomes of Swedish snus and US ST use with those of cigarette smoking.

A study of a British physicians cohort found that men born between 1900 and 1930 who smoked cigarettes died an average of about 10 years earlier than lifelong non-smokers (Doll et al., 2004). Data for health outcomes associated with ST use are far less defined and for some disease endpoints are less conclusive compared with the large body of data on adverse health effects of cigarette smoking. Nevertheless, STs generally appear to be less harmful than cigarettes (Foulds et al., 2003; Rodu, 1994). However, even though all STs are non-combusted tobacco products, they also contain nicotine, a potently addictive substance. ST use results in local oral lesions, although the risk of oral cancers is lower for ST users than for smokers (Rodu, 1994). According to Foulds et al. (2003), some cardiovascular risks may be associated with ST use, but these CVD risks are lower than those related to smoking. ST users also have increased risk of pancreatic cancer compared to non-tobacco users. Compared with cigarette smokers, ST users have markedly lower risks of both LC and COPD. However, data on lower risks may be confounded by differences in health risks associated with diverse ST products and toxic constituents, and by differences in risks associated with concurrent use of several types of tobacco products.
The diversity of ST product composition makes general statements about the health effects of all ST forms impossible (Asplund, 2003). Well-designed, large-scale, long-term studies on health effects of STs versus cigarette smoking are lacking. Existing research was not designed to evaluate the health risks of STs alone. Also, sample sizes are inadequate to evaluate associated risk and are poorly controlled for confounding factors such as smoking history and alcohol use (Broadstock, 2007; Critchley & Unal, 2003).

Use of ST products is more prevalent in Sweden than any other country (discussed in detail in Chapter 6). Although health risks of Swedish *snus* are relatively well described, findings from the Swedish experience cannot be extrapolated to other countries because of societal and cultural differences.

During the past several decades, Swedish men have switched from smoking to using *snus*. Despite this switch, most health effects assessments treat this group as exclusive *snus* users rather than ex-smokers who switched to *snus*. Therefore, only indirect assumptions can be made about these ex-smokers who are current exclusive *snus* users.

A number of researchers have concluded that the use of *snus* played a major role in the decline of smoking rates among Swedish men [see (Bates *et al.*, 2003; Rodu *et al.*, 2003)]. In Europe, estimates suggest that almost 200,000 deaths attributable to smoking could be avoided each year if all European Union countries had the smoking prevalence of Sweden (Rodu & Cole, 2004). In 2003, a panel of experts estimated that low-nitrosamine ST products (such as Swedish *snus* or Ariva®) have a 90% reduction in health risk compared with cigarettes (Levy *et al.*, 2004). However, in many environments these ST products are not the dominant one. International products (*i.e.*, ST products other than those used in the US and Sweden) are discussed in Chapter 8.

This chapter evaluates published scientific evidence on health outcomes of ST use versus cigarette smoking and considers US ST products and Swedish *snus*. This chapter is limited to disease endpoints and does not discuss contributing factors. These factors are described in Chapter 5. Studies were evaluated by using a weight of evidence approach in accordance with Hill’s criteria and the US Environmental Protection Agency Information Quality Guidelines (Hill, 1971; U.S. Environmental Protection Agency & Science Policy Council, 2003). A summary table of the health effects of US STs and Swedish *snus* can be found in Appendix D of this report.
Differentiating the Health Risks of Categories of Tobacco Products

7.2 COMPARING SWEDISH SNUS AND US SMOKELESS TOBACCO WITH CIGARETTES

7.2.1 Lung Cancer

Since 1985, LC has been the most common cause of cancer death worldwide (International Agency for Research on Cancer, 2002; Parkin et al., 2005). The connection between LC and cigarette smoking is strengthened by the link between duration of lifetime smoking and the number of cigarettes smoked per day (Doll et al., 2004; Doll & Hill, 1954; Thun et al., 1995). In 2000, an estimated 85% of LC in males and 47% of LC in females worldwide was the consequence of cigarette smoking (Parkin et al., 2005). The Centers for Disease Control and Prevention (CDC) estimated that smoking causes 124,000 deaths/year from LC in the US (Centers for Disease Control and Prevention, 2002). After several reports with numerous confounders are excluded (Accortt et al., 2002, 2005a; Henley et al., 2005) no evidence that ST use can cause LC has been published (Boffetta et al., 2005; Luo et al., 2007; Williams & Horm, 1977) (Figure 7.1). A meta-analysis also concluded that no association exists between LC and ST use (Thornton & Lee.P.N., 2007). In addition, given the different routes of administration for cigarette smoking and ST and the known differences in mutagenicity of the two products, no plausible biological mechanism could lead to LC in ST users.

7.2.1.1 Swedish snus

Data from a cohort of Swedish construction workers found no elevated risk of LC in exclusive snus users compared with non-tobacco users (RR=0.8; 95% CI: 0.4–1.3) (Luo et al., 2007). However, an elevated risk of LC (RR=7.2; 95% CI: 6.0–8.5) was found in smokers from this cohort. Another study also found no association between current snus use and LC (RR=0.80; 95% CI: 0.58–1.11) (Boffetta et al., 2005). At this time, no other published studies have investigated an association between Swedish snus and LC.

7.2.1.2 US smokeless tobacco products

The Third National Cancer Survey (1969–71) reported no increased risk of lung cancer in US chewing tobacco or snuff users (OR=0.65; 95% CI: not reported), of less than 50 years, compared to non-tobacco users, after controlling for cigarette smoking (Williams & Horm, 1977). This data should be interpreted with caution because it was collected around the early 70’s and ST usage patterns have changed over time. Data from the National Health and Nutrition Examination Survey and Epidemiologic Follow-up Study (NHANES) suggest that male ever users of ST (category not specified) had no increased risk of mortality from LC compared with non-tobacco users.
Health Outcomes

Figure 7.1 Lung Cancer Incidence and Mortality Associated with Cigarette Smoking and Smokeless Tobacco Use

- Thun et al. 1995 (CPSII males)  
- Thun et al. 1995 (CPSII females)  
- Thun et al. 1995 (CPSI males)  
- Thun et al. 1995 (CPSI females)  
- Luo et al. 2007  
- Boffetta et al. 2005  
- Henley et al. 2005 (CPSII) (snuff or chewing tobacco)  
- Henley et al. 2005 (CPSI) (snuff or chewing tobacco)  
- Accortt et al. 2005a (males) (snuff or chewing tobacco)  
- Accortt et al. 2005a (females) (snuff or chewing tobacco)  
- Accortt et al. 2002 (males) (ST product unspecified)  
- Accortt et al. 2002 (females) (ST product unspecified)  

Horizontal lines represent the 95% CI. CI: confidence interval; CPS: Cancer Prevention Study; DRR: death rate ratio (death rate for smokers divided by that for nonsmokers); HR: hazard ratio; RR: relative risk; *: not stated.

(HR=0.0; CI: not reported) (Accortt et al., 2002). Conversely, female ST users, reportedly never smokers, appeared to have a statistically significant increased risk of LC mortality (HR=9.1; 95% CI: 1.1–75.4), although these results were based on only 3 deaths (Accortt et al., 2002). Another report using NHANES data found that male exclusive ST users did not have an increased risk of LC above that of non-tobacco users (Accortt et al., 2005a). However, female exclusive ST users also showed a significantly increased risk of LC (HR=6.8; 95% CI: 1.6–28.5); this result was based on 3 deaths in women 65 years old and older. LC incidence is increasing in the female population in general (Parkin et al., 2005).

An interesting finding from NHANES I data is that users of both ST and cigarettes had an increased risk of LC (HR=22.6; 95% CI: 6.4–80.3) compared with exclusive smokers (HR=13.2; 95% CI: 4.5–38.2) (Accortt et al., 2002). In this population, dual users smoked more than exclusive smokers (42.3 and 35.1 mean pack-years, respectively). Accortt et al. (2002)
suggested that this increase in cigarette smoking, and not ST use, contributed to the increase in LC.

NHANES I relied on self-reported ST use, smoking, and exposure to potential confounders. In addition, non-tobacco user controls included pipe and cigar smokers, and ST users were ever users, including individuals who used ST only once. Information about tobacco use at follow-up was not collected. Therefore, analysis of exclusive habitual ST users and possible inclusion of smokers could confound results. Furthermore, NHANES was not designed to examine exclusive ST users, and inclusion of only small numbers of ST users could lead to potential bias and confounders. These complications lend further doubt to the LC risk for ST users reported above (Accortt et al., 2002) and further research is needed to rule out an effect of ST use in populations of smokers.

Henley et al. (2005) analyzed two large prospective studies conducted by the American Cancer Society (ACS): Cancer Prevention Study (CPS) I and CPS II. Data from CPS I indicated no increased risk of mortality from LC in male exclusive ST users (chewing tobacco or spit tobacco) compared with non-tobacco users (HR=1.08; 95% CI: 0.64–1.83). However, exclusive male ST users from CPS II had an increased risk of mortality from LC (HR=2.00; 95% CI: 1.23–3.24). The authors raised questions about the accuracy of this finding and stated that they did not control for previous smoking in the exclusive ST population. Lack of a clear mechanism between ST and LC and a dose-response relationship between either frequency, or duration of ST use may cast doubt on the likelihood that the relationships between ST and LC represent causal effects. The most likely confounder was potential differences in exposure to tobacco smoke. ST use was recorded only at baseline and was not updated at follow-ups, and tobacco use behavior could have changed over time. The confounders of CPS I and II were described previously (Foulds & Ramström, 2006). These studies were not population-based surveys of the US population; volunteers from the ACS enrolled participants, which skewed the demographic variables toward mostly white, well-educated, middle-class participants.

7.2.2 Chronic Obstructive Pulmonary Disease

COPD, which includes emphysema and chronic bronchitis, is characterized by pathophysiological inflammatory changes resulting in airflow obstruction and destruction of lung parenchyma (Burns, 2004; Demedts et al., 2006;  

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9 A pack-year is a unit of measure of smoking exposure. One pack-year represents consumption of 20 cigarettes (1 pack) per day for 1 year by 1 person.
Fenderson et al., 2007). The incidence of COPD is highly related to smoking history, particularly for males (Thun et al., 1995). The CDC estimated that smoking causes more than 100,000 deaths/year from pulmonary diseases (Centers for Disease Control and Prevention, 2002). Several theoretical constructs have been created to mechanistically explain how cigarette smoking contributes to the pathobiology of COPD, in both airways and lung parenchyma, and several of these constructs are supported by experimental animal exposure data (Spurzem & Rennard, 2005). Both gas and particulate phases of cigarette smoke are toxic to respiratory tract epithelium and result in cell injury and subsequent inflammatory and respiratory remodeling processes that compromise host defenses, airflow, and gas exchange functions of the lungs (Fenderson et al., 2007; Rubin et al., 2005). Another mechanistic theory of COPD pathophysiology is the protease-antiprotease imbalance hypothesis. A previous Life Sciences Research Office (LSRO) report discussed this hypothesis in detail (Life Sciences Research Office, 2007a). In short, it postulates that emphysema is caused by degradation of connective tissue components in lung parenchyma by various proteases and that, in COPD, production of antiproteases may be insufficient to neutralize the effects of multiple proteases. No mechanistic evidence that ST use contributes to pathobiology of COPD has been published (Foulds et al., 2003).

To date, no published studies have examined an association between Swedish snus and COPD. Data from CPS I indicated that ST users had a significantly increased risk of death from COPD (HR=1.86; 95% CI: 1.12–3.06) (Henley et al., 2005). CPS II data did not show such an increased risk of mortality (HR=1.28; 95% CI: 0.71–2.32) in ST users but also did not contradict the findings of CPS I. Male smokers from CPS I and CPS II had increased HR values of 9.3 (95% CI: 6.6–12.9) and 11.7 (95% CI: 9.1–15.0) compared with that for non-tobacco users (Thun et al., 1995). As stated previously, this dataset had significant confounders. Tobacco use information was collected at baseline but not updated during follow-up, which casts doubt on whether this population was limited to exclusive ST users. ST users were not compared with smokers enrolled in the same study. The inclusion of cigarette smokers in the ST group in CPS I could indicate that former smokers, or dual users, could to some degree have a residual incidence of COPD above that of lifetime non-smokers. It can be concluded that ST is an unlikely contributing cause to COPD pathobiology, especially in view of the lack of plausible biological mechanisms at this time. Biological mechanisms need not be apparent for an association to exist between a disease and a risk, but in this case, scant evidence links COPD to ST use. Although no direct
comparisons of cigarette smokers and ST users have been made, the risk of COPD in ST users is far less than that of smokers (Figure 7.2).

**Figure 7.2 Chronic Obstructive Pulmonary Disease Mortality Associated with Cigarette Smoking and Smokeless Tobacco Use**

Horizontal lines represent the 95% CI. CI: confidence interval; CPS: Cancer Prevention Study; DRR: death rate ratio (death rate for smokers divided by that for nonsmokers); HR: hazard ratio; RR: relative risk.

### 7.2.3 Cardiovascular Disease

CVD includes diseases of the heart and/or vascular (blood vessel) system: atherosclerosis, coronary artery disease, carotid artery disease, peripheral arterial disease, aortic aneurysm, and myocardial infarction (MI). Figure 7.3 shows the association of first MI with ST and cigarette use. Cigarette smokers have an increased risk of fatal MI (Bolinder et al., 1994; Hergens et al., 2005; Huhtasaari et al., 1992; Huhtasaari et al., 1999; Johansson et al., 2005; Teo et al., 2006; Thun et al., 1995). According to the CDC, smoking causes 132,000 deaths/year from CVD (Centers for Disease Control and Prevention, 2002). One study reported a weak association between Swedish snus and CVD (Bolinder et al., 1994), but several other studies found no association (Asplund et al., 2003; Hergens et al., 2005; Huhtasaari et al., 1992; Huhtasaari et al., 1999; Wennberg et al., 2007). Henley et al. (2005) reported an association between US snuff and CVD (increased risk of dying from CVD), whereas Accortt et al. (2002) observed no such association. A
case-control study conducted in 52 countries found an increased risk of non-fatal MI related to chewing tobacco use (OR=2.23; 95% CI: 1.41–3.52) compared with non-tobacco users (Teo et al., 2006). However, the study included small numbers of each type of chewing ST and therefore no analysis by type could be made. At this time, no clear evidence is available that ST itself increases the risk of CVD, but more research is needed. Cardiovascular changes such as electrical cardiac activity and hemodynamics, endothelial function, atherosclerosis, inflammation, and lipid metabolism, are discussed in Chapter 5 of this report.

Figure 7.3 First Myocardial Infarction Incidence Associated with Cigarette Smoking and Smokeless Tobacco Use

Horizontal lines represent the 95% CI. CI: confidence interval; CPS: Cancer Prevention Study; HR: hazard ratio; OR: odds ratio; RR: relative risk.

7.2.3.1 Swedish snus

Several health effects assessments found no increased risk of CVD associated with Swedish snus use (Asplund et al., 2003; Hergens et al., 2005; Huhtasaari et al., 1992; Huhtasaari et al., 1999; Johansson et al., 2005; Wennberg et al., 2007). Wennberg et al. (2007) reported that Swedish snus users, never smokers (OR=0.82; 95% CI: 0.46–1.43), and smokers who had switched to snus (OR=1.25; 95% CI: 0.80–1.96) did not have an elevated risk of first MI. Former smokers who quit using tobacco had a
reduced risk of first MI (OR=1.18; 95% CI: 0.82–1.70). Certain of the studies with negative findings mentioned controlled for possible confounders such as CVD risk factors but included small numbers (<50) of snus users who had never smoked (Hergens et al., 2005; Huhtasaari et al., 1999). Because many snus users are former smokers, finding populations of exclusive Swedish snus users (i.e., never smokers) is challenging.

Two studies reported an increased risk of CVD for Swedish snus users (Bolinder et al., 1994; Hergens et al., 2007). The Bolinder et al. study (Bolinder et al., 1994) of male Swedish construction workers reported that snus users had an increased risk of CVD mortality (RR=1.4; 95% CI: 1.2–1.6) compared with non-tobacco users. Although this study did not directly compare Swedish snus users with smokers, the risk was less than that of smokers of 15 or more cigarettes/day (RR=1.9; 95% CI: 1.7–2.2). This study also did not control for alcohol consumption or overall lifetime smoking history. The inclusion of ex-smokers in groups of snus users would be expected to increase their overall risk of CVD. Dose-response effects for snus were not investigated, which further limits inferences about causality. Swedish snus composition has changed over time, and this study evaluated an older product containing previously higher levels of many toxic compounds related to tobacco processing (e.g., nitrosamines) (Nilsson, 1998). The risk may therefore not apply to products on the current Swedish market. As a group, construction workers had an unfavorable risk factor profile at the beginning of the survey in the 1970s, with a higher body mass index and higher resting blood pressure levels than non-tobacco users. These findings cannot be ignored, but given the caveats about the study, questions remain about the applicability of these data to present-day Swedish snus users.

Heavy Swedish snus users (>50 g/day) had an increased risk of mortality from CVD compared with non-tobacco users (RR=1.96; 95% CI: 1.08–3.58) (Hergens et al., 2007). This study reported that ever snus use was not associated with an increased risk of MI (RR=0.99; 95% CI: 0.90–1.10). This population was also derived from the Swedish construction workers cohort, with 200,000 new subjects, and therefore has limitations similar to those of the Bolinder et al. study (1994).

Little evidence seems to be available for increased CVD in male Swedish snus users. Studies finding a positive association included study subjects from certain specific socioeconomic groups, did not evaluate a dose-response effect, and had confidence intervals greater than 1 (Bolinder et al., 1994). More recent studies applied more broadly to the snus-using population failed to find any association (Wennberg et al., 2007). The mechanism underlying
any excess risk for fatal MI is unclear because snus does not affect many biochemical factors implicated in CVD (Asplund, 2003). A review by Fagerström and Schildt concluded that Swedish snus users have a lower risk of CVD than cigarette smokers (Fagerström & Schildt, 2003). However, more research is needed to rule out an increased risk of CVD related to Swedish snus use. In contrast, all studies that evaluated smokers found a link between smoking and CVD compared with never smoking (Thun et al., 1995).

7.2.3.2 US smokeless tobacco products

As is the case for Sweden, reports on the association between ST and CVD in the US have provided conflicting results. Accortt et al. (2002) reported no higher CVD mortality associated with ST use (HR=1.1; 95% CI: 0.8–1.5) compared with no tobacco use. Henley et al. (2005), however, reported that male ST users enrolled in CPS I had an increased risk of dying from CVD (HR=1.18; 95% CI: 1.11–1.26) and stroke (HR=1.46; 95% CI: 1.31–1.64) compared with non-tobacco users. Similar findings were reported for ST users in the CPS II for mortality from both CVD (HR=1.23; 95% CI: 1.09–1.39) and stroke (HR=1.40; 95% CI: 1.10–1.79). Weaknesses of data from CPS I and II were discussed in Section 7.2.1.

7.2.3.3 Cardiovascular effects of pure nicotine

Nicotine replacement therapies (NRTs) have been described as safer than smoking, even for the chronically ill (McRobbie & Hajek, 2001). Nicotine did not seem to represent significant risk factors in CVD and was believed to have no contributions to cancer or COPD (McNeill et al., 2001). Certain studies, however, have implicated nicotine in CVD (Bolinder et al., 1997). Other studies did not find an increased risk for cardiac patients treated with nicotine patches (Hubbard et al., 2005; Joseph et al., 1996; Kimmel et al., 2001). A population-based case-control study found no association between nicotine patches and risk of first MI (OR=0.46; 95% CI: 0.09–1.47) (Kimmel et al., 2001). Hubbard et al. (2005) reported no association of NRT with an increased risk of death (incidence ratio=0.86; 95% CI: 0.60–1.23) in the first 56 days of treatment. No increased risk of MI (incidence ratio=1.27; 95% CI: 0.82–1.97) or stroke (incidence ratio=1.30; 95% CI: 0.77–2.19) was also found in this relatively short-term study. NRT used for limited periods thus seems safe for patients with CVD, and in fact the US Public Health Service Clinical Practice Guideline concluded that NRT products could be used safely by patients with CVD (Fiore et al., 2000). However, it should be emphasized that NRT is usually used for relatively short periods (i.e., weeks to months).
7.2.4 Oral Cancer

Oral cancer includes cancer of the tongue, gum, floor of mouth, and other unspecified parts of the mouth (National Center for Health Statistics & World Health Organization, 1977). These unspecified parts include the cheek, vestibule, palate, uvula, and retromolar region (Rodu & Cole, 2002). Squamous cell carcinoma is the most common oral cancer diagnosed (Nair et al., 2004). The ACS estimated that approximately 34,000 new oral and pharyngeal cancer cases would be diagnosed in the US in 2007, with almost 7,600 deaths (Jemal et al., 2007). The rise of oral cancer in India (Nair et al., 2004) is a topic covered in depth in Chapter 8.

ST causes oral mucosal lesions and gingival recession (Axéll, 1993; Grady et al., 1990; Shulman et al., 2004). ST is estimated to pose only half the risk of oral cancer associated with cigarette smoking (Rodu & Cole, 1994). Cancer of the pharynx includes cancer of the oropharynx and hypopharynx; some studies also included cancer of the larynx in this category (Rodu & Cole, 2002). Only oral cavity and oropharyngeal cancers are covered here; laryngeal cancer is covered in Section 7.2.6.1.

Cigarette use is associated with increased risk of oral cancer (Blot et al., 1988; Luo et al., 2007; Mashberg et al., 1993; Schildt et al., 1998). No studies directly compare cigarettes with ST with regard to oral cancer risk. No association has been reported between Swedish snus use and oral cancer (Boffetta et al., 2005; Lewin et al., 1998; Luo et al., 2007; Rosenquist et al., 2005; Schildt et al., 1998). Some evidence has been published that use of US chewing tobacco and dry snuff increases oral cancer risk, but these data have limitations because confounders such as smoking and alcohol consumption were often ignored in the analysis (Blot et al., 1988; Stockwell & Lyman, 1986; Winn et al., 1981). Other studies from the US reported no association (Accortt et al., 2005a; Mashberg et al., 1993; Sterling et al., 1992). One review suggested that if all smokers switched to ST, oral cancer risk could be reduced by 43% (Rodu, 1994). A 2001 review reported that increasing oral cancer rates in the Western world are related to rising alcohol use (Johnson, 2001). Both tobacco and alcohol are risk factors for oral cancer and are likely to potentiate each other’s effects (International Agency for Research on Cancer, 2002; Parkin et al., 2005). Studies that do not account for alcohol use are likely to be confounded by inclusion of the alcohol-using population and this complex potentiating effect. A more detailed discussion of oral cancer risks for users of Swedish snus, US ST products, and other tobacco products follows (Figure 7.4).
7.2.4.1 Swedish snus

As stated above, several studies reported no association between Swedish snus and oral cancer (Boffetta et al., 2005; Lewin et al., 1998; Luo et al., 2007; Rosenquist et al., 2005; Schildt et al., 1998). Data from the Swedish construction workers cohort found no elevated risk of oral cancer in exclusive snus users (RR=0.8; 95% CI: 0.4–1.7) (Luo et al., 2007). Boffeta et al. (2005) found no increased risk of oral or pharyngeal cancer (RR=1.10; 95% CI: 0.50–2.41). A population-based control study (Schildt et al., 1998) found no increased risk of oral cancer among snus users (OR=0.7; 95% CI: 0.4–1.2). These studies controlled for smoking and alcohol use (Boffetta et al., 2005; Luo et al., 2007; Schildt et al., 1998).

A population-based case-control study in southern Sweden examining the role of snus use, smoking, and alcohol consumption in the etiology of oral and oropharyngeal squamous cell carcinoma (OOSCC) found that smoking and alcohol consumption were risk factors for OOSCC (Rosenquist et al., 2005). The use of moist snuff had no effect on OOSCC risk (OR=1.1; 95% CI: 0.5–2.5) compared with non-tobacco users, after adjustments were made.
for both smoking and alcohol use (Rosenquist et al., 2005). The risks for exclusive Swedish snus users, compared with smokers and alcohol users, were minimal (Rosenquist et al., 2005).

### 7.2.4.2 US smokeless tobacco products

Inconsistent results were obtained from studies of US ST products and oral cancer. Some studies reported that US ST products (snuff, chewing tobacco, loose-leaf tobacco) were associated with oral cancer (Blot et al., 1988; Stockwell & Lyman, 1986; Williams & Horm, 1977; Winn et al., 1981; Zahm et al., 1992). However, other US studies reported no association (Mashberg et al., 1993; Sterling et al., 1992).

US veterans who used chewing tobacco and snuff and who were studied from 1954 to 1980 had a significantly increased risk of mortality from cancer of the buccal cavity (RR=3.0; 95% CI: 2.0–4.5) and pharynx (RR=8.7; 95% CI: 4.1–18.3), in a dose-dependent manner, compared with non-tobacco users (Zahm et al., 1992). This study did not adjust for cigarette smoking or alcohol use, however. The Third National Cancer Survey (1969–1971) reported that users of chewing tobacco or snuff had a significantly increased risk of gum or mouth cancer (OR=3.88; 95% CI: not reported) compared with non-tobacco users; this study controlled for cigarette smoking (Williams & Horm, 1977). The authors also reported that chewing tobacco or snuff users had no increased risk of lip or tongue cancer (OR=0.36; 95% CI: not reported) or pharyngeal cancer (OR= 0.45; 95% CI: not reported).

Winn et al. (1981) found a 4-fold increase in oral and pharyngeal cancer associated with dry snuff use (snuff dipping OR=4.2; 95% CI: 2.6–6.7) among white women living in rural North Carolina. Significant dose-response relationships were observed for women who had used snuff for 50 years or more. Another Winn et al. (1984) study of women in North Carolina also found increased risk of cancer of the oral cavity and pharynx for snuff dippers (RR=3.8; 95% CI: 2.6–6.3) compared with non-tobacco users. These studies were well controlled for confounders including smoking and alcohol. Because these studies were limited to women, who are not the dominant ST users in the US, and were carried out in the 1970s, questions about applicability to the present day may be raised inasmuch as the types of snuff used and patterns of use have changed over time.

Blot et al. (1988) reported risks of oropharyngeal cancer for US female chewing tobacco users and found that exclusive users had a non-significantly increased risk compared with non-tobacco users (OR=6.2; 95% CI: 1.9–19.8). This study did control for both smoking and alcohol use but included
only 10 subjects who were ST users and did not have as its primary goal the assessment of the risk of ST use related to oropharyngeal cancer. Kabat et al. (1994b) reported that female exclusive snuff users had an increased risk of oral and pharyngeal cancer (OR=34.5; 95% CI: 8.49–140.1) compared with non-tobacco users. However, this study did not account for smoking and alcohol use. Another study reported similar findings for chewing tobacco and loose-leaf tobacco users, but it also had similar confounders and a small sample size (n=9) (Spitz et al., 1988). Studies with small sample sizes may not be representative of the population and add bias to the study. These studies found that ST use was associated with increased risk of oral and pharyngeal cancer, but some studies finding such positive associations did not account for the contributions of smoking and alcohol use.

In a population of US veterans, with control for both alcohol use and cigarette smoking, neither snuff (type not specified) use (OR=0.8; 95% CI: 0.4–1.9) nor chewing tobacco use (OR=1.0; 95% CI: 0.7–1.4) (Mashberg et al., 1993) was associated with oral cancer. A study by Sterling et al. (1992) that controlled for confounders found no elevated risk of oral cancer in ST users (chewing tobacco or unspecified snuff) compared with non-tobacco users (OR=1.21; 95% CI: 0.32–4.63); however, the sample size was small.

A study of people in North Carolina reported that cigarette smokers had no increase in overall risk of nasal cancer but a significantly elevated risk of squamous cell tumors of the oropharynx (RR=1.8; 95% CI: 1.0–3.2) (Brinton et al., 1984). Chewing tobacco users had no increased risk of nasal cancer (RR=0.74; 95% CI: 0.4–1.5). However, users of snuff (type not specified) had a non-significantly increased risk of cancer of the nasal cavity and sinuses (RR=1.47; 95% CI: 0.8–2.8). Snuff users (n=14) also had a non-significant increase in risk of cancer of the nasal cavity and sinuses (RR=1.9; CI: not reported). There was an increased risk of adenocarcinoma of the nasal cavity and sinuses, however, this finding was based on 6 cases (RR = 3.1; CI: not reported). This study controlled for both smoking and alcohol use.

Data from CPS I showed a non-significantly increased risk of oropharyngeal cancer in US ST users (HR=2.02; 95% CI: 0.53–7.74) compared with non-tobacco users, but this result was based on only 3 deaths (Henley et al., 2005). Data from CPS II indicated no increased risk of oropharyngeal cancer (HR=0.90; 95% CI: 0.12–6.71) compared with non-tobacco users, but this finding too was based on only 1 death. In view of the previously mentioned criticism of this sample group and the small number of deaths, the evidence of any ST effect is not compelling.
In addition to studies that performed risk assessments of the relationship between oral and pharyngeal cancer and ST products, some studies have merely associated disease with ST use. Vogler et al. (1962) reported that 14.7%, 24.6%, and 4.8% of tobacco chewers had cancer of the lip, buccal cavity, and pharynx, respectively. This study did not control for cigarette smoking. Another study associated cancer type—squamous cell carcinoma and verrucous carcinoma—with ST use (Link et al., 1992). Of squamous cell cancers in the buccal mucosa, 1.4% occurred in ST users (type not specified), and 7.7% of patients with verrucous carcinoma used ST. In a Swedish study of users of snuff (type not specified), 2 patients with lower lip cancer used ST (Blomqvist et al., 1991). Another study reported similar findings: 1.3% of patients with oral cancer used snuff or chewing tobacco (Muscat et al., 1996). Although these findings associate ST with oral cancer, the association is not statistically significant.

Most US studies reporting a positive relationship between ST use and oral cancer did not control for concurrent smoking, which is an established risk factor for oral and pharyngeal cancer. Sample sizes were often too small, and specific oral cancer sites (e.g., lip, tongue) were not assessed. Overall, the data provide only equivocal evidence that US ST products promote oral cancer, whereas it has been been established that cigarettes promote oral cancer. One significant drawback of most studies is a lack of specificity when reporting information on oral cancer sites associated with ST use. Neither histopathological confirmation of cancer diagnosis nor the specific oral cancer site is often reported. Identification of ST type is also missing from the literature. Some evidence has been provided that dry snuff carries a higher risk of oral cancer than other STs such as chewing tobacco and moist snuff, but more research is needed (Rodu & Cole, 2002).

### 7.2.5 All-Cause Mortality

Smokers have a dose-dependent excess risk of all-cause mortality compared with non-tobacco users (Bolinder et al., 1994; Thun et al., 1995) (Figure 7.5). One study found no association between all-cause mortality and ST users compared with non-tobacco users (Accortt et al., 2002). Two studies, which included several confounders, found an increased risk of mortality for ST users (Bolinder et al., 1994; Henley et al., 2005). A recently published meta-analysis of these studies determined no significantly increased RR of mortality (1.18; 95% CI: 1.13–1.23) for ST users compared with non-tobacco users (Thornton & Lee, 2007).
7.2.5.1 Swedish snus

A study of male Swedish construction workers reported that snus users had an increased risk of all-cause mortality (RR=1.4; 95% CI: 1.3–1.8) compared with non-tobacco users (Bolinder et al., 1994). Limitations of this study were discussed previously. No other reports have been published on the association between Swedish snus and all-cause mortality.

7.2.5.2 US smokeless tobacco products

Accortt et al. (2002) analyzed data from NHANES I, which included 414 ST users, and found no association between ST users and all-cause mortality (HR=1.1; 95% CI: 0.9–1.3) when compared with non-tobacco users. Limitations of the Accortt et al. (2002) study are inclusion of pipe and/or cigar smokers in the non-tobacco group and inclusion of one-time ST users. These limitations result in further challenges in interpreting this study, in view of the previously described confounders (see Section 7.2.1).

In their analysis of the CPS I and CPS II studies, Henley et al. (2005) found that men who currently used snuff or chewing tobacco had a statistically
significant higher all-cause mortality than did non-tobacco users (CPS I HR=1.17; 95% CI: 1.11–1.23; and CPS II HR=1.18; 95% CI: 1.08–1.29). No relationships were seen for frequency and duration of ST use in current users, which leads to calls into question the accuracy of this study given previously discussed confounders (see Section 7.2.1).

Currently, published data comparing the effects of using ST alone with effects of using cigarettes on all-cause mortality are scarce.

7.2.6 Other Cancers

7.2.6.1 Laryngeal cancer

Laryngeal cancer is consistently associated with cigarette smoking (Doll et al., 2004; Lewin et al., 1998). In 2007, an estimated 11,300 new cases of laryngeal cancer were diagnosed in the US, with 3,700 deaths (Jemal et al., 2007). Given the different routes of administration of ST products, it seems implausible that ST users would have an increased risk of laryngeal cancer. In Sweden, current users of snuff had no increased risk of laryngeal cancer (RR=1.0; 95% CI: 0.5–1.9) compared with non-tobacco users, with data adjusted for cigarette smoking and alcohol use (Lewin et al., 1998). The third National Cancer Survey (1969–1971) reported that users of US chewing tobacco or snuff did not have an increased risk of cancer of the larynx (OR=1.75; 95% CI: not reported) compared with non-tobacco users and controlling for cigarette smoking (Williams & Horm, 1977). These data should be interpreted with caution because it was collected around the early 1970’s and ST usage patterns have changed over time. One US study reported that users of ST (type not specified) had an increased laryngeal cancer risk (OR=7.3; 95% CI: 2.9–18.3) compared with non-tobacco users and adjusting for cigarette smoking but not alcohol consumption (Stockwell & Lyman, 1986). This study obtained information about tobacco use only from hospital records, and the quality of this information is doubtful (International Agency for Research on Cancer, 2007b).

At this time, the risk of laryngeal cancer and its relationship to ST use are poorly understood and additional research is needed.

7.2.6.2 Esophageal and gastric cancer

Tobacco and alcohol are among the major known causative factors of esophageal squamous cell cancer in Europe and North America and perhaps contribute to more than 90% of cases worldwide (Lagergren et al., 2000; Parkin et al., 2005). Estimates predicted approximately 15,560 new cases
of esophageal cancer in the US in 2007, with almost 13,940 deaths (Jemal et al., 2007). Smokers have an increased risk of esophageal squamous cell carcinoma compared with non-tobacco users, but not of esophageal adenocarcinoma (Lagergren et al., 2000) (Figure 7.6). A dose-response increased risk of esophageal squamous cell carcinoma correlated with intensity and duration of smoking has also been reported. Alcohol is also a risk factor for esophageal cancer and should be controlled for in well-designed health effects assessments (Negri, 2004). A recent meta-analysis of 13 studies found a significantly increased risk of esophageal cancer associated with ST use (RR estimate=1.37; 95% CI: 1.10–1.71) (Thornton & Lee, 2007).

Smokers have a clearly increased risk of gastrointestinal cancer compared with non-tobacco users (Lewin et al., 1998). Approximately 21,000 cases of gastric cancer were diagnosed in the US in 2007, with the number of deaths estimated at 11,000 (Jemal et al., 2007). Gastric cancer is the fourth most common cancer in the world and is the second most common cause of death from cancer (Parkin et al., 2005).

**Figure 7.6 Esophageal Squamous Cell Carcinoma Incidence Associated with Cigarette Smoking and Smokeless Tobacco Use**

Horizontal lines represent the 95% CI. CI: confidence interval; CPS: Cancer Prevention Study; OR: odds ratio; RR: relative risk; *: not stated.
Tobacco smoking is associated with gastric cancer (Doll et al., 2005; Hansson et al., 1994; International Agency for Research on Cancer, 2002; Lagergren et al., 2000; Parkin et al., 2005; Ye et al., 1999). [For a review, see Zaridze, (2004).] One meta-analysis revealed that smoking increased the risk of gastric cancer 1.5–1.6 fold (Tredaniel et al., 1997). The risk of gastric cancer was 50–60% higher, on average, for smokers than for non-smokers (Vineis et al., 2004) (Figure 7.7). As is the case for most smoking-related cancers, smoking cessation was associated with a decreased risk of gastric cancer (Lagergren et al., 2000).

**Figure 7.7 Gastric Cancer Incidence Associated with Cigarette Smoking and Smokeless Tobacco Use**

The notation “Other ST products” refers to the fact that this study included Swedish moist snuff and chewing tobacco. Horizontal lines represent the 95% CI. **CI**: confidence interval; **CPS**: Cancer Prevention Study; **OR**: odds ratio; **RR**: relative risk.
7.2.6.2.1 Swedish snus

Most studies have reported no increased risk of esophageal cancer in Swedish snus users (Boffetta et al., 2005; Lagergren et al., 2000; Lewin et al., 1998), except for a recent report on the Swedish construction workers cohort (Zendehdel et al., 2008). No elevated risk of esophageal cancer (RR=1.4; 95% CI: 0.61–3.24) or gastric cancer (RR=1.11; 95% CI: 0.83–1.48) was found for a population of male Norwegian ever snus users compared with non-tobacco users (Boffetta et al., 2005). ST use was not associated with esophageal squamous cell carcinoma (OR=1.4; 95% CI: 0.9–2.3) or esophageal adenocarcinoma (OR=1.2; 95% CI: 0.7–2.0) (Lagergren et al., 2000).

Compared with non-tobacco users, Swedish snuff users had no significantly increased risk of gastric cancer (OR=0.70; 95% CI: 0.47–1.06), but the gastric area was not specified (Hansson et al., 1994). Snus use was not described well, and the accuracy of this study is difficult to determine (Broadstock, 2007). One recent report on the Swedish construction workers cohort found an increased risk of non-cardia gastric cancer (RR=1.4; 95% CI: 1.1–1.9) compared with non-tobacco users (Zendehdel et al., 2008). However, this study has confounders of smoking and alcohol use. Another study from Sweden that examined chewing tobacco and snuff (not snus) did not report a positive association between ST users and gastric cardia adenocarcinoma (OR=0.6; 95% CI: 0.3–1.2) (Ye et al., 1999) compared with non-tobacco users and adjusted for confounders. The conclusion, albeit from few studies is that snus use is not associated with an increased incidence of gastric cancer.

7.2.6.2.2 US smokeless tobacco products

Two reports indicating no increased risk of esophageal cancer in US users of chewing tobacco or snuff (type not specified) have been published (Brown et al., 1988; Williams & Horm, 1977). Williams and Horm (1977), controlling for cigarette smoking, found no increased risk of esophageal cancer in users of US chewing tobacco or snuff (OR=0.90; 95% CI: not reported) compared with non-tobacco users. The other study reported no increased risk of esophageal cancer in users of US chewing tobacco or snuff (type unspecified) (OR=1.2; 95% CI: 0.1–13.3) compared with non-tobacco users and adjusted for alcohol use but not cigarette smoking (Brown et al., 1988). At this time, not enough evidence exists to determine the risks of commercially available US ST products as associated with esophageal cancer.

A significantly increased risk of gastric cancer (type not specified) was found in ST users (chewing tobacco or snuff) (RR=3.8; 95% CI: 1.00–14.32) compared with non-tobacco users; this assessment controlled for alcohol
use and cigarette smoking but was based on only 3 cases (Kneller et al., 1991). Exclusive current users of chewing tobacco or snuff had a statistically significant increased risk of mortality from gastric cancer (type not specified) (RR=1.58; 95% CI: 0.76–3.28) compared with non-tobacco users; this study was based on only 8 deaths (Chao et al., 2002). Sterling et al. (1992) reported that neither ST users (OR=0.61; 95% CI: 0.34–1.10) nor smokers (OR=1.02; 95% CI: 0.82–1.27) had an elevated risk of digestive tract cancer. This study controlled for smoking and alcohol use and had an adequate sample size. A case-control study by Williams and Horm (1977) reported an increased risk of gastric cancer (type not specified) in heavy users (duration of use >50 years) of ST (snuff and chewing tobacco) (OR=1.73; 95% CI: not reported), but no increase in risk for moderate users (0–50 years duration) (OR=1.00; 95% CI: not reported) compared with non-tobacco users; these authors adjusted for cigarette smoking and alcohol use. Data from NHANES I indicated no increased risk of death from gastrointestinal cancer in females (HR=0.8; 95% CI: 0.3–2.7) or males (HR=0.9; 95% CI: 0.3–2.3), but this study has several limitations as mentioned previously (Accortt et al., 2002).

7.2.6.3 Pancreatic cancer

Pancreatic cancer is the eighth most common cause of cancer death worldwide, and accounts for more than 33,000 deaths/year in the US (Jemal et al., 2007; Parkin et al., 2005). Cigarette smoking is the major recognized risk factor for pancreatic cancer (Fuchs et al., 1996; Gallicchio et al., 2006; Harnack et al., 1997; Hassan et al., 2007; Silverman et al., 1994; Thun et al., 2000). Current smokers have a dose-dependent increase in RR compared with non-tobacco users, and quitting smoking can reduce pancreatic cancer risk by an estimated 50% (MacLeod & Chowdhury, 2006). Alcohol consumption also increases the risk of pancreatic cancer, so well-designed studies should control for alcohol use (Harnack et al., 1997). Removal of estimated excess risks of pancreatic cancer in smokers would reduce the overall population risk of pancreatic cancer by 25% (Fuchs et al., 1996; Silverman et al., 1994).
7.2.6.3.1 Swedish snus

Use of Swedish snus has been associated with an increased risk of pancreatic cancer compared with non-tobacco use (Boffetta et al., 2005; Luo et al., 2007). Norwegian male snus users, enrolled in a prospective cohort study, had a significantly increased risk of pancreatic cancer (RR=1.67; 95% CI: 1.12–2.50) (Boffetta et al., 2005). The higher RR of pancreatic cancer was similar in both current and former Swedish snus users; however, no doses or durations of use were reported. This study controlled for smoking and alcohol use. The report on Swedish construction workers cohort also showed that exclusive snus users had an elevated pancreatic cancer risk (RR=2.0; 95% CI: 1.2–3.3), with an increased risk for users of more than 10 g/day (Luo et al., 2007). However, this study did not control for alcohol use or measure Swedish snus consumption at follow-up interviews.

7.2.6.3.2 US smokeless tobacco products

Some studies have found an association between US snuff use and increased pancreatic cancer risk (Alguacil & Silverman, 2004; Muscat et al., 1997),...
Differentiating the Health Risks of Categories of Tobacco Products

whereas others have not (Farrow & Davis, 1990; Hassan et al., 2007; Williams & Horm, 1977). Alguacil and Silverman (2004), in a population-based case-control study of pancreatic cancer, found that users of ST (chewing tobacco and unspecified snuff combined) had a non-significant increase in pancreatic cancer risk (OR=1.1; 95% CI: 0.4–3.1) compared with non-tobacco users. ST users of more than 2.5 ounces/week had an increased OR of 3.5 (95% CI: 1.1–10.6), but long-term users (more than 20 years) did not have additional risk (OR=1.5; 95% CI: 0.6–4.0). In this population, tobacco chewers had higher risk (OR=1.7; 95% CI: 0.6–4.5) than did snuff users (OR=1.1; 95% CI: 0.4–3.5). A case-control study in New York City found a non-significant increase in pancreatic cancer among chewing tobacco users (OR=3.6; 95% CI: 1.0–12.8) (Muscat et al., 1997); however, the small number of ST users (n=11) makes these results difficult to interpret. These two studies indicate a tentative association between US ST products and an increased risk of pancreatic cancer. Clearly, a larger well-controlled study of the incidence of pancreatic cancer in an exclusive ST user population is needed to clarify possible associations between ST use and this type of cancer.

A recent case-control study by Hassan et al. (2007) found no association between ST use and pancreatic cancer; non-tobacco users were compared with users of chewing tobacco (RR=0.6; 95% CI: 0.3–1.4) and users of snuff (unspecified type) (RR=0.5; 95% CI: 0.1–1.5). Individuals who used both chewing tobacco and cigarettes had no increased risk (RR=0.7; 95% CI: 0.4–1.2). However, smokers of more than 20 cigarettes/day had an increased risk of 1.4 (95% CI: 1.1–2.0) compared with non-tobacco users (Hassan et al., 2007). The lack of statistical significance of this study may be due to the small sample size of ST users (n=88). Similarly, a cohort study, based on only 16 deaths and adjusted for alcohol use but not smoking, reported that users of US ST (type not specified) had an RR of 1.7 (95% CI: 0.9–3.1) compared with non-tobacco users (Zheng et al., 1993). A population-based case-control study from Washington state reported no increased risk of pancreatic cancer for chewing tobacco users (OR=0.8; 95% CI: not reported) compared with non-tobacco users (Farrow & Davis, 1990). This study had a small sample of ST users and did not control for cigarette smoking. Thus, although several studies indicated no increased risk of pancreatic cancer in US ST users (chewing tobacco and unspecified snuff), these studies analyzed small sample sizes and were often not adjusted for cigarette smoking, the result being a weak association.

7.2.6.4 Bladder cancer

An estimated 67,160 cases of bladder cancer occurred in the US in 2007 (Jemal et al., 2007). Smokers have an elevated risk of bladder cancer
compared with non-tobacco users in a frequency and dose-dependent
fashion (Burch et al., 1989; Mommsen & Aagaard, 1983; Slattery et al., 1988;
Thun et al., 2000; Wynder et al., 1963).

7.2.6.4.1 Swedish snus
An association between Swedish snus and bladder cancer has not been
reported. Snus users had no increased risk of bladder cancer compared
with non-tobacco users (RR=0.72; 95% CI: 0.52–1.06) (Boffetta et al., 2005).

7.2.6.4.2 Chewing tobacco
Two studies reported an increased risk of bladder cancer in ST users in
Denmark and the US compared with non-tobacco users (Mommsen &
Aagaard, 1983; Slattery et al., 1988), although one study reported no
increased risk for ST users (Hartge et al., 1985). Chewing tobacco users in
Denmark had an increased risk of bladder cancer (RR=2.0; 95% CI: 1.2–
3.4) versus non-tobacco users, but this risk was non-significantly lower than
that of smokers (RR=2.7; 95% CI: 1.5–4.9) (Mommsen & Aagaard, 1983). A
Utah study that adjusted for cigarette smoking found that snuff users
(OR=2.73; 95% CI: 0.48–15.57) and chewing tobacco users (OR=2.78; 95%
CI: 0.38–20.20) had a non-significantly increased risk of bladder cancer
compared with non-tobacco users (Slattery et al., 1988). However, another
US study also reported no association between bladder cancer and chewing
tobacco users who never smoked cigarettes (RR=1.02; 95% CI: 0.67–1.54)
and between bladder cancer and snuff users (RR=0.77; 95% CI: 0.38–1.56)
compared with non-tobacco users (Hartge et al., 1985). These latter two
studies had small sample sizes (Hartge et al., 1985; Slattery et al., 1988).

The Third National Cancer Survey (1969–1971), which controlled for cigarette
smoking, reported no increased risk of bladder cancer in users of US chewing
tobacco or snuff (OR=1.61; 95% CI: not reported) compared with non-tobacco
users (Williams & Horm, 1977). With adjustment made for cigarette smoking,
another study determined that US male chewing tobacco users had no
increased risk of bladder cancer (RR=0.9; 95% CI: 0.5–1.6) compared with
non-tobacco users (Howe et al., 1980). A Canadian study found no
association between bladder cancer and tobacco chewing (RR=0.6, 95%
CI: 0.34–1.06) or snuff use (type not specified) (RR=0.47; 95% CI: 0.21–
1.07) compared with non-tobacco users (Burch et al., 1989).

At this time, it is not clear whether ST use promotes bladder cancer compared
with never smoking; however, the studies showing a positive association
reported an RR of lesser magnitude than that for smokers (Figure 7.9).
Figure 7.9 Bladder Cancer Incidence Associated with Cigarette Smoking and Smokeless Tobacco Use

The notation “Other ST products” refers to the fact that this study did not include Swedish snus or a US ST product. Horizontal lines represent the 95% CI. CI: confidence interval; CPS: Cancer Prevention Study; OR: odds ratio; RR: relative risk; *: not stated.

7.2.6.5 Kidney cancer

There were an estimated 13,000 deaths out of 51,000 kidney cancer cases in the US in 2007 (Jemal et al., 2007). The relationship between cigarette smoking and renal cell cancer is controversial (Doll, 1996). US male chewing tobacco users had an increased OR for renal cell cancer of 4.0 (95% CI: 1.1–14.2) compared with non-tobacco users (Goodman et al., 1986). The authors pointed out that although this finding was significant it was based on only 17 subjects. The same study reported no association between cigarette smoking and renal cell cancer compared with non-tobacco use (OR=1.1; 95% CI: 0.7–1.8); again, this result may be due to the small sample size. A similar study reported an increased risk of renal cell carcinoma in male chewing tobacco users (OR=3.2; 94% CI: 1.1–8.7) compared with non-tobacco users, but it did not adjust for smoking and had a small sample size as well (Muscat et al., 1995). This study also reported an elevated risk for cigarette smokers (OR=1.4; 95% CI: 1.02–2.0).
US men who used snuff had a significantly increased risk of renal cell carcinoma (OR=3.6; 95% CI: 1.2–13.3) compared with non-tobacco users; this study controlled for cigarette smoking (Asal et al., 1988). A study that combined results from five countries (Australia, Denmark, Germany, Sweden, and the US) reported a non-significantly increased risk of renal cell cancer in ST users (type not specified) (RR=1.3; 95% CI: 0.6–3.1) compared with non-tobacco users (McLaughlin et al., 1995). However, this conclusion was based on a small number of users (n=23), and no dose-response effect was detected. One study controlling for cigarette smoking reported that use of ST (chewing tobacco or snuff) had no effect on risk of renal cell cancer (OR=1.02; 95% CI: 0.56–1.85) (Yuan et al., 1998) compared with non-tobacco use. The same study, with an adequate sample size, reported an increased risk of renal cell carcinoma in cigarette smokers (OR=1.37; 95% CI: 1.13–1.66).

ST use is thus weakly associated with renal cell carcinoma. The studies presented here often did not control for confounders and had inadequate sample sizes.

### 7.2.7 Other Health Effects

#### 7.2.7.1 Pregnancy outcomes

A 2001 study estimated that 19–27% of US women smoked during pregnancy (Dempsey & Benowitz, 2001). Women who smoke during pregnancy have an increased risk of pregnancy complications, premature birth, low birth weight (LBW) infants, stillbirth, and infant mortality (Shea & Steiner, 2008; U.S. Department of Health and Human Services, 2001). Pregnant women who smoke have reduced-weight and small-for-gestation-age infants (England et al., 2003). Nicotine readily crosses the placental barrier, regardless of consumption form. Exposure to maternal smoking has been implicated in sudden infant death syndrome (SIDS) and intrauterine growth retardation. [See Anderson and Cook (1997) for review.] The toxicity of smoking during pregnancy is attributed to the cardiovascular effects of nicotine that result in reduced blood flow to the placenta, which creates a state of hypoxia and malnutrition for the fetus (Birnbaum et al., 1994; Dempsey & Benowitz, 2001; Xiao et al., 2007). However, smokers have a reduced risk for pre-eclampsia as compared with non-tobacco users (U.S. Department of Health and Human Services, 2001). If nicotine is obtained from a source other than cigarettes, fetal exposure to carbon monoxide and other toxins that could contribute to pregnancy complications is reduced. ST use during pregnancy reduced infant birth weight and increased risk of pre-eclampsia and preterm delivery (England et al., 2003). LSRO does not support the use of ST products by pregnant women.
7.2.7.1.1 Swedish snus

Women who used Swedish snus during pregnancy gave birth to reduced-birth-weight infants (mean weight change = -39 g; 95% CI: 6–72) compared with non-tobacco users (England et al., 2003). Snus users did not have a significantly increased risk of giving birth to small-for-gestational-age infants (OR=1.25; 95% CI: 0.72–2.17), whereas cigarette smokers had an increased risk (OR=2.99; 95% CI: 2.48–3.61) (England et al., 2003). Swedish snus users also had an increased risk of preterm delivery (OR=1.98; 95% CI: 1.46–2.68), as did smokers (OR=1.57; 95% CI: 1.38–1.80). The risk of pre-eclampsia was increased for Swedish snus users (OR=1.58; 95% CI: 1.09–2.27) but was reduced for smokers (OR=0.63; 95% CI: 0.53–0.75). This study failed to control for alcohol use (England et al., 2003).

7.2.7.1.2 Pure nicotine and pregnancy outcomes

Nicotine carries pregnancy risks and has been implicated in LBW infants and SIDS. [See Benowitz and Dempsey (2004) for review.] However, cigarette smoking during pregnancy carries a far greater risk than does exposure to pure nicotine (Birnbaum et al., 1994; Dempsey & Benowitz, 2001; Xiao et al., 2007). A small study of pregnant women wearing a transdermal nicotine patch for 4 days found that fetal heart rate was reduced compared with when the same subjects were smoking (MacLeod & Chowdhury, 2006). Another study reported higher infant birth weights for smokers also using transdermal nicotine patches than for smokers receiving a placebo for 2–9 weeks (Wisborg et al., 2000). Despite some risks to the fetus, the US Public Health Service Clinical Practice Guidelines concluded, that NRT is preferable to continued smoking (Fiore et al., 2000).

7.2.7.2 Inflammatory bowel disease

Smoking increases the risk of Crohn’s disease but decreases the risk of ulcerative colitis (Birrenbach & Bocker, 2004; Vessey et al., 1986). Compared with never smokers, current smokers had approximately half the risk of ulcerative colitis but paradoxically more than double the risk of Crohn’s disease. The biological mechanisms underlying these opposite effects in the two most common inflammatory bowel diseases (IBDs) are unknown. Nicotine is metabolized to a number of metabolites or degradation products, some of which may be responsible for protective effects. Swedish snus users had no increased risk of Crohn’s disease (RR=0.9; 95% CI: 0.3–3.1) or ulcerative colitis (RR=1.1; 95% CI: 0.4–3.1) (Persson et al., 1993). This study was limited by a lack of statistical power, given the relatively low incidence of IBD and thus small comparison groups. Clinical trials studying the effects of nicotine in patients with ulcerative colitis are complicated by side effects
from high systemic nicotine concentrations used in the trials (McGilligan et al., 2007).

### 7.2.7.3 Diabetes

Cigarette smokers have an increased risk of non-insulin-dependent diabetes mellitus, with a dose-response trend toward higher risk for heavier smokers, compared with non-tobacco users (Kawakami et al., 1997; Manson et al., 2000; Rimm et al., 1995; Will et al., 2001).

#### 7.2.7.3.1 Swedish snus

In a population study of cigarette smokers and Swedish snus users, Persson et al. (2000) found that snus users had a non-significantly increased risk of type 2 diabetes compared with non-tobacco users (OR=1.5; 95% CI: 0.8–3.0). For users of 3 or more boxes (approximately 50 g/box) of snus per week, the comparative OR increased further to 2.7 (95% CI: 1.3–5.5). This study controlled for both smoking and alcohol use. A more recent prospective study showed that current Swedish snus users did not have an increased risk (OR=1.06; 95% CI: 0.43–2.64) (Eliasson et al., 2004). In this study, neither Swedish snus users nor smokers had abnormal glucose tolerance. A link between ST use and diabetes may exist, but no biological mechanism for the relationship is currently known.

### 7.2.7.4 Amyotrophic lateral sclerosis

Female smokers enrolled in CPS II had an increased risk of dying from amyotrophic lateral sclerosis (ALS) (RR=1.67; 95% CI: 1.24–2.24), whereas males had a reduced risk (RR=0.69; 95% CI: 0.49–0.99) (Weisskopf et al., 2004). A dose and duration effect of cigarette smoking and increased risk of ALS was found in a population-based case-control study in the US (OR=2.0; 95% CI: 1.3–3.2) (Nelson et al., 2000). This study also controlled for alcohol use and found no association between alcohol and risk of ALS. The authors speculated that toxic constituents of cigarette smoke could damage motor neuron components. In the Swedish construction worker cohort, neither Swedish snus users (OR=0.6; 95% CI: 0.3–1.5) nor smokers (OR=0.7; 95% CI: 0.5–1.1) had an elevated risk of ALS (Fang et al., 2006). The literature is inconclusive on whether cigarette smoke contributes to ALS, some studies report no association (Fang et al., 2006). However, as would be true for all neurodegenerative diseases, more research is needed to rigorously define any contributions of ST to the pathophysiology of these diseases.
Differentiating the Health Risks of Categories of Tobacco Products

7.3 CONCLUSIONS

The literature on health effects of ST use versus cigarette smoking is suboptimal. Much of the literature that does exist is confounded by well-recognized limitations. Most important is the lack of information that rigorously compares the effects of cigarette smoking and the risks of ST products. Small sample size; inadequate control of factors such as ever smoking and alcohol; socioeconomic status; and ill-defined quantitation of ST exposure (type, dose, and frequency) are important challenging confounders. Little consideration has been given to residual effects of previous smoking or to exposure to environmental tobacco smoke. Many of these studies were performed in different countries and used various tobacco products, which lessens the comparative value of the findings. Tobacco manufacturing methods, tobacco species, and added ingredients may play a role in health effects of ST use, but the literature often neglects these issues.

Increased risk of LC in the smoking population is well established, and compared with ST, the evidence of increased risk is overwhelming. Given the mechanism of development of LC, this cancer does not seem to represent a plausible outcome of ST use, however.

Nevertheless, ST use is not free of risk. Despite the reduced risk of many smoking-related diseases such as LC and COPD in ST users, ST still carries risks of oral disease, pre-eclampsia for pregnant women, pancreatic cancer, and possibly CVD.

Smokers have an increased risk of dying from CVD. Scant evidence links Swedish snus or US ST products to increased CVD, but these data suffer from various confounders. All studies reporting positive associations for ST use and CVD determined risk values to be lower than those for smokers, although these two groups were compared with non-tobacco users, not compared directly with each other.

Cigarette smokers also have a dose-dependent increased risk of all-cause mortality compared with non-tobacco users. Studies with numerous confounding factors indicated that Swedish snus users and US ST users had an increased risk of all-cause mortality compared with non-tobacco users. One study reported no increased risk of all-cause mortality in US ST users compared with non-tobacco users.

Oral cancer risk is greater for cigarette smokers than for non-smokers. No evidence of increased oral cancer risk was found for Swedish snus users compared with non-tobacco users. Several studies of the US population
reported an increased risk of oral or pharyngeal cancer in ST users. However, some of these studies did not control for the aforementioned likely confounders. Swedish *snus* and US ST products do not seem to be related to an increased risk of oral cancer and almost certainly confer a lesser risk to oral health than cigarettes. Laryngeal cancer is not increased in US ST users.

Smokers have an increased risk of esophageal squamous cell carcinoma and gastric cancer. Compelling evidence suggests that Swedish *snus* is not associated with esophageal or gastric cancer. Mixed results came from evaluations of US snuff use and gastric cancer risk: some studies reported increased risk, others reported no increased risk. Swedish *snus* was not associated with any of these cancers.

Smokers also have an increased risk of bladder cancer, and ST users in Denmark and in one US study had an increased risk of bladder cancer. In Canada, no association between bladder cancer and ST use was found, but sample sizes were small. Smokers, Swedish *snus* users, and US ST users have greater pancreatic cancer risk than non-tobacco users, but the risk for ST users compared with that for smokers was significantly reduced.

Smoking is associated with adverse events in pregnant women, including low-birth-weight infants and preterm delivery. Tentative conclusions suggest that Swedish *snus* also has adverse effects in pregnant women, including low-birth-weight infants and pre-eclampsia, although the magnitude of the increase compared with that of smokers remains to be fully clarified.

Smoking may be associated with increased risk of diabetes, and reports of diabetes risk and *snus* use offer persuasive evidence of an association. Both smokers and ST users have a decreased risk of Parkinson’s disease, but this finding was based on only one study.

### 7.4 SECONDARY LITERATURE

Differentiating the Health Risks of Categories of Tobacco Products

and concluded that ST products are carcinogenic to humans and that ST exposure is associated with higher incidences of oral cancers compared with healthy populations who never smoked (Cogliano et al., 2004; International Agency for Research on Cancer, 2004b). A 2001 US Institute of Medicine report found an association between ST and oral cancer (Institute of Medicine, 2001). This report also concluded that ST is safer than cigarettes, particularly as used in Sweden and North America. ST use was also related to non-cancerous oral diseases and other adverse health effects including CVD, diabetes, poor pregnancy outcomes, and overall mortality (World Health Organization Scientific Advisory Committee on Tobacco Products Regulation, 2003).

Several reviews on health effects of ST have appeared (Critchley & Unal, 2003, 2004; Fagerström & Schildt, 2003). Critchley and Unal (2003) concluded that recent studies from the US and Scandinavia reporting an increased oral cancer risk for ST users were not statistically significant because of a lack of statistical power in view of the small number of never tobacco users. Roth et al. (2005) concluded that health risks associated with Swedish snus are considerably lower than those associated with smoking. A review by Fagerström and Schildt (2003) also concluded that Swedish snus does not cause oral cancer and that adverse effects on the cardiovascular system are less than those of smoking.

Several health effects assessments have reviewed data on the risk of oral cancer in exclusive Swedish snus users (Critchley & Unal, 2003; Fagerström & Schildt, 2003; 2003). Fagerström & Schildt (2003) concluded that published health effects assessments of snus users indicate no increased risk of oral cancer. However, other reviewers (Critchley & Unal, 2003) concluded that although the Scandinavian studies of oral and oropharyngeal cancer consistently show no effect, these studies do not have sufficient sample sizes to detect a significantly increased OR. Foulds et al. (2003) concluded that 5 large studies examining snus in relation to oral cancer were consistent in finding no increased oral cancer risk among snus users. On the basis of their review, Foulds et al. (2003) concluded that the absence of a relationship between Swedish snus and oral cancer was unlikely to be due to methodological problems such as large confidence intervals.

The type of US ST product used will have an impact on health effect findings. A review of cancer of the upper respiratory tract cancer evaluated different types of ST and various locations of cancer (Rodu & Cole, 2002). The authors reported that dry snuff, which is used mostly by women in the southern US, was associated with the highest risk of oral and pharyngeal cancer. However,
chewing tobacco or moist snuff use did not seem to be associated with an increased risk of oropharyngeal cancer. Future research should differentiate tobacco products by type, as classified in this report. Information on the precise incidence of cancer at specific sites should also be collected.
INTERNATIONAL SMOKELESS TOBACCO PRODUCTS

8.1 ANALYSIS OF INTERNATIONAL SMOKELESS TOBACCO PRODUCTS

The use of smokeless tobacco (ST) is growing worldwide. Afghanistan, Bangladesh, Sri Lanka, Thailand, and the Central Asian republics have a high prevalence of ST use (International Agency for Research on Cancer, 1986). An estimated 100 million people of the Indian-Pakistani subcontinent consume ST (National Cancer Institute & Smoking and Tobacco Control Program, 1992). People in Africa, Asia, and Latin America use a wide variety of different forms of ST. Table 8-1 provides examples of international STs including product constituents and commercial brand names (National Cancer Institute & Centers for Disease Control and Prevention, 2002).

Most international ST products differ considerably from the standard ST products manufactured and used in the US and Sweden (National Cancer Institute & Centers for Disease Control and Prevention, 2002). They vary in terms of tobacco species, aging, fermentation and production, processing, packaging, pH, moisture, nicotine and tobacco-specific nitrosamine (TSNA) contents, additives, and method of use (Idris et al., 1998a). Many products are made by individual farmers and small companies, the result being little control over fermentation and curing processes and thus effects on TSNA content (Rodu & Godshall, 2006). International ST products are often combined with additives (e.g., betel leaf, areca nut, and powdered lime) that may themselves enhance the toxicity and psychotropic effects of tobacco (Thomas & MacLennan, 1992; Wary & Sharan, 1988). Some of these additives, such as areca nut, were shown to be toxic by themselves (Dave et al., 1992; Wary & Sharan, 1988). Many ST users abroad also smoke concurrently, which complicates assessments of the health impact of their ST use (Hirayama, 1966; Jayant et al., 1977).
<table>
<thead>
<tr>
<th>Product Type</th>
<th>Brand Names</th>
<th>Geographic Origin</th>
<th>Constituents</th>
<th>Method of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chimó</em></td>
<td>San Carleño, El Tovareño, El Tigrito, El Sabroso, El Gran Búfalo, El Dragón, El Morichal</td>
<td>Venezuela</td>
<td>Tobacco leaf, sodium bicarbonate, brown sugar, ashes from the Mamón tree (<em>Melicocca bijuga</em>), and vanilla and anisette flavorings; regional variation of ingredients</td>
<td>Small amount placed between the lip or cheek and gum, left for about 30 minutes. Mixture of <em>chimó</em> and saliva spit out.</td>
</tr>
<tr>
<td><em>Gul</em> (gudakhu)</td>
<td>None</td>
<td>Central and eastern India</td>
<td>Paste containing tobacco powder, and other ingredients</td>
<td>Used to clean teeth.</td>
</tr>
<tr>
<td><em>Gutkha</em></td>
<td>Manikchand, Moolchand, Tulsi, Shimla, Sikandar, Pan Parag</td>
<td>India, Southeast Asia, UK</td>
<td>Areca nut, catechu, tobacco, slaked lime, saffron, and flavoring</td>
<td>Held in mouth and chewed. Saliva spit out, sometimes swallowed.</td>
</tr>
<tr>
<td><em>Iq’mik</em></td>
<td>None</td>
<td>Alaska (US)</td>
<td>Fire-cured tobacco leaves, and punk ash (ash generated by burning punk fungus that grows on birch tree bark)</td>
<td>Small piece pinched off and chewed. Piece possibly pre-chewed and placed in small box for later use by others.</td>
</tr>
<tr>
<td><em>Khaini</em></td>
<td>Raja, Kuber</td>
<td>Western and central states of India</td>
<td>Tobacco, slaked lime paste (liquid calcium hydroxide), and sometimes areca nut</td>
<td>Basic ingredients formed into ball at time of use. Held in mouth and sucked for 10–15 minutes.</td>
</tr>
</tbody>
</table>
### Table 8-1. International Smokeless Tobacco Brand Names, Geographic Origins, Constituents, and Methods of Use (continued)

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Brand Names</th>
<th>Geographic Origin</th>
<th>Constituents</th>
<th>Method of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qiwam (kimam)</td>
<td>None</td>
<td>India</td>
<td>Tobacco, spices (cardamom, saffron, and/or anise), and additives such as musk</td>
<td>Paste placed in mouth and chewed. Also used in betel quid.</td>
</tr>
<tr>
<td>Mawa</td>
<td>None</td>
<td>India</td>
<td>Tobacco flakes, slaked lime, and sun-cured areca nut</td>
<td>Placed in mouth and chewed for 10–20 minutes.</td>
</tr>
<tr>
<td>Mishri (masheri, misheri)</td>
<td>None</td>
<td>India</td>
<td>Toasted powdered tobacco</td>
<td>Applied to teeth and gums to clean teeth, then held in mouth.</td>
</tr>
<tr>
<td>Nass</td>
<td>None</td>
<td>Central Asia, Iran, Afghanistan, Pakistan, India</td>
<td>Tobacco leaf, ash, cottonseed or sesame oil, water, slaked lime, and sometimes gum</td>
<td>Held in mouth for 10–15 minutes.</td>
</tr>
<tr>
<td>Naswar, niswar</td>
<td>None</td>
<td>Central Asia, Iran, Afghanistan, Pakistan, India</td>
<td>Tobacco, slaked lime, indigo, cardamom, oil, menthol, and water</td>
<td>Held in mouth for 10–15 minutes, sometimes chewed slowly.</td>
</tr>
<tr>
<td>Pan masala (betel quid, pan-tobacco)</td>
<td>Manikchand, Mahak, Pan Parag #1, Vimal, Crane, Rajdarbar, Kuber, Yamu, Badshah, Tulsi, Rahat, Pan King, Jubilee, Kanchan</td>
<td>India, Sri Lanka, Pakistan, Bangladesh, Myanmar, Thailand, Cambodia, Malaysia, Singapore, Indonesia, Philippines, New Guinea, Taiwan, China</td>
<td>Slaked lime and catechu smeared on betel leaf; leaf folded into a funnel shape, with tobacco, areca nut, and other ingredients (menthol, camphor, sugar, rosewater, aniseed, mint, or other spices) added; top of funnel folded over to make a quid</td>
<td>Quid placed in mouth between gum and cheek, then gently sucked and chewed.</td>
</tr>
</tbody>
</table>
### Table 8-1. International Smokeless Tobacco Brand Names, Geographic Origins, Constituents, and Methods of Use (continued)

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Brand Names</th>
<th>Geographic Origin</th>
<th>Constituents</th>
<th>Method of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creamy snuff</td>
<td>Ipco, Denobac, Tona, Ganesh</td>
<td>India</td>
<td>Tobacco, clove oil, glycerin, spearmint, menthol, and camphor</td>
<td>Used to clean teeth.</td>
</tr>
<tr>
<td>Toombak</td>
<td>None</td>
<td>Sudan</td>
<td>Tobacco and sodium bicarbonate</td>
<td>Product rolled into 10 g ball called <em>saffa</em>. <em>Saffa</em> is held between gum and lip or cheek, or under tongued slowly for 10–15 minutes, then saliva spit out (males) or swallowed (females). Mouth usually rinsed with water after removal.</td>
</tr>
<tr>
<td>Red tooth powder</td>
<td>Dabur</td>
<td>India</td>
<td>Tobacco</td>
<td>Used to clean teeth.</td>
</tr>
<tr>
<td>Zarda</td>
<td>Baba, Bharat, Gopal</td>
<td>India, Arab countries</td>
<td>Tobacco, lime, spices, vegetable dyes, and areca nut</td>
<td>Often used in betel quid or chewed.</td>
</tr>
<tr>
<td>Shammah</td>
<td>None</td>
<td>Saudi Arabia, Yemen</td>
<td>Powdered tobacco, lime, ash, oils, black pepper, and flavoring</td>
<td>Chewed and then placed between lower lip and cheek.</td>
</tr>
</tbody>
</table>

Adapted from National Cancer Institute and Centers for Disease Control and Prevention (2002) and World Health Organization (2004b).
8.2 MANUFACTURING, GEOGRAPHIC ORIGIN, AND CHEMICAL COMPOSITION

8.2.1 Tobacco Types, Curing Methods, and Production Processes

Many international ST products are made locally via non-standardized and poorly characterized manufacturing processes. The few companies that manufacture international ST products do not detail their methods. This situation makes a careful review of curing and production methods impossible. Table 8-2 provides a brief summary of manufacturing processes of selected international ST products. Table 8-3 presents an overview of available data for a small number of tobacco constituents, pH, nicotine, polyaromatic hydrocarbons, and heavy metals in zarda.

Table 8-2. Manufacturing Processes of International Smokeless Tobacco Products

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Tobacco Used</th>
<th>Type of curing</th>
<th>Manufacturing Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toombak</td>
<td>Dark Nicotiana rustica</td>
<td>Air/sun</td>
<td>Dried in field, bundled, moistened, fermented, ground, aged, and mixed with sodium bicarbonate (20%)</td>
</tr>
<tr>
<td>Gutkha</td>
<td>Dark Nicotiana tabacum</td>
<td>Air/sun</td>
<td>Cut tobacco mixed with crushed areca nut, slaked lime, saffron, catechu, and flavorings</td>
</tr>
<tr>
<td>Betel quid</td>
<td>Dark Nicotiana tabacum</td>
<td>Air/sun</td>
<td>Leaf roasted, chopped, powdered, mixed with flavoring into paste and added to boiled or roasted areca nut, slaked lime, and catechu and wrapped in betel leaf</td>
</tr>
<tr>
<td>Nass</td>
<td>Dark Nicotiana tabacum</td>
<td>Sun/heat</td>
<td>Leaf mixed with slaked lime, ash, and flavoring, added to water, rolled in a ball</td>
</tr>
<tr>
<td>Gul</td>
<td>Dark Nicotiana tabacum</td>
<td>Air/sun</td>
<td>Leaf roasted, ground to powder, mixed with flavoring (paste used to clean teeth)</td>
</tr>
</tbody>
</table>

Adapted from Davis and Nielsen (1999), Rodu and Godshall (2006), and National Cancer Institute and Centers for Disease Control and Prevention (2002).

8.2.2 Tobacco-Specific Nitrosamines

International ST products have higher TSNA levels than do most US and Swedish products (Table 8-4). Sudanese toombak, an ST product consisting of tobacco and sodium bicarbonate rolled into a ball, has exceptionally high levels of TSNAs, up to 100 times the levels measured in US and Swedish snuff (Idris et al., 1994). Both the type of tobacco used (Nicotiana rustica) and the manufacturing process likely contribute to these high TSNA levels (Idris et al., 1991).
Table 8-3. Chemical Comparison of the Smokeless Tobacco Product Zarda

<table>
<thead>
<tr>
<th>Product</th>
<th>Brand</th>
<th>Moisture (% w/w)</th>
<th>pH</th>
<th>Nicotine (mg/g)</th>
<th>B[a]P (ng/g)</th>
<th>Cr (µg/g)</th>
<th>Ni (µg/g)</th>
<th>Pb (µg/g)</th>
<th>As (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zarda, India</td>
<td>Baba 120</td>
<td>13.18</td>
<td>4.88</td>
<td>55</td>
<td>2.83</td>
<td>2.08</td>
<td>2.94</td>
<td>1.56</td>
<td>0.4</td>
</tr>
<tr>
<td>Zarda, UK</td>
<td>Baba Zarda</td>
<td>7.88</td>
<td>5.32</td>
<td>48.4</td>
<td>2.04</td>
<td>2.34</td>
<td>5.88</td>
<td>1.18</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Adapted from McNeill et al. (2006).

B[a]P: benzo[a]pyrene, Cr: chromium; Ni: nickel; Pb: lead; As: arsenic.

8.3 PRECLINICAL STUDIES

8.3.1 Genotoxicity and Cytotoxicity Assays

As stated in Chapter 4, standardized methods should be applied to genotoxicity and cytotoxicity testing of ST products.

8.3.1.1 Salmonella mutagenicity assay

Similar to extracts of ST products commonly used in India and Saudi Arabia, increase the number of revertants in Salmonella typhimurium (Hannan et al., 1986; Kulkarni et al., 1987; Niphadkar et al., 1996; Rickert et al., 2007; Shah et al., 1985; Shirname et al., 1983).

These Indian and Saudi Arabian products include betel quid with tobacco (betel leaf, areca nut, and slaked lime), gutkha (areca nut, tobacco, catechu, slaked lime, saffron, and flavorings), shammah (powdered tobacco, lime, ash, black pepper, oils, and flavorings), zarda (tobacco, lime, spices, vegetable dyes, and areca nut), and mishri (toasted powdered tobacco, used as a dentifrice).

Smokeless tobacco extracts (STE) made from chewing tobacco and mishri caused frameshift mutations in strain TA98 that required dose-dependent metabolic activation (Kulkarni et al., 1987; Shah et al., 1985). Betel quid with tobacco caused revertants both with and without metabolic activation in strain TA1535, which indicated a mutation by base-pair substitution (Shirname et al., 1983). Mishri and shammah only increased mutagenicity with metabolic activation. Mishri caused a frameshift mutation in strain TA98 with metabolic activation and base-pair substitutions in strains TA102 and TA100 with nitrosation (Niphadkar et al., 1996). Shammah caused frameshift mutations in several strains (TA100, TA97, TA98) but no base-pair substitution in TA102 (Hannan et al., 1986).
### Table 8-4. Tobacco-Specific Nitrosamine Levels in International Smokeless Tobacco Products

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toombak</td>
<td>Manufacturer A</td>
<td>Idris et al., 1991</td>
</tr>
<tr>
<td></td>
<td>Manufacturer B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Manufacturer C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Manufacturer D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Manufacturer F</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khani</td>
<td>Raja</td>
<td>Stepanov et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Hans Chhap</td>
<td>Stepanov et al., 2005</td>
</tr>
<tr>
<td>Zarda</td>
<td>Goa 1000</td>
<td>Stepanov et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Moolchand Super</td>
<td>Stepanov et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Sanket 999</td>
<td>Stepanov et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Baba 120</td>
<td>Stepanov et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Baba 120 Hakim Pury</td>
<td>Stepanov et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Dalal Misti</td>
<td>Stepanov et al., 2005</td>
</tr>
<tr>
<td>Qwam</td>
<td>10 samples</td>
<td>Tricker &amp; Preussmann, 1989</td>
</tr>
<tr>
<td></td>
<td>Shahin</td>
<td>Stepanov et al., 2005</td>
</tr>
<tr>
<td>Misrhi</td>
<td></td>
<td>Stepanov et al., 2005</td>
</tr>
</tbody>
</table>

**Notes:**
- All data except toombak reported as μg/g wet weight.
- Tobacco-specific nitrosamines [N'-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosoanabasine (NAB)].
- NAB + NAT.
- NA: not available; ND: not detected (detection limit 50 pmol/g tobacco).

**Legend:**
- **NNN:** 630–7,870 μg/g dry weight.
- **NNK:** 1,140–2,790 μg/g dry weight.
- **NAB:** 1,700–3,530 μg/g dry weight.
- **NAT:** 1,610–5,990 μg/g dry weight.
- **Total TSNAs:** 1,610–1,680 μg/g dry weight.

**Sources:**
- McNeill et al., 2006
- Tricker & Preussmann, 1989
- Stepnov et al., 2005
Chewing tobacco from India caused frameshift mutations in strains TA98 and TA100 with nitrosation (Niphadkar et al., 1996). In the same study, chewing tobacco with lime (a common additive in India) was mutagenic in TA98, TA100, and TA102 without metabolic activation. The lack of conformity of results in these products suggests that ST may contain more than one kind of mutagen, which leads to various lesions in different cell lines.

### 8.3.1.2 Mammalian cell mutagenicity

Chromosomal damage can be assayed with Chinese hamster ovary (CHO) cells. *Mishri*, betel quid with tobacco, and *pan masala* with tobacco (similar to betel quid) increased chromosomal aberrations in this assay (Jaju et al., 1992; Kulkarni et al., 1987; Patel et al., 1994; Shirname et al., 1984). However, *pan masala* without tobacco also produced the same effect (Jaju et al., 1992).

The micronucleus assay is used to screen for chemicals that are clastogens (*i.e.*, agents that cause structural alterations) and aneugens (*i.e.*, agents that cause the loss or gain of one or more chromosomes) (Organization for Economic Cooperation and Development, 1997c). *Pan masala* with and without tobacco increased the occurrence of sister chromatid exchange and micronucleated cells (Jaju et al., 1992; Patel et al., 1994). STE made from betel quid increased the micronucleated cell frequency with V79 cells (Shirname et al., 1984).

Betel quid with tobacco and lime increased the number of micronucleated cells in exfoliated human oral mucosal cells compared with oral cell samples from non-tobacco users *in vitro* (Nair et al., 1992). Other studies of Indian ST products reported similar findings (Desai et al., 1996; Kayal et al., 1993). In these studies, increased numbers of micronucleated cells were found in exfoliated buccal mucosal cells of users of betel quid with tobacco, dry snuff, *mishri*, and areca nut alone. No statistically significant differences were found among the various products. Indian users of *khaini* (tobacco mixed with slaked lime) also had increased numbers of micronucleated cells (Stich et al., 1992). These reports suggest that Indian ST products increase the frequency of micronucleated cells.

STE made from *khaini* reduced expression of the DNA repair enzyme methylguanine methyltransferase and caused deregulation of genes involved in cell growth, cell cycle regulation, and apoptosis *in vivo* in an oral epithelial cell line (AMOL-III) (Rohatgi et al., 2005). Commercially available *pan masala* with tobacco mixtures increased cytochrome c reduction, lipid peroxidation, and DNA fragmentation in cultured human oral keratinocytes (Bagchi et al., 2002). STE from both research grade moist snuff and *pan masala* with
tobacco produced DNA fragmentation and increased cytochrome c reduction (Bagchi et al., 2002). These results demonstrate that ST products used in India cause DNA damage in vitro.

Taken together, these studies indicate that many forms of international ST can damage chromosomes. Indian ST products produce DNA damage and increase the frequency of micronucleated cells in vitro. These studies lend support to the significantly increased health risks of many of these ST products compared with Swedish snus and US ST products.

### 8.3.2 Animal Models and Tumor Formation

Many Indian ST products are carcinogenic in vivo. STE made from betel quid with tobacco induced lung tumors in male Swiss mice (Shirname et al., 1983). Betel leaf alone was not tumorigenic. Another study in which betel quid with tobacco extract was applied on the buccal pouch of male Syrian golden hamsters found increased squamous cell carcinomas of the cheek pouch (Suri et al., 1971). This study was the first in vivo report confirming the tumorigenicity of betel quid. Another report found that Indian tobacco alone was not tumorigenic when painted on the check pouch of golden hamsters 3 times/week for the lifespan of the animal (18–24 months) (Ranadive et al., 1976). Areca nut extract caused malignant changes but was not tumorigenic. Indian chewing tobacco extract was tumorigenic (i.e., lung adenocarcinomas and hepatocellular carcinomas) in male Swiss mice (Shah et al., 1985). Although none of these studies identified the carcinogenic components of STE, these data suggest that Indian ST products are tumorigenic.

### 8.4. HUMAN EXPOSURE AND BIOLOGICAL EFFECTS

Urine and saliva TSNA levels were measured in users of toombak and betel quid with tobacco (Tables 8-5 and 8-6, respectively). Extremely high levels of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) were detected in urine and saliva of male Sudanese toombak users (Carmella et al., 2002; Idris et al., 1994; Murphy et al., 1994). Total TSNA levels were also increased in toombak users (Idris et al., 1994), which may play a role in the induction of oral tumors in this population.
Considerable data are available for a widely used Indian ST product known as betel quid with tobacco (International Agency for Research on Cancer, 2004). Betel quid is a combination of areca nut, slaked lime, and betel leaf, with spices and sweeteners sometimes added depending on regional differences (National Cancer Institute & Centers for Disease Control and Prevention, 2002). Betel quid can be made with or without tobacco in the mixture; when tobacco is added, it is specified throughout the following analysis. Other names include pan masala and pan. Pan masala refers to a commercially manufactured preparation, whereas pan refers to freshly prepared preparations of betel quid (National Cancer Institute & Centers for Disease Control and Prevention, 2002).

When health effects of ST versus cigarettes are considered, it is important to note the sociocultural preferences for tobacco smoking products. In India, the bidi cigarette, a temburni tree leaf-wrapped unfiltered cigarette, accounts for 50% of tobacco use (Mudur, 2001). Bidis are more commonly smoked by the lower socioeconomic classes because of low cost (Narayan et al., 1996). Given that the bidi is the dominant smoking product in India, many studies from India have evaluated risk for bidi smoking rather than cigarette smoking.

Table 8-5. Tobacco-Specific Nitrosamine Levels in Biological Fluids – Urine

<table>
<thead>
<tr>
<th>Reference</th>
<th>Product (Number of Subjects)</th>
<th>NNAL(^a)</th>
<th>NNAL-Gluc(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carmella et al., 2002</td>
<td>Cigarettes (10)</td>
<td>0.462 ± 0.214</td>
<td>0.322 ± 0.161 ((N)) 0.434 ± 0.343 ((O))</td>
</tr>
<tr>
<td></td>
<td>Snuff (10)</td>
<td>1.48 ± 1.13</td>
<td>0.59 ± 0.60 ((N)) 2.13 ± 2.55 ((O))</td>
</tr>
<tr>
<td></td>
<td>Toombak (4)</td>
<td>254.8 ± 187.2</td>
<td>32.6 ± 17.4 ((N)) 231.8 ± 264.4 ((O))</td>
</tr>
<tr>
<td>Murphy et al., 1994</td>
<td>Toombak (7)</td>
<td>210–620</td>
<td>360–1,500</td>
</tr>
</tbody>
</table>

\(^a\)NNAL measurements given as pmol/mg creatinine.

NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL-Gluc: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol glucuronide conjugates; \((N)\): N-linked glucuronide conjugates; \((O)\): O-linked glucuronide conjugates.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Product (Number of Subjects and Sex)</th>
<th>NNN</th>
<th>NAT</th>
<th>NAB</th>
<th>NNK</th>
<th>Total TSNAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nair <em>et al.</em>, 1985</td>
<td><em>Betel quid</em> + tobacco (12)</td>
<td>1.6–14.7</td>
<td>1.0–10.9</td>
<td>NR</td>
<td>ND–2.3</td>
<td>2.6–27.9</td>
</tr>
<tr>
<td></td>
<td><em>Betel quid</em> (12)</td>
<td>ND</td>
<td>ND</td>
<td>NR</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Tobacco (3)</td>
<td>16.5–59.7</td>
<td>13.5–51.7</td>
<td>NR</td>
<td>ND</td>
<td>30–111.4</td>
</tr>
<tr>
<td>Bhide <em>et al.</em>, 1986</td>
<td><em>Betel quid</em> + tobacco (7M)</td>
<td>15–50</td>
<td>NR</td>
<td>ND–40</td>
<td>ND–8.3</td>
<td>15–98.3</td>
</tr>
<tr>
<td></td>
<td><em>Betel quid</em> + tobacco (10F)</td>
<td>3–85.7</td>
<td>NR</td>
<td>ND–14</td>
<td>ND–14.3</td>
<td>3–114</td>
</tr>
<tr>
<td></td>
<td>Tobacco + lime (10)</td>
<td>10–430</td>
<td>ND–133</td>
<td>NR</td>
<td>ND–28.5</td>
<td>10–591.5</td>
</tr>
</tbody>
</table>

*Nitrosamine measurements given as ng/ml saliva.

**F**: female; **M**: male; **ND**: not detected; **NAT**: *N*-nitrosoanatabine; **NAB**: *N*-nitrosoanabasine; **NNK**: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; **NNN**: *N*-nitrosonornicotine; **NR**: not reported; **TSNAs**: tobacco-specific nitrosamines.
8.5.1 Lung Cancer

Indian users of betel quid with tobacco had no increased risk of lung cancer (LC) (OR=0.7, 95% CI: 0.4–1.2) compared with non-tobacco users after data were adjusted for smoking (Dikshit & Kanhere, 2000). In the same study, users of more than 20 bidis/day (OR=33.2; 95% CI: 13.9–79.2) or 20 cigarettes/day (OR=26.8; 95% CI: 6.0–120.2) had an increased risk of LC compared with non-tobacco users.

8.5.2 Cardiovascular Disease

A case-control study conducted in 52 countries reported that chewing tobacco users had an OR of 2.23 (95% CI: 1.41–3.52) for non-fatal myocardial infarction compared with non-tobacco users (Teo et al., 2006). However, the authors noted that their sample size was too small (specific numbers not reported) to draw any conclusions.

Data from the Nutrition and Health Survey in Taiwan (NAHSIT, 1993–1996) suggested that female daily users of betel quid with tobacco (10 or more/day) had an elevated incidence of cardiovascular disease (CVD) (OR=1.37; 95% CI: 1.1–1.6) (Guh et al., 2007). Men had no elevated risk of CVD with daily betel quid use (OR=0.5; 95% CI: 0.14–1.9). This study controlled for the confounders smoking, alcohol use, and diet. The authors speculated that these findings may be related to overall increased risk of CVD in females and that perhaps betel quid affects the sympathetic nervous system differently in females.

8.5.3 Oral Cancer

Studies from India, Pakistan, and Sudan reported a very significantly increased risk of oral cancer related to the use of betel quid with tobacco, chewing tobacco, toombak, and shammah (Balaram et al., 2002; Dikshit & Kanhere, 2000; Nandakumar et al., 1990; Wasnik et al., 1998) (Figure 8.1). A review by Critchley and Unal (2003) concluded that chewing betel quid (pan) and tobacco, a mixture of tobacco with slaked lime and areca nut wrapped in a betel leaf, was associated with “substantial risk of oral cancer.”

This finding is consistent with the increased occurrence of oral cancer in India compared with the rest of the world (Nair et al., 2004). Oral cancer risk is increased for betel quid with tobacco users compared with non-tobacco users (Balaram et al., 2002; Dikshit & Kanhere, 2000; Jayant et al., 1977; Rao et al., 1994; Sankaranarayanan et al., 1989a; Wasnik et al., 1998). Increased risk of oral cancer was reported for male chewers of 10 or more
Differentiating the Health Risks of Categories of Tobacco Products

Betel quid with tobacco/day in Kerala, India (RR=5.52; 95% CI: 2.85–10.67) compared with non-tobacco users (Sankaranarayanan et al., 1989a). A similar effect was reported for female chewers of 10 or more betel quid with tobacco/day (RR=9.27; 95% CI: 3.11–27.59), compared with non-tobacco users. Smokers of more than 21 bidi/day (RR=4.62, 1.98–10.76), but not cigarette smokers (RR=0.49; 0.21–an1.10), had an increased risk of oral cancer. This study could not determine significance because too few cigarette smokers were included. The authors also reported an RR of 3.02 (95% CI: 0.94–9.60) for users of nasal snuff (fine home ground tobacco powder), but there were too few users (n=14) to allow detection of significance. Several studies reported an increased risk of oral cancer associated with use of betel quid with tobacco, with dose and duration response adding strength to the association.

Chewing betel quid with tobacco is also specifically associated with cancer of the gingiva. In a population in Kerala, India, chewing more than 10 betel quid with tobacco/day led to an increased risk of gingival cancer in males
(RR=15.1; 95% CI: 7.83–28.99) and females (RR=13.69; 95% CI: 4.41–42.49), with a dose-response and duration effect (Sankaranarayanan et al., 1989b). Users of more than 10 quid/day had the highest risk. Similar to Sankaranarayanan et al. (1989a), smokers of more than 21 bidi/day (RR=3.20; 95% CI: 1.33–7.73) but not cigarette smokers (RR=0.53; 95% CI: 0.18–1.51) had an increased risk of gingival cancer. Both studies controlled for smoking and alcohol, and the frequency and dose-response association lend strength to the relationship between chewing betel quid with tobacco and oral cancer.

Another Indian study of betel quid with tobacco chewers found an increased risk of oral cancer (RR=2.95; 95% CI: 2.34–3.71) compared with controls (Rao et al., 1994). This study did not control for confounders (such as alcohol use) and observed a reduced risk for exclusive cigarette smokers (RR=0.56; 95% CI: 0.38–0.81). They reported an increased risk of oral cancer associated with bidi smoking (RR=1.59; 95% CI: 1.25–2.03), the more popular product in India, with a larger population of bidi smokers than cigarette smokers.

Balaram et al. (2002) reported an increased risk of oral cavity cancer for chewers of betel quid with tobacco in southern India for both males (OR=6.10; 95% CI: 3.84–9.71) and females (OR=45.89; 95% CI: 25.02–84.14), compared with non-tobacco users and adjusting for confounders (Balaram et al., 2002). Although the magnitude of the OR is unlikely to be accurate, this finding is consistent with results from other studies of chewing betel quid with tobacco. This study also reported an increased risk of oral cancer in bidi smokers (more than 20/day) (OR=2.50; 95% CI: 1.41–4.42) but not in cigarette smokers (ever users) (RR=1.08; 95% CI: 0.56–2.09) compared with non-tobacco users. Another study from southern India (Bangalore) also determined an increased risk of oral cancer for chewers of betel quid with tobacco (RR=14.6; 95% CI: 8.2–25.9) compared with non-tobacco users and adjusted for confounders (Nandakumar et al., 1990). In this study, cigarette smokers had an increased risk of oral cancer (RR=2.1; 95% CI: 1.1–4.2) but bidi smokers did not (RR=1.4; 95% CI: 0.6–3.0), compared with non-tobacco users and adjusted for confounders. This particular study included few bidi smokers and a greater number of cigarette smokers.

An early report associated chewing tobacco with oral cancers in India (RR=6.0, CI not reported) (Jayant et al., 1977) compared with non-tobacco users and controlling for cigarette smoking. In the same population, smokers had an increased risk of 2.8. ST users had an increased risk of cancer of the oropharynx (RR=3.3), hypopharynx (RR=6.2), and larynx (RR=4.6). An additive risk effect was found for users of both cigarettes and ST: oral cavity
Differentiating the Health Risks of Categories of Tobacco Products

(RR=10.1), oropharynx (RR=31.7), hypopharynx (RR=16.9), and larynx (RR=20.1). However, without CI values reported, the strength of these data is difficult to evaluate.

A study from central India reported that both tobacco chewing (OR=7.98; 95% CI: 4.11–13.58) and cigarette smoking (OR=2.25; 95% CI: 1.22–3.70) were associated with oropharyngeal cancer, with a dose and duration effect, compared with non-tobacco users and adjusted for confounders (Wasnik et al., 1998). Another study from central India reported an increased risk of oral cavity cancer in tobacco quid chewers (OR=5.8; 95% CI: 3.6–9.5) and a non-significantly increased risk of oropharyngeal cancer (OR=1.2; 95% CI: 0.8–1.8) compared with non-tobacco users and adjusted for confounders (Dikshit & Kanhere, 2000). Chewing tobacco quid for more than 30 years resulted in an increased oral cavity cancer risk (OR=23.9; 95% CI: 12.0–47.3) and an increased risk of oropharyngeal cancer (OR=3.1; 95% CI: 1.6–5.7) compared with non-tobacco users. Smokers (bidi or cigarette) also had an increased oral cavity cancer risk (OR=4.3; 95% CI: 2.0–9.1) and an increased risk of oropharyngeal cancer (OR=18.6; 95% CI: 10.0–34.5) compared with non-tobacco users (Dikshit & Kanher, 2000).

A more recent study also determined an increased risk of pharyngeal cancer in Taiwanese betel quid users (OR=6.9; 95% CI: 3.4–14.3) compared with non-tobacco users; the effect was dose-dependent, with users of more than 20 quids/day having the greatest risk (Lee et al., 2005). This study adjusted for the confounders of alcohol use and cigarette smoking. However, this study found no increased risk of laryngeal cancer for betel quid users (OR=1.3; 95% CI: 0.6–2.7) but reported an increased risk for cigarette smokers (OR=8.3; 95% CI: 3.9–18.6). This finding makes sense in terms of route of administration for betel quid. Users who swallow quid juice would expose the pharyngeal mucosa but not the larynx (upper airway). Another study confirmed this finding; chewing more than 10 betel quid with tobacco/day was not associated with laryngeal cancer (RR=0.73, 95% CI: 0.36–1.46) compared with non-tobacco users and controlling for confounders (Sankaranarayanan et al., 1990a).

Toombak use was associated with oral cancer (Elbeshir et al., 1989; Idris et al., 1995a). Ever users of toombak had an increased risk of squamous cell carcinoma of the lip, buccal cavity, and floor of mouth compared with controls (OR=3.9; 95% CI: 2.9–5.3) (Idris et al., 1995a). Risk was also elevated with a greater duration of use, with toombak users of more than 11 years having an OR of 4.3 (95% CI: 2.9–6.3) (Idris et al., 1995a). Risk of cancer of the tongue, palate, and maxillary sinus was also elevated (OR=1.4, 95% CI:
0.8–2.5) but not statistically significant (Idris et al., 1995a). This study did not adjust for confounders and failed to find any association between oral cancer and cigarette smoking, which weakens the findings. The other study associating toombak use and oral cancer did not perform statistical analyses and included only the proportion of oral cancer patients who were also toombak users (Elbeshir et al., 1989).

Shammah users in Saudi Arabia had mucosal lesions at the site of shammah placement; these studies did not control for confounders (Allard et al., 1999; Salem et al., 1984). To date, no statistical studies of the risk of oral cancer in shammah users have been reported. It should be noted that shammah is illegal in Saudi Arabia, and conducting health effects assessments of this product is difficult (International Agency for Research on Cancer, 2007b).

A study from Pakistan found that ever users of naswar (a tobacco and lime mixture that is chewed and held in the mouth) or betel quid with tobacco chewers had increased risk of oral submucous fibrosis (a precancerous lesion) (OR=9.53; 95% CI: 1.73–52.53 and OR=8.42; 95% CI: 2.31–30.64, respectively) compared to non-tobacco users and adjusted for smoking and alcohol use (Merchant et al., 2000). Additionally this study reported that betel quid alone, without tobacco, increased risk for oral cancer (OR=9.90; 95% CI: 1.76–55.62). As mentioned previously, areca nut (a component of betel quid) itself is carcinogenic. Jafarey et al. (1977) found an increased risk of oral cavity cancer and oropharynx cancer in Karachi, India in users of chewing tobacco, betel quid with tobacco nass, and naswar compared with non-tobacco users, but this study did not adjust for cigarette smoking.

In summary, strong and consistent evidence exists for a greater risk of oral cancers associated with the types of ST used in India (betel quid with tobacco) and Sudan (toombak) compared with cigarettes and bidis (International Agency for Research on Cancer, 2004a). Studies from various regions with different chewing practices have consistently found statistically significant and clinically important associations for chewing betel quid with tobacco and oral cancers (International Agency for Research on Cancer, 2004a). Chewing betel quid is thus a significant world health problem.

8.5.4 Esophageal Cancer

Indian smokers have shown an increased risk of esophageal cancer (Jayant et al., 1977; Nandakumar et al., 1996; Phukan et al., 2001). Several reports of Indian users of chewing tobacco and betel quid have also been published, some of which indicated increased risks of esophageal cancer (Jayant et al., 1977; Nandakumar et al., 1996; Phukan et al., 2001), whereas one study did not (Sankaranarayanan et al., 1991). The Jayant et al. (1977) study of
Indian tobacco chewers showing such an increased risk compared with non-tobacco users (RR=2.5; CI: not reported) controlled the analysis for smoking.

A study of users of betel quid with tobacco in Kerala, India, however, found no increased risk of esophageal cancer (RR=0.64; 95% CI: 0.31–1.31) and no increased risk for cigarette smokers (RR=0.56; 95% CI: 0.26–1.19) (Sankaranarayanan et al., 1991). They also reported that bidi smokers had an increased risk of esophageal cancer, with users of 21 bidis or more/day having the highest risk (RR=5.22; 95% CI: 2.72–10.00). In contrast, another study reported a significantly increased risk of esophageal cancer (RR=2.9; 95% CI: 1.5–5.4) for chewers of betel quid with tobacco in Bangalore, India, compared with non-tobacco users and adjusted for confounders (Nandakumar et al., 1996). Further analysis showed that the increased risk related to chewing betel quid with tobacco was associated with cancer of only the lower third of the esophagus (RR=6.6; 95% CI: 2.1–21.2). These authors also reported an increased risk for bidi smokers (RR=3.5; 95% CI: 2.1–5.6). These two investigations were case-control studies with matched controls and took place during a similar time period (1982–1985) but were carried out independently. Sankaranarayanan et al. (1991) reported that the absence of risk may have been due to the regional habit of spitting rather than swallowing the quid. Nandakumar et al. (1996) reported that both spitters and swallowers had an increased risk, but the risk was greater in the swallowers (OR=2.7; 95% CI: 1.7–4.0). Sankaranarayanan et al. (1991) did not report such an analysis.

Phukan and coworkers (2001) evaluated the risk of esophageal cancer associated with various ways of chewing and preparing betel quid with tobacco. They found that preparations including fermented areca nut with tobacco (OR=7.1; 95% CI: 3.5–6.7) and without tobacco (OR=2.3; 95% CI: 0.7–8.4) increased risk. This suggests that fermented betel quid alone increases the risk of esophageal cancer. Non-fermented areca nut with tobacco also non-significantly elevated the risk of esophageal cancer (OR=1.9; 95% CI: 0.08–6.3). Together, these data show that Indian ST products (betel quid-like preparations) promote esophageal cancer and that the practice of holding the quid in the mouth and preparation method of the ST product contribute to carcinogenicity.

### 8.5.5 All-Cause Mortality

Data for Indian tobacco chewers have demonstrated an increased risk of mortality (Gupta et al., 1984; Gupta & Mehta, 2000). Gupta et al. (1984) analyzed mortality associated with chewing tobacco and smoking in the Ernakulam district of Kerala, India, during a 10-year period. Consumption of
betel quid with tobacco resulted in significantly higher all-cause mortality (RR=1.3) compared with non-tobacco users. Male tobacco chewers did not have an elevated mortality risk (RR=1.2). Because no confidence intervals were reported, these results are difficult to evaluate. A baseline study of the association between tobacco use and mortality in Mumbai, India, found an increased risk for female ST users (RR=1.35) compared with non-tobacco users (Gupta & Mehta, 2000). Similar findings were reported for male ST users (RR=1.22). Again, no confidence intervals were reported. This study did not control for potential confounders such as alcohol, education, and diet. Not enough information is available on Indian tobacco chewers over time to conclude whether Indian ST products increase mortality.

8.5.6 Pregnancy Outcomes

Pregnant female users of mishri (toasted powdered tobacco, often used as a dentifrice) and snuff had a greater risk of increased stillbirth, reduced birth weight, and increased preterm delivery (Gupta & Sreevidya, 2004; Gupta & Subramoney, 2006; Krishna, 1978). A study of mishri use, birth weight, and gestational age in Mumbai, India, found an increased OR of 1.6 (95% CI: 1.1–2.4) for low birth weight, a birth weight reduction of an average of 105 g, and increased risk of preterm delivery (Gupta & Sreevidya, 2004). Another study from Mumbai, India, assessed the association of ST use with risk of stillbirth (Gupta & Subramoney, 2006). Approximately 81% of ST users in this study typically used mishri and had an increased stillbirth HR of 2.6 (95% CI: 1.4–4.8) compared with non-tobacco users. Higher daily use of mishri—5 or more times/day—increased the risk of stillbirth (HR=3.8; 95% CI: 1.5–10.1). This study did not collect data on birth weight.

A South African study of maternal snuff and cigarette use revealed that both smokers and snuff users had differences in infants' mean birth weight, small-for-gestational-age infants, and preterm infants compared with non-tobacco users (Steyn et al., 2006). Infants' mean birth weight for snuff users was not significantly reduced (-29.4 g), but smokers had infants with significantly reduced birth weight (-165 g). Smoking was associated with small-for-gestational age infants and preterm delivery, but snuff use was not. The amount of tobacco use was not quantified in this study, which makes it impossible to determine dose effects.

Indian ST products increased risks of bearing low birth weight infants and preterm delivery. South African toombak reduced birth weight and increased risk of preterm delivery.
Taken together, these data suggest that use of *mishri* and snuff by pregnant women is harmful. These products differ from US STs and Swedish *snus* and contain many additives with unknown effects on pregnancy.

8.6 BEHAVIOR

8.6.1 Population Use Patterns

Different types of ST products are preferred in various parts of the world. For example, *toombak* is popular in Sudan. A study population of approximately 21,600 Sudanese individuals older than 4 years was sampled to determine the prevalence of *toombak* use and cigarette smoking in the Nile state (Idris et al., 1998b). The prevalence of *toombak* use (12.6% of the study population) was significantly higher than the prevalence of cigarette smoking (6.6% of the study population). *Toombak* use is more common in rural areas (35%) than in urban areas (24%); in contrast, cigarette smoking is more common in urban areas (18%) than in rural areas (12%). Users are predominantly male (23.0% of males versus 17% of females) and older adults.

The prevalence of ST use in South Asia varies considerably, with 1% of Thai and Indonesian populations reporting ST use and 25% of Bangladeshi individuals reporting ST use (Dobe et al., 2006). Within India, the prevalence of ST use shows a wide disparity in different regions of the country, with certain regions reporting 7.2% prevalence and another region, 80.3%. ST accounts for 35–40% of the tobacco used in India by both males and females (Dobe et al., 2006). In India, ST consumption increased among economically disadvantaged people between 1961 and 2000 (National Sample Survey Organization, 2001). Traditionally, Indian women and men were equally likely to be tobacco chewers (Gupta & Ray, 2004). However, these patterns are changing. Young women in urban areas prefer smoking, whereas women in rural areas continue to prefer ST in various forms (Dobe et al., 2006). Rates of ST use in Mumbai, India, are highest among unemployed and unskilled workers, both men and women, which suggests a socioeconomic difference in tobacco use in India (Gupta, 1996; Sorensen et al., 2005).

8.6.2 Nicotine Pharmacokinetics

Nicotine is a primary reason for the maintenance of a tobacco use habit. The pH of the product determines its ratio of unprotonated to free nicotine. The pH of Swedish *snus* is 7.5–8.5 (Andersson, 1991; Hirsch & Thilander, 1981). The pH of Sudanese *toombak* is estimated at 8–11 (Idris et al., 1998a), and *nass* (tobacco mixed with ash, cottonseed or sesame oil, and slaked
lime) has a pH of 11–11.8. The high pH of *toombak* and *nass* makes more nicotine available to the user.

### 8.6.3 Concomitant Use of Smokeless Tobacco and Cigarettes

Many South Asians use a variety of tobacco products indigenous to South Asia, such as betel quid with tobacco (Croucher *et al.*, 2007). An estimated 600 million people worldwide chew betel quid with tobacco (Gupta & Warnakulasuriya, 2002).

Concomitant use of cigarettes and ST may be higher in South Asian populations than in populations in other parts of the world. In a study of UK resident Bangladeshi men, 36% used cigarettes as their only source of tobacco, 8% chewed only betel quid with tobacco, and 22% used both products (Croucher *et al.*, 2007). Concomitant tobacco users were more likely to be married to women who also chewed tobacco. Another study of Bangladeshi men reported a 10% dual use prevalence (Pearson *et al.*, 2001), whereas a study of South Asians reported a 12% prevalence (Erens *et al.*, 1999). Many Sudanese *toombak* users also smoked cigarettes (Idris *et al.*, 1998b). Concurrent use has been reported for 5.5% of 11,068 men and 0.3% of 10,526 women.

### 8.7 CONCLUSIONS

International ST products vary in a number of respects such as species of tobacco, method of use, and added ingredients (Idris *et al.*, 1998a). Most international ST products are not standardized because individual farmers and small companies produce them. These variations result in products that have disparate chemical and mutagenic profiles.

Despite this variability, it is still possible to conclude that *pan masala*, betel quid with tobacco, and *khaini* increase the frequency of micronucleated cells. *Mishri*, betel quid with tobacco, and *pan masala* cause chromosomal aberrations in the CHO cell assay, increased occurrence of sister chromatid exchange, and increased frequency of micronucleated cells.

Reduced birth weight infants and preterm delivery were also associated with ST use in India and South Africa. More rigorous research is needed on exclusive ST users versus cigarette smokers for various health risks of tobacco use. Given the diversity of ST products worldwide, careful attention should be paid to ST type, added ingredients, and methods of preparation and use to provide valuable information on possible health effects associated with these products.
Dual use of ST and cigarettes has been reported throughout Asia and Sudan and ranges from 5% to 20% depending on the country and ST product.

Epidemiological studies have verified that betel quid is a significant world health problem and has attracted the interest of the World Health Organization (International Agency for Research on Cancer, 2007b). Health effects assessments indicate a high risk of oral cancer associated with these products.
9.1 RISK ASSESSMENT OBJECTIVES FOR THE DIFFERENTIATING TOBACCO RISKS PROJECT

The risk assessment objectives for the Differentiating Tobacco Risks (DTR) project were to review scientific evidence about smokeless tobacco (ST) and conventional cigarettes to:

- Develop an independent consensus opinion about whether ST meets the criteria for a reduced-risk or reduced-harm tobacco product with a focus on comparisons with cigarettes;
- Identify and characterize the critical characteristics that contribute to the evaluation of risk; and
- Develop an independent consensus opinion about whether sufficient evidence exists to stratify categories of ST products according to risk.

The Life Sciences Research Office (LSRO) approached the DTR project by adapting the evaluative framework described in *Scientific Methods to Evaluate Potential Reduced-Risk Tobacco Products* (2007b) to comparative risk assessments for (1) conventional cigarettes and ST and (2) categories of ST. LSRO’s evaluative process included conducting a systematic review of studies to compare individual and population risks resulting from cigarette smoking and ST use. The types of studies that provide information about tobacco product-related risk to the individual are those evaluating product characteristics, ST product and cigarette smoke chemistry studies, genotoxicity and cytotoxicity assays, animal studies, and clinical studies of exposure and biological effects. Information about population risk is gained from clinical, epidemiological, and behavioral studies.

LSRO’s evaluative framework utilized a weight of evidence approach. Each study was classified on the basis of its experimental design and quality, and the number, size, consistency, and relevance of the studies were determined. If, after assessing all available data, LSRO concluded that risk reduction was likely, LSRO estimated the magnitude of the probable risk reduction. LSRO also determined a level of confidence that risk would decrease for individuals using ST instead of cigarettes. This chapter describes LSRO’s
risk characterization for ST compared with that for cigarettes. Chapter 10 describes LSRO’s comparative risk assessment for different categories of ST products.

9.2 DISEASE RISKS FOR CIGARETTES AND SMOKELESS TOBACCO

Because lung cancer (LC), chronic obstructive pulmonary disease (COPD), and cardiovascular disease (CVD) account for approximately more than 80% of smoking-attributable causes of death (Centers for Disease Control and Prevention, 2005b), LSRO focused its literature review on scientific evidence on the risk of these diseases for individuals who switch from cigarette smoking to using ST. LSRO also considered the potential for ST use to reduce the risk of oral cancer and other adverse health outcomes linked to both ST use and cigarette smoking. In addition, LSRO compared all-cause mortality rates for cigarette smokers and ST users.

9.3 INDIVIDUAL AND POPULATION RISK ASSESSMENT

9.3.1 Risk Reduction

To determine whether ST products reduce risk to individuals compared with conventional cigarettes, LSRO addressed the following specific questions:

- Do preclinical studies (including product characterization, ST and cigarette smoke chemistry studies, in vitro tests, and animal studies) suggest reduced exposure and toxicity for ST compared with cigarettes?
- Do clinical studies show reduced levels of biomarkers of exposure for tobacco-specific nitrosamines (TSNAs), nicotine, polycyclic aromatic hydrocarbons (PAHs), and other biomarkers of tobacco smoke exposure in ST users compared with cigarette smokers?
- Do clinical studies show evidence of fewer cigarette smoking-related adverse biological effects associated with LC, CVD, and COPD for ST users compared with cigarette smokers?
- Do clinical studies report a decrease in adverse biomarkers of effect related to other cancers and diseases associated with ST and cigarette smoking for smokers who switch to ST?
- What is the overall weight of evidence that risks of LC, COPD, and CVD are lower for ST users than for continuing cigarette smokers?
What is the overall weight of evidence that risks of other cancers and diseases associated with cigarette smoking are reduced for ST users?

What are the magnitudes of the risk reductions?

What is the degree of confidence in the answers to the above questions?

### 9.3.2 Harm Reduction

LSRO sought answers to the following questions to determine whether ST products reduce *harm to individuals* compared with conventional cigarettes:

- Do cigarette smokers who switch to ST have lower all-cause mortality and morbidity than continuing smokers?
- Do concurrent users of cigarettes and ST have lower all-cause mortality and morbidity than individuals who use cigarettes exclusively?
- What are the magnitudes of the harm reductions?
- What is the degree of confidence in the answers to the above questions?

### 9.3.3 Population Risk Assessment

To address whether ST use reduces *population risks*, LSRO considered the following questions:

- Are non-users of tobacco likely to initiate tobacco use because of the increased visibility of ST as reduced-risk products?
- Does ST use serve as a gateway to smoking?
- Is ST use less, more, or equally addictive compared with cigarette smoking?
- Are cigarette smokers who switch to ST to help them to stop smoking more, less, or equally likely to quit smoking than are cigarette smokers who do not use ST as a smoking cessation aid?
- What is the degree of confidence in the answers to the above questions?

### 9.4 SMOKELESS TOBACCO CATEGORIES

Traditionally, ST products have been classified into three categories on the basis of mode of use, manufacturing, and processing: moist snuff (including Swedish *snus*), loose-leaf chewing tobacco, and dry snuff. A new category of ST product is hard snuff, which is a dissolvable, compressed, dried tobacco lozenge intended for oral use. LSRO chose to emphasize the categories of
moist snuff, loose-leaf chewing tobacco, plug/twist chewing tobacco, dry
snuff, and Swedish snus. Because of a lack of data on hard snuff compared
with other US and Swedish ST products LSRO did not discuss this product
in extensive detail. This report also addresses international ST products,
which herein refer to ST products other than those used in the US or Sweden.

9.5 SCIENTIFIC UNCERTAINTY

For any risk assessment, conclusions about risk are made by considering
the uncertainties related to scientific information and using scientific judgment.
Many uncertainties made risk comparisons related to use of cigarettes and
many different types of ST products challenging. Below is a description of
the uncertainties that LSRO encountered in determining the relative risk of
cigarettes and ST products.

9.5.1 Risks to Different Populations

Most epidemiological evidence about decreased risk of morbidity and
mortality for individuals who switch from cigarette smoking to using ST comes
from studies of the Swedish population. Questions exist, however, about
whether decreased morbidity and mortality from cigarette smoking-related
disease, observed largely for Swedish men who switched from cigarette
smoking to using snus, would translate into decreased illness and death for
smokers in the US who used Swedish snus and cigarettes. One reason for
this is that some individuals continue to smoke cigarettes after they begin to
use ST. Concurrent use of cigarettes and ST can reduce such a user's
exposure to smoke constituents if fewer cigarettes per day are smoked and
are not smoked more intensely. However, the extent of substitution of ST for
cigarettes necessary to reduce risk of cigarette smoking-related disease is
uncertain. A much higher percentage of US tobacco users are dual users of
cigarettes and ST when compared with Swedish tobacco users (Centers for
Disease Control and Prevention, 1993; Ramström & Foulds, 2006; Tomar et
al., 2003). This finding introduces additional uncertainty about the potential
for scientific evidence-based evaluations of the magnitude of any health risk
reduction.

9.5.2 Different Smokeless Tobacco Manufacturing Conditions

Swedish snus, other Swedish STs, and US STs are manufactured via
standardized methods. In contrast, manufacturing of many STs in other parts
of the world is often not standardized. Farmers or small companies make many
international STs without regulation of the fermentation and curing of tobacco,
which reportedly results in increased TSNA levels (Brunnemann et al., 1985). The lack of standardized manufacturing processes increases the potential for variability in the composition of ST products, so that commenting on composition and, ultimately, drawing conclusions about health risks are difficult.

9.5.3 Information Availability for Smokeless Tobacco

Data quality and quantity are critical for the assessment of risk. The Swedish National Health Service has extensive population data and excellent recording services. Swedish snus has been used for more than 100 years. Although the Swedish population is significantly less diverse than the US population, the Swedish experience serves as a model for studying the health effects of Swedish snus. Although an expansive database exists on the health effects of Swedish snus, considerably less information has been published about other STs. Information gaps make it difficult to compare risks for different categories of STs.

9.5.4 Residual Risk for Former Smokers Who Switch to Smokeless Tobacco

Smoking cessation favorably modifies the risk of diseases such as LC (International Agency for Research on Cancer, 2004c). The risk declines further with each year of abstinence. However, many respiratory tract symptoms associated with COPD do not improve. Once such damage has occurred, it is irreparable. Although it is generally accepted that CVD risk falls over time after smoking cessation, residual risk may remain.

9.5.5 Switching Between Products

Another source of uncertainty in comparing risks of tobacco products is that ST users may switch between product types. This behavior makes it difficult to determine the relative contribution of each specific ST product to the reduction of risk of diseases related to tobacco smoking.
9.6 RISK CHARACTERIZATION: CIGARETTES VERSUS SMOKELESS TOBACCO

LSRO reviewed preclinical, clinical, and epidemiological studies related to individual risk reduction and developed a comparative risk characterization of ST versus cigarettes. Tables 9-1 through 9-4 describe the risk characterization for LC, CVD, COPD, and oral cancer. All-cause mortality is discussed separately. LSRO's risk characterization of these causes of death and other cancers (i.e., laryngeal, gastric, esophageal, pancreatic, and bladder), which are discussed in detail in Chapter 7, is summarized in Table 9-5.

LSRO used the descriptors low, moderate, and high to summarize its levels of confidence in the potential for risk reduction related to ST compared with cigarettes. These definitions of levels of confidence in risk reduction were adapted from the Manual for ACC/AHA Guideline Writing Committees (American Heart Association, 2008).

**Low confidence in risk reduction:** Evidence is insufficient to assess risk reduction and/or there is general agreement of the DTR Committee that the weight of evidence indicates that ST does not reduce the risk of disease.

**Moderate confidence in risk reduction:** The weight of evidence supports risk reduction, but critical evidence is lacking and/or there is general agreement of the DTR Committee that available data are inconsistent about whether ST reduces the risk of disease compared with cigarettes.

**High confidence in risk reduction:** Available evidence is sufficient to assess risk reduction and there is general agreement of the DTR Committee that the weight of evidence indicates that ST reduces disease risk compared with cigarettes.
Table 9-1. Risk Characterization Summary for Lung Cancer

<table>
<thead>
<tr>
<th>Exposure Assessment</th>
<th>Biological Effects Assessment</th>
<th>Epidemiological Studies</th>
<th>Risk Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST does not generate combustion products when used as intended.</td>
<td>An extract of betel quid with tobacco produced lung tumors (Shirname et al., 1983).</td>
<td>Several studies showed that smoking caused LC (Doll et al., 2004; Doll &amp; Hill, 1954; Luo et al., 2007; Parkin et al., 2005; Thun et al., 1995).</td>
<td>Confidence that ST use presents a lower risk of LC than cigarette smoking is high.</td>
</tr>
<tr>
<td>Swedish snus extracts were not mutagenic in Salmonella assays or V79 cells, whereas cigarette smoke was cytotoxic and mutagenic (Jansson et al., 1991).</td>
<td></td>
<td>Several studies reported that ST users did not have an increased risk of LC (Accortt et al., 2002, 2005; Boffetta et al., 2005; Dikshit &amp; Kanhere, 2000; Luo et al., 2007).</td>
<td></td>
</tr>
<tr>
<td>Chewing tobacco caused base-pair substitutions and frameshifts in Salmonella strains (Guttenplan, 1987; Stamm et al., 1994; Whong et al., 1985).</td>
<td></td>
<td>HR for female never smokers/ST users was significantly elevated (HR = 9.1; 95% CI: 1.1–95.4) (Accortt et al., 2002). (Authors noted that this could be due to the small number of subjects having large sample weights.) HR (0.0) for male never smokers/ST users was not significantly elevated (Accortt et al., 2002).</td>
<td></td>
</tr>
<tr>
<td>US dry and moist snuff caused base-pair substitutions in Salmonella assays (Rickert et al., 2007).</td>
<td></td>
<td>Female ST users had an increased risk of LC but this result was based on very few deaths (Accortt et al., 2005).</td>
<td></td>
</tr>
<tr>
<td>Biomarker of exposure studies showed that moist ST (non-Swedish snus) users had a significantly higher median level of total NNAL/ml urine than smokers did (Hecht et al., 2007).</td>
<td></td>
<td>CPS I reported no increased risk of LC for dual users; CPS II showed an increased risk of LC for ST users (Henley et al., 2005).</td>
<td></td>
</tr>
</tbody>
</table>
Differentiating the Health Risks of Categories of Tobacco Products

Table 9-1. Risk Characterization Summary for Lung Cancer (continued)

<table>
<thead>
<tr>
<th>Exposure Assessment</th>
<th>Biological Effects Assessment</th>
<th>Epidemiological Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST users who switched from other moist ST products to</td>
<td></td>
<td>Dual users had an increased risk of LC (Accortt et al., 2002). However, non-user controls</td>
</tr>
<tr>
<td>Swedish snus had lower TSNA metabolite levels (Hatsukami</td>
<td></td>
<td>included pipe and cigar smokers, ST users included people who used ST only once, and dual</td>
</tr>
<tr>
<td>et al., 2004).</td>
<td></td>
<td>users may have included more than exclusive cigarette smokers.</td>
</tr>
<tr>
<td>Levels of the hemoglobin-4-hydroxy-1-(3-pyridyl)-1-</td>
<td></td>
<td>A meta-analysis showed that ST users did not have an increased risk of LC (Thornton &amp;</td>
</tr>
<tr>
<td>butanone (HPB) adduct were significantly higher in ST</td>
<td></td>
<td>Lee, 2007).</td>
</tr>
<tr>
<td>users than in cigarette smokers and non-users of tobacco</td>
<td></td>
<td>Betel quid users had no increased risk of LC (Dikshit &amp; Kanhere, 2000).</td>
</tr>
<tr>
<td>(Schäffler et al., 1993).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST users had significantly lower thiocyanate levels than</td>
<td></td>
<td></td>
</tr>
<tr>
<td>did cigarette smokers (Shah et al., 1985). ST users’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>levels were not significantly different from non-users’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>levels (Holiday et al., 1995).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST users’ urine mutagenicity was significantly lower than</td>
<td></td>
<td></td>
</tr>
<tr>
<td>that of cigarette smokers but was not significantly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>different from that of non-users of tobacco (Benowitz</td>
<td></td>
<td></td>
</tr>
<tr>
<td>et al., 1989; Curvall et al., 1987).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 9-1. Risk Characterization Summary for Lung Cancer (continued)

<table>
<thead>
<tr>
<th>Exposure Assessment</th>
<th>Biological Effects Assessment</th>
<th>Epidemiological Studies</th>
<th>Risk Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conclusions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exclusive users of ST products were not exposed to the toxic combustion products in cigarette smoke. Biomarkers of exposure for hydrogen cyanide were reduced but levels of TSNA metabolites were higher for ST users than for smokers. ST users are likely to have lower exposure to carcinogens produced in cigarette smoke compared with smokers.</td>
<td><strong>Conclusions</strong></td>
<td>Studies linking ST use and LC in animals are limited. However, one study of an international product showed the product causing LC. There is weak evidence that ST causes LC in animals.</td>
<td>Most studies reported ST users not having an increased risk of LC, whereas cigarette smoking consistently and significantly increased LC risk. ST use poses a significantly lower risk of LC compared with cigarette smoking.</td>
</tr>
</tbody>
</table>

*Adjust hazard ratio derived from 0/6 where the numerator represents the number of cases among exclusive smokeless tobacco users, and the denominator represents the number of cases among non-tobacco users* Accortt et al., 2002.

**CPS**: Cancer Prevention Study; **HR**: hazard ratio; **LC**: lung cancer; **NNAL**: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; **ST**: smokeless tobacco; **TSNA**: tobacco-specific nitrosamine.
### Table 9-2. Risk Characterization Summary for Chronic Obstructive Pulmonary Disease

<table>
<thead>
<tr>
<th>Exposure Assessment</th>
<th>Biological Effects Assessment</th>
<th>Epidemiological Studies</th>
<th>Risk Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>No combustion products are produced when ST products are used as intended, whereas smokers and others in their environment are exposed to cigarette smoke.</td>
<td>Cigarette smoke can cause COPD in animals (Spurzem &amp; Rennard, 2005).</td>
<td>Numerous studies showed that cigarette smoking caused COPD (Thun et al., 1995). No studies were conducted to investigate whether Swedish snus increases the risk of COPD. CPS showed significantly increased risk of COPD for US ST users; CPS II showed no increased risk (Henley et al., 2005). The CPS I dataset had significant problems, including non-collection of tobacco use information at follow-up, potential inclusion of smokers, and lack of comparisons between smokers and ST users in the same study. These studies compared ST users with non-users, and the risk ratios, although statistically significant, were smaller than those observed when smokers were compared with non-users. Accortt et al. (2002) reported no increase in respiratory disease mortality for ST users compared with non-users.</td>
<td>Confidence that ST use presents no significant risk of COPD is high.</td>
</tr>
</tbody>
</table>
Table 9-2. Risk Characterization Summary for Chronic Obstructive Pulmonary Disease (*continued*)

<table>
<thead>
<tr>
<th>Exposure Assessment</th>
<th>Biological Effects Assessment</th>
<th>Epidemiological Studies</th>
<th>Risk Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conclusions</td>
<td>Conclusions</td>
<td>Conclusions</td>
<td></td>
</tr>
<tr>
<td>Exclusive users of ST products were not exposed to the toxic combustion products in cigarette smoke.</td>
<td>Animal studies have shown that cigarette smoke exposure can cause COPD.</td>
<td>Limited information is available linking ST and COPD, and no biologically plausible relationship exists between ST use and COPD. ST use is likely to pose a substantially lower risk of COPD compared with cigarettes.</td>
<td></td>
</tr>
</tbody>
</table>

*COPD*: chronic obstructive pulmonary disease; *CPS*: Cancer Prevention Study; *ST*: smokeless tobacco.
Table 9-3. Risk Characterization Summary for Cardiovascular Disease

<table>
<thead>
<tr>
<th>Exposure Assessment</th>
<th>Biological Effects Assessment</th>
<th>Epidemiological Studies</th>
<th>Risk Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomarker of effect studies</td>
<td>Smoking and ST use resulted in similar maximal increases in heart rate (Benowitz et al., 1988). Integrated heart rate and systolic blood pressure responses to ST use were significantly higher than responses to cigarette smoking (Benowitz et al., 1988). Daytime ambulatory heart rates were significantly elevated in both ST users and smokers compared with never users (Bolinder &amp; de Faire, 1998). No significant differences were found between heart rates of ST users and smokers. Swedish moist snuff users had significantly less severe atherosclerotic changes than did smokers (Bolinder et al., 1997; Wallenfeldt et al., 2001).</td>
<td>Smoking significantly increased MI risk (Bolinder et al., 1994; Hergens et al., 2005; Huhtasaari et al., 1992, 1999; Johansson et al., 2005; Teo et al., 2006; Thun et al., 1995). Swedish snus users had slightly higher CVD risk than did non-tobacco users (Bolinder et al., 1994; Hergens et al., 2007). No association was found between Swedish snus and CVD, but smoking increased CVD risk (Asplund et al., 2003; Hergens et al., 2005; Huhtasaari et al., 1992, 1999; Wennberg et al., 2007). One study reported an increased risk of death from CVD for US snuff product users (Henley et al., 2005), but another report found no increased risk (Accortt et al., 2002).</td>
<td>Confidence that ST use presents a lower risk of CVD than smoking is moderate.</td>
</tr>
</tbody>
</table>
No significant differences were observed in carotid bulb IMT (Bolinder et al., 1997; Wallenfeldt et al., 2001) and common carotid IMT for non-tobacco and moist snuff users (Bolinder et al., 1997), as well as in femoral artery thickness (Wallenfeldt et al., 2001).

Plasma fibrinogen levels were significantly lower in snuff users than in smokers and were equal to levels in non-tobacco users (Bolinder et al., 1997; Eliasson et al., 1991, 1995).

Users of Swedish moist snuff and non-tobacco users had significantly lower CRP levels than smokers (Wallenfeldt et al., 2001).

ST users and non-tobacco users had similar total serum cholesterol, HDL-C, LDL-C, and apolipoprotein levels (Bolinder et al., 1997; Eliasson et al., 1991; Wallenfeldt et al., 2001).

Snuff users had elevated triglyceride levels compared to never snuff users (Wallenfeldt et al., 2001).

Chewing tobacco used in 52 countries increased users' risk of non-fatal MI (Teo et al., 2006).
Table 9-3. Risk Characterization Summary for Cardiovascular Disease (continued)

<table>
<thead>
<tr>
<th>Exposure Assessment</th>
<th>Biological Effects Assessment</th>
<th>Epidemiological Studies</th>
<th>Risk Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conclusions ST does not lower smokers' exposures to nicotine and other alkaloids.</td>
<td>Conclusions ST use and smoking have similar effects on some aspects of hemodynamics, and ST use more favorably affects certain biomarkers of effect. ST users have reduced levels of inflammatory biomarkers (CRP and fibrinogen) and lipids (HDL-C, LDL-C) and less atherosclerotic thickening.</td>
<td>Conclusions Although ST use appears to increase CVD risk above that of non-tobacco users, it appears to have reduced risk compared with smoking.</td>
<td></td>
</tr>
</tbody>
</table>

CVD: cardiovascular disease; CRP: C-reactive protein; HDL-C: high-density lipoprotein cholesterol; IMT: intima-media thickness; LDL-C: Low-density lipoprotein cholesterol; MI: myocardial infarction; ST: smokeless tobacco.
Table 9-4. Risk Characterization Summary for Oral Cavity Cancer

<table>
<thead>
<tr>
<th>Exposure Assessment</th>
<th>Biological Effects Assessment</th>
<th>Epidemiological Studies</th>
<th>Risk Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>No combustion products were produced when ST was used as intended.</td>
<td>Swedish snus extracts were significantly less mutagenic and cytotoxic than cigarette smoke (Jansson et al., 1991). Two animal studies reported that moist snuff did not cause oral tumors (Barley et al., 2004; Chen, 1989). ST users and non-users of tobacco had significantly lower peripheral lymphocyte sister chromatid exchange frequency in oral mucosa tissue than did cigarette smokers (Livingston et al., 1990).</td>
<td>Cigarette smoking significantly increased risk of oral cancer (Accortt et al., 2002; Boffetta et al., 2005; Franceschi et al., 1990; Luo et al., 2007; Mashberg et al., 1993; Rosenquist et al., 2005; Schildt et al., 1998; Sterling et al., 1992). Swedish snus did not increase risk of oral cancer (Luo et al., 2007; Schildt et al., 1998) for heavy users (&gt;50 g/week) (Boffetta et al., 2005; Lewin et al., 1998; Rosenquist et al., 2005). Some studies reported that US ST products increased oral cancer risk (Spitz et al., 1988; Stockwell &amp; Lyman, 1986; Winn et al., 1981), but other studies reported no increase in risk (Accortt et al., 2005; Mashberg et al., 1993; Sterling et al., 1992). Some international products significantly increased oral cancer risk (Balaram et al., 2002; Elbeshir et al., 1989; Jayant et al., 1977; Rao et al., 1994; Sankaranarayanan et al., 1989; Wasnik et al., 1998).</td>
<td>LSRO’s confidence that use of US and Swedish STs presents a lower risk of oral cavity cancer than cigarette smoking is moderate. LSRO’s confidence that use of some international STs presents a higher risk of oral cavity cancer than cigarette smoking is moderate.</td>
</tr>
</tbody>
</table>

Conclusions
ST reduces exposure to carcinogens that are products of combustion.

Conclusions
ST users have fewer adverse biological effects than cigarette smokers.

Conclusions
Risk varies with different ST products.

ST: smokeless tobacco.
A brief discussion of the studies that contributed to the risk characterization of ST versus conventional cigarettes is presented below.

9.6.1 Preclinical Studies

9.6.1.1 Product characteristics

Product characteristics refer to the composition, manufacturing, processing, and method of use of tobacco products. Inhalation of mainstream cigarette smoke exposes smokers to thousands of smoke constituents (Green & Rodgman, 1996) and, compared with never smokers, increases smokers’ risk of COPD, CVD, LC, and many other diseases. Oral and gastroesophageal tract exposures result when saliva is swallowed during ST use, but not during nasal use of dry snuff. Because no pulmonary exposure to cigarette smoke occurs during use of oral ST products, ST is highly likely to present a very significantly lower risk of LC and COPD to users compared with cigarettes (U.S. Department of Health and Human Services, 2004). However, ST contains nicotine and other substances linked to cardiovascular effects. As a result, questions remain about the degree to which ST use increases the risk of CVD compared with never users of tobacco. The potential for ST to increase risk of other cancers also exists.

9.6.1.2 Chemistry

Comparing substances in cigarette smoke and ST extracts can provide qualitative and quantitative information about exposures from using these products. Cigarettes and STs contain, in addition to tobacco, additives such as flavorings, conditioners, and other substances, some of which can increase nicotine uptake. Cigarette smoke has more than 4,800 constituents (Green & Rodgman, 1996; Rodgman & Green, 2002) including more than 60 carcinogens (Hoffmann & Hecht, 1990; International Agency for Research on Cancer, 2004c). The tobacco leaf contains more than 3,000 substances (Roberts, 1988) and more than 28 carcinogens (Brunnemann & Hoffmann, 1992). Growing and manufacturing processes lead to tobacco leaves’ containing many other harmful substances such as carcinogenic aldehydes, metals, and PAHs (Hecht, 2008).

The Swedish Match Company voluntarily developed the GothiaTek® standard for maximal permissible limits of some substances in their ST products (Swedish Match, 2007). Certain companies appear to be voluntarily following this GothiaTek® standard. Overall, the chemical characterization of ST products appears to be improving because the list of substances measured by ST companies is becoming more inclusive. In general, there is no clear rationale for inclusion or exclusion of ST analytes.
No specific link between ST product constituents or smoke constituents with disease has been made. How differences in specific contents of ST extracts and cigarette smoke affect disease risk remains to be fully characterized. Because ST is not combusted when used as intended and does not produce mainstream smoke or environmental tobacco smoke, it eliminates exposure to the combustion products in cigarette smoke. ST undoubtedly confers lower risk of lung disease than cigarettes and does not expose bystanders to environmental tobacco smoke.

### 9.6.1.3 Genotoxicity and cytotoxicity assays

Many studies have shown that cigarette smoke is mutagenic (DeMarini, 2004). LSRO reviewed results of genotoxicity and cytotoxicity assays to compare the toxicity of cigarettes and STs. In summary, results of mutagenesis assays are highly variable and difficult to interpret. As discussed in Chapter 4 of this report, numerous issues related to experimental design and conduct of in vitro assays of ST products, such as the failure to modify International Organization for Standardization methods for preparation of test materials (International Organization for Standardization, 2007) and the absence of appropriate control ST products, rendered arriving at conclusions about risk challenging.

### 9.6.1.4 Animal studies

Most animal studies that have assessed the carcinogenicity of US STs have produced negative results (Rodu & Jansson, 2004). Commercial moist snuff did not increase gastric cancer risk (Barley et al., 2004) or induce tumor formation in Syrian gold hamsters or rats (Chen, 1989). Swedish snus, however, increased risk of gastric cancer in transgenic mice that were infected with *Helicobacter pylori* (Stenström et al., 2007). Some international products such as betel quid extracts (Shirname et al., 1983; Suri et al., 1971) and Indian chewing tobacco (Shah et al., 1985) induced experimental tumor formation.

LSRO concludes that animal studies provide limited evidence of carcinogenicity for ST products, in particular US and Swedish ST products. In contrast, total particulate matter from cigarette smoke promoted dermal tumor development (Meckley et al., 2004), and inhaled sidestream smoke and mainstream smoke both caused respiratory tract lesions in different rodent models (Hutt et al., 2005; Mauderly, 2006; Witschi et al., 2004; Witschi et al., 1997).
9.6.2 Clinical Studies

9.6.2.1 Biomarkers of exposure

Few studies of biomarkers of exposure in ST users have been published. TSNAs are some more commonly measured biomarkers of exposure to tobacco. Hecht et al. (2007) reported that ST users had significantly higher urinary levels of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), which is a metabolite of the TSNA 4-(methylNitrosamino)-1-(3-pyridyl)-1-butanone (NNK), than did smokers. Levels of the adduct of hemoglobin and 4-hydroxy-1-(3-pyridyl)-1-butanol (HPB), a TSNA, were significantly higher in ST users than in cigarette smokers and non-users of tobacco (Schäffler et al., 1993).

However, use of different STs results in different degrees of exposure to tobacco product constituents. In one study, users of Swedish snus had lower TSNA metabolite levels compared with users of other moist STs (Hatsukami et al., 2004b). Use of some classes of ST resulted TSNA levels that were not significantly different from levels measured after use of a medicinal nicotine product. Individuals who were trying to quit smoking who used a hard snuff tobacco lozenge had urinary TSNA levels that were not significantly different from levels when they used a medicinal nicotine lozenge (Mendoza-Baumgart et al., 2007). These data on human exposure to TSNAs indicate that use of some STs reduced exposure to TSNAs compared with cigarette smoking, but use of other STs elevated TSNA levels above those related to cigarette smoking. Results were different for another biomarker of exposure, serum thiocyanate, a measure of hydrogen cyanide exposure. Compared with cigarette smokers, ST users had lower levels of serum thiocyanate (Holiday et al., 1995; Shah et al., 1985).

9.6.2.2 Biomarkers of effect

Benowitz et al. (1988) reported that systolic blood pressure and integrated heart rate “tended to be greater” for users of ST (US moist snuff and chewing tobacco) than for cigarette smokers and users of nicotine gum. The same study reported that cigarette smoking and ST use resulted in similar maximal increases in heart rate (Benowitz et al., 1988). A study that conducted 24-hour blood pressure monitoring showed that both ST and cigarettes significantly elevated daytime ambulatory heart rate (69 ± 14 beats/minute and 74 ± 13 beats/minute, respectively) compared with never users of tobacco (63 ± 12 beats/minute) (Bolinder & de Faire, 1998).

In two studies, users of Swedish snus had less severe atherosclerotic changes than did smokers (Bolinder et al., 1997; Wallenfeldt et al., 2001). The carotid bulb intima-media thickness (0.80 ± 0.13 mm) and common
carotid artery IMT (0.67 ± 0.11 mm) of Swedish snus users were not significantly different from those of never users of tobacco (0.78 ± 0.12 mm and 0.68 ± 0.11 mm, respectively) (Bolinder et al., 1997). Compared with never users of tobacco, smokers had a significantly higher carotid bulb IMT (0.87 ± 0.19 mm) and common carotid artery IMT (0.74 ± 0.13 mm, \( p=0.03 \)) (Bolinder et al., 1997). Another study reported similar results: no significant difference between Swedish snuff users and never users with regard to carotid bulb IMT (1.04 ± 0.26 mm versus 0.99 ± 0.26 mm) and common carotid artery IMT (0.82 ± 0.12 mm versus 0.80 ± 0.13) or femoral artery thickness (1.12 ± 0.43 versus 1.05 ± 0.49 mm) (Wallenfeldt et al., 2001). In contrast, compared with values for never smokers, smokers’ carotid bulb IMT (1.05 ± 0.35 mm versus 0.95 ± 0.22 mm, \( p<0.05 \)) and femoral artery thickness (1.31 ± 0.62 versus 0.87 ± 0.28 mm, \( p<0.001 \)) were significantly higher.

Levels of markers of inflammation—plasma fibrinogen (Bolinder et al., 1997; Eliasson et al., 1991, 1995) and C-reactive protein (Wallenfeldt et al., 2001)—were higher in smokers than in never users of tobacco, whereas levels of these markers in Swedish snus users were not higher than levels in never users of tobacco. Swedish snus users did not demonstrate altered lipid metabolism \( i.e., \) total serum cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, or apolipoprotein A-I levels\] compared with never users (Bolinder et al., 1997; Eliasson et al., 1991; Hirsch et al., 1992; Wallenfeldt et al., 2001). However, one study reported an association of Swedish snus with elevated triglyceride levels (Wallenfeldt et al., 2001).

In the weight of evidence approach, changes in lipids, biomarkers of inflammation, and measures of atherosclerosis are weighted more heavily than are changes in systolic blood pressure and heart rate. Therefore, on the basis of a selected panel of biomarkers of effect for CVD, ST users appear to have a lower degree of CVD risk compared with cigarette smokers.

### 9.6.3 Health Effects Studies

#### 9.6.3.1 Lung cancer

An overwhelming amount of evidence has firmly established the high incidence of LC in smokers (US Department of Health and Human Services, 2004). The excess risk of LC for smokers compared with the risk for never users of tobacco has been quantitated \( [RR=7.2; \text{95% CI: } 6.0–8.5\) (Luo et al., 2007); HR=13.2; 95% CI: 4.5–38.2 (males) (Accortt et al., 2002); HR=13.2; 95% CI: 5.5–31.8 (males) (Accortt et al., 2005a)]. In the Cancer Prevention
Study II (CPS II), the death rate ratio from LC for men who were smokers was significantly higher than that for men who were never smokers (23.2; 95% CI: 19.3–27.9) (Thun et al., 1997). Fewer studies have explored the relationship between ST and the risk of LC. However, several indicated no increased risk of LC for ST users compared with non-users of tobacco [RR=0.8; 95% CI: 0.4–1.3 (Luo et al., 2007); HR=0.0 (Accortt et al., 2005a); RR=0.8; 95% CI: 0.58–1.11 (Boffetta et al., 2005)]. Two studies with significant methodological problems reported an increased LC risk for users of chewing tobacco or spit tobacco compared with non-users of ST products [HR=2.00; 95% CI: 1.23–3.24 in CPS II (Henley et al., 2005); HR=0.0 for male exclusive tobacco users (Accortt et al., 2002)].

In a study conducted by Accortt et al. (2002), women older than 65 years who were exclusive ST users and never smoked had an increased risk of LC (HR=9.1; 95% CI: 1.1–75.4). These authors later remarked that this result may have been due to confounding from exposure to environmental tobacco smoke, misclassification of smokers as ST users, and/or effects of a specific brand or type of ST (i.e., dry snuff used orally) (Accortt et al., 2005a). In addition, this finding was based on very few deaths.

The TSNA NNK is a potent lung tumorigen in rodents (Belinsky et al., 1990; Prokopczyk et al., 1991; Rivenson et al., 1988) and a putative causal factor of lung adenocarcinoma in humans (Hecht, 1998a; Hecht, 1999). Although clinical studies assessing exposure showed that US moist snuff users had higher urinary TSNA levels than did smokers (Hecht et al., 2007), epidemiological studies, which are weighted more heavily than are biomarker studies, showed that ST users had a significantly lower risk of LC than did cigarette smokers. Swedish snus users did not have an elevated risk of LC (Boffetta et al., 2005; Luo et al., 2007).

The Scientific Committee on Emerging and Newly Identified Health Risks reported that there is no evidence that any category of ST causes LC (Scientific Committee on Emerging and Newly Identified Health Risks, 2007). LSRO’s confidence in significant reduction of risk of LC by replacement of cigarettes with exclusive ST use is high.

**9.6.3.2 Chronic obstructive pulmonary disease**

Epidemiological studies have shown that cigarette smoking increases the risk of COPD (Thun et al., 1995). Exposure to cigarette smoke results in COPD in animals (Spurzem & Rennard, 2005). Few studies have examined the relationship between ST use and COPD. However, Accortt et al. (2002) found no excess risk of respiratory disease for ST users in their study population.
Risk of COPD appears to be less for ST users than for smokers. Because there is no plausible biological mechanism by which ST could increase risk of COPD (Foulds et al., 2003) and because CPS I and II datasets that showed increased risk of COPD had serious confounding difficulties (Henley et al., 2005), LSRO concludes that ST poses no risk of COPD beyond any risk that can be attributed to prior cigarette smoking. LSRO’s level of confidence in reduction of risk of COPD for ST compared with cigarettes is high.

9.6.3.3 Cardiovascular disease

Cigarette smoking significantly increases the risk of CVD (Bolinder et al., 1994; Hergens et al., 2005; Huhtasaari et al., 1992, 1999; Johansson et al., 2005; Teo et al., 2006; Thun et al., 1995). Cigarette smokers had a 2- to 4-fold higher risk of developing coronary heart disease compared with non-users of tobacco.

Six studies reported that CVD risk was significantly lower for Swedish snus users than for cigarette smokers (Asplund et al., 2003; Bolinder et al., 1994; Hergens et al., 2005; Huhtasaari et al., 1992, 1999; Wennberg et al., 2007). However, two studies found that Swedish snus increased the risk of myocardial infarction above that for non-users of tobacco (Bolinder et al., 1994; Hergens et al., 2007). In the study by Bolinder et al. (1994), the risk of CVD for snus users (RR=1.4; 95% CI: 1.2–1.6) was significantly lower than that for smokers (RR=1.9; 95% CI: 1.7–2.2). This study did not control for alcohol consumption, nor did it correct for Swedish snus users who were ex-smokers. Hergens et al. (2007) observed an increased risk of myocardial infarction for heavy snus users (RR=1.96; 95% CI: 1.08–3.58) but not for ever snus users (RR=0.99; 95% CI: 0.90–1.10).

One study found that US snuff and chewing tobacco was not associated with an increased risk of CVD (Accortt et al., 2002) (HR=1.1; 95% CI: 0.8–1.5). However, a different study reported a link between the two (HR=1.18; 95% CI: 1.11–1.26) (Henley et al., 2005). As noted previously, significant methodological issues were associated with this second study. An international study noted a significantly higher risk of myocardial infarction for lifetime exclusive ST users (OR=2.23, 95% CI: 1.41–3.52) and smokers OR=2.95, 95% CI: 2.77–3.14) (Teo et al., 2006).

After a review of relevant studies, Fagerström and Schildt (2003) concluded that Swedish snus users did not have an increased risk of myocardial infarction compared with non-smokers. Although some studies reported an increased risk of CVD for ST users compared with never users, these values were lower than those for cigarette smoking. Roth et al. (2005) estimated
that ST use presented at least a 50% lower risk of CVD than cigarette smoking. A challenge in determining risk relates to the lack of direct comparisons between ST users and cigarette smokers. In addition, many study populations are small (Broadstock, 2007), and certain studies did not correct for a relatively large number of study populations consisting of combined ST and cigarette users.

Although data are limited, biomarker of effect studies comparing Swedish snus users with cigarette smokers have shown no significant increase in thickening of the common carotid, carotid bulb, and femoral arteries; lower levels of the markers of inflammation C-reactive protein and fibrinogen; and reduced levels of HDL, LDL, and apolipoprotein in ST users. These biomarkers are weighted more heavily than are transient hemodynamic changes that have shown, in some instances, a more significant effect for ST. In summary, current evidence suggests an elevated CVD risk for ST users compared with non-users of tobacco but a significantly lower CVD risk for ST users than for cigarette smokers. On the basis of available data, LSRO's confidence that ST use poses a lower risk of CVD than cigarette smoking is moderate.

9.6.3.4 Oral cancer

Research has demonstrated that cigarette smoking increases the risk of oral cancer (Blot et al., 1988; Franceschi et al., 1990; Luo et al., 2007; Mashberg et al., 1993; Rodriguez et al., 2004; Schildt et al., 1998). Although ST use has also been associated with increased risk of oral cancer, some investigators have estimated that ST use poses half the risk of cigarette smoking (Rodu & Cole, 2002; Roth et al., 2005).

Various STs present different risks of oral cancer. Swedish snus users do not appear to have an increased risk of oral cancer compared with non-tobacco users [OR= 0.7; 95% CI: 0.4–1.1 (Schildt et al., 1998); RR=0.8; 95% CI: 0.4–1.7 (Luo et al., 2007); OR=1.1; 95% CI: 0.5–2.5 (Rosenquist et al., 2005); RR=1.10; 95% CI: 0.50–2.41 (Boffetta et al., 2005)]. However, a systematic review of recent studies observed no statistically significant risk of oral cancer from US and Scandinavian STs but also stated that these studies lacked the statistical power to measure “moderate positive associations” (Critchley & Unal, 2003).

Some older US studies reported that STs increased the risk of oral cancer [RR=4.2; 95% CI: 2.6–6.7 (Winn et al., 1981); OR=3.4; 95% CI: 1.0–10.9 (Spitz et al., 1988)], but other studies reported no increased risk [HR=0.0, for exclusive ST users (no observed cases) (Accortt et al., 2005a); OR=0.8,
Comparative Risk Assessment of Smokeless Tobacco and Cigarettes

95% CI: 0.4–1.9 (snuff) and OR=1.0; 95% CI: 0.7–1.4 (chewing tobacco) (Mashberg et al., 1993); RR=1.2; 95% CI: 0.32–4.63 (Sterling et al., 1992)]. Therefore, older US ST products may have posed a higher risk of oral cancer than newer US ST products and Swedish snus. However, most studies that involved US STs did not control for smoking and alcohol use.

The potential for reduced risk of oral cancer for ST products compared with cigarettes does not apply to all STs. As discussed in Chapter 8, the relative risk of oral cancer for users of certain international ST products was higher than that for cigarette smokers. Users of betel quid with chewing tobacco had a very significantly elevated oral cancer risk compared with non-users of tobacco (Critchley & Unal, 2003). The relative risk of cancers of the lip, buccal cavity, and floor of the mouth for toombak users compared with non-users of tobacco was 7.3 (95% CI: 4.3–12.4), with the corresponding value for cigarette smokers being 1.0 (95% CI: 0.5–1.9) (Idris et al., 1995a).

In summary, LSRO considers oral cancer risk to be lower for users of US and Swedish STs than for users of international STs. LSRO’s confidence that US and Swedish ST products present a lower risk of oral cancer compared with cigarettes is moderate. LSRO’s confidence that some international STs (e.g., toombak) are associated with a higher risk of oral cancer than cigarette smoke is moderate (Idris et al., 1995a).

9.6.3.5 Other adverse health effects

LC, COPD, CVD, and oral cancer account for more than 80% of smoking-attributable mortality (Centers for Disease Control and Prevention, 2005b). Because all other adverse health effects account for a significantly lower proportion of this mortality, LSRO did not discuss them in detail in the conclusions (see Chapter 7 of this report for an extensive discussion of the studies reviewed). Table 9-5 summarizes LSRO’s confidence in risk reduction by switching to ST and risk characterization of some adverse health effects of smoking.
9.6.3.6 All-cause mortality

Cigarette smoking has been demonstrated to increase the risk of mortality from all causes (Centers for Disease Control and Prevention, 2005b). Bolinder et al. (1994) found that users of Swedish snus had an increased risk of all-cause mortality compared with non-users of tobacco (RR=1.4; 95% CI: 1.3–1.8). Study participants came from a group of construction workers who had a higher body mass index and resting blood pressure than non-tobacco users, and the study did not control for alcohol consumption or history of smoking. The relative risks for men who smoked fewer than versus more than 15 cigarettes/day were 1.7 (95% CI: 1.6–1.9) and 2.2 (95% CI: 2.0–2.4), respectively (Bolinder et al., 1994). Because the Swedish snus used by the men was different from present-day snus, the degree of risk may not be the same as that for users of current Swedish snus.

In the US CPS I study (1958–1965), the death rate ratios for men and women who were smokers were 1.7 (95% CI: 1.7–1.8) and 1.2 (95% CI: 1.2–1.3), respectively compared to non-smokers (Thun et al., 1995). Corresponding values for CPS II (1982–1988) were 2.3 (95% CI: 2.3–2.4) and 1.9 (95% CI: 1.9–2.0). In the US, men who used snuff or chewing tobacco had a statistically significant higher risk of all-cause mortality than did non-users, as reported in CPS I (HR=1.17; 95% CI: 1.11–1.23) and CPS II (HR=1.18; 95% CI: 1.08–

<table>
<thead>
<tr>
<th>Disease</th>
<th>LSRO’s Confidence in Risk Reduction</th>
<th>Estimated Percentage of Smoking-Attributable Mortality</th>
</tr>
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<tbody>
<tr>
<td>Lung cancer</td>
<td>High</td>
<td>28</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>High</td>
<td>21</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>Moderate</td>
<td>32</td>
</tr>
<tr>
<td>Lip, oral cavity, and pharyngeal cancer</td>
<td>Moderate</td>
<td>1</td>
</tr>
<tr>
<td>Laryngeal cancer</td>
<td>Moderate</td>
<td>1</td>
</tr>
<tr>
<td>Esophageal and gastric cancer</td>
<td>Moderate</td>
<td>2</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>Low</td>
<td>1</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>Low</td>
<td>1</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>High</td>
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</tbody>
</table>

aData from Centers for Disease Control and Prevention (2005b)
Comparative Risk Assessment of Smokeless Tobacco and Cigarettes

1.29) (Henley et al., 2005). However, as noted previously, this study had problems with confounders. Henley et al. (Henley et al., 2007) compared mortality rates of smokers who switched to ST with those of smokers who quit tobacco. Twenty years after quitting, former smokers had a higher rate of death from all causes (HR=1.08; 95% CI: 1.01–1.15 than did smokers who switched to ST. A different study by Accortt et al. (2002) reported that exclusive use of US ST did not increase risk of mortality from all causes relative to non-tobacco users (HR=1.1; 95% CI: 0.9–1.3).

Users of some international ST products had an increased risk of mortality compared with non-users of tobacco. Indian chewers of betal quid with tobacco showed a significant increase in all-cause mortality (RR=1.3; 95% CI: not reported) (Gupta et al., 1984), and Indian women who exclusively used chewing tobacco including mishri, betel quid, and other STs had an increased risk of all-cause mortality (RR=1.35; 95% CI: not reported) (Gupta & Mehta, 2000).

Although some studies reported that all-cause mortality was elevated relative to non-users, LSRO concludes that ST is a reduced-mortality product compared with cigarettes. LSRO’s confidence in risk reduction from replacement of cigarettes with Swedish snus or US STs is high. International products may confer a higher risk of mortality compared with US and Swedish products.

9.6.4 Behavioral Studies and Surveys

9.6.4.1 Abuse liability

Cigarettes and ST products both contain nicotine (U.S. Department of Health and Human Services, 1986a, 1988). Nicotine is the primary reason for continued use of tobacco products (U.S. Department of Health and Human Services, 1988), although other factors may contribute to maintenance of a tobacco habit (Rose, 2006). Nicotine absorption is slower during ST use than during cigarette smoking (Benowitz et al., 1988). A “dose” of ST results in a venous nicotine concentration of approximately 15 ng/ml after 30 minutes, and continued ST use produces steady-state levels of 30 ng/ml (Holm et al., 1992). In contrast, plasma nicotine concentrations peak at 25 ng/ml approximately 5 minutes after smoking and decline to 10 ng/ml after 30 minutes (Foulds et al., 2003). Cigarette smokers and ST users have similar overall maximal nicotine levels (Fant et al., 1999; Pitsiu et al., 2002).

Multiple definitions of nicotine addiction exist, but some hallmarks of this addiction are psychoactive effects of the drug, drug-reinforced behavior,
Differentiating the Health Risks of Categories of Tobacco Products

and highly controlled or compulsive use of the drug (U.S. Department of Health and Human Services, 1988). Similar to cigarette smokers, ST users experience withdrawal symptoms when they abstain from tobacco use (Hatsukami et al., 1987; Pitsiu et al., 2002). LSRO concluded that ST is not likely to decrease the risk of nicotine addiction compared with cigarettes.

9.6.4.2 Smokeless tobacco as a smoking cessation aid

An estimated 2 million individuals in the US have used ST as a smoking cessation aid (Centers for Disease Control and Prevention, 1993). A small pilot study (63 subjects) reported that 25% of study participants were able to quit smoking by using ST instead of cigarettes, and 6.7% reduced cigarette smoking by at least 50% by replacing some cigarettes with ST (Tilashalski et al., 1998). Tomar (2007) reported a lack of data on the efficacy of ST as an aid for smoking cessation.

9.7 CONCLUSIONS

Table 9-5 summarizes LSRO’s conclusions about the risk of ST compared with cigarettes. On the basis of available scientific evidence, LSRO concludes that Swedish snus and US STs pose lower individual risk for LC and COPD than cigarette smoking. No reliable data indicate any risk of respiratory disease from ST, and no biological mechanism associating ST consumption with respiratory diseases is plausible. Evidence that ST users have an elevated CVD risk is available, but the estimated risk is lower than the risk for cigarette smokers.

If all smokers were to switch to ST and there were no increase in population use of cigarettes and ST, smoking-attributable deaths from LC (approximately 120,000 deaths/year) and COPD (approximately 90,000 deaths/year) would be reduced or eliminated, which would result in a marked decrease in deaths per year. In reality, not all current smokers would switch to ST and inevitably, an unpredictable percentage of individuals would use cigarettes and ST concurrently.

Although epidemiological studies have reported increased risks of oral and pancreatic cancers for some ST products, this risk was typically lower than the risk for cigarette smoking. All-cause mortality was greatly reduced for ST users compared with cigarette smokers. The lack of smoke production from STs would also eliminate morbidity and mortality from environmental tobacco smoke (Rickert et al., 2007). However, ST appears to be as addictive as cigarettes, and it has been argued that ST serves as a gateway for cigarette
smoking in the US. In contrast, *snus* use does not appear to have a gateway effect in Sweden. Swedish *snus* has been used as a smoking cessation aid, but its efficacy in this capacity is not well characterized. Information about ST use as a cessation aid in the US is limited. Therefore, the population risks from ST use are unknown.

Although additional research is needed about disease risk for ST compared with conventional cigarettes, LSRO’s confidence that ST is a reduced-risk product compared with cigarettes is high.
10.1 RISK CHARACTERIZATION OF CATEGORIES OF SMOKELESS TOBACCO

The second component of the Differentiating Tobacco Risks project was to:

- Identify and characterize the critical characteristics that contribute to the evaluation of risk, and
- Develop an independent consensus opinion about whether sufficient evidence exists to stratify categories of smokeless tobacco (ST) products according to risk.

To address these issues, the Life Sciences Research Office (LSRO) reviewed the scientific literature including preclinical studies (ST product characteristics, manufacturing and chemical composition, in vitro assays, and animal studies), clinical studies, health effects studies, and behavioral studies. In evaluating whether sufficient evidence exists to stratify categories of ST according to risk, LSRO addressed the following questions:

- What is the overall weight of evidence that individuals who use one category of ST would experience greater risk reduction than individuals who use a different type of ST?
- What are the specific characteristics of STs that allow determination of relative risk?
- What characteristics of STs are most important for assessing risk?
- What is the degree of confidence in the answers to these questions?

A discussion follows of the scientific evidence relevant to a comparative risk assessment of STs. LSRO focused on data on moist snuff, loose-leaf chewing tobacco, plug/twist chewing tobacco, dry snuff, and Swedish snus. Hard snuff products were addressed to the extent that data were available. LSRO also considered ST products other than US STs and non-Swedish ST products (called international products in this report). This report addresses products that are currently available and may not be accurate for products that are developed in the future.
10.1.1 Product Characteristics

Tobacco species, fertilization practices, pesticide usage, soil conditions and climate during growth of the tobacco, and tobacco-curing method may all contribute to the risk of disease for users of different STs. Non-tobacco ingredients incorporated into the ST product and other features of the manufacturing, production, and post-production processes for STs may also influence risk. Swedish *snus* and traditional US moist snuff products\(^\text{10}\) differ in the method of tobacco curing (Foulds *et al.*, 2003). Typically, Swedish *snus* contains only sun- or air-cured tobacco, whereas US moist snuff has historically contained tobacco blends that include fire-cured tobacco (Johnson, 2007). Fire-cured tobacco likely exposes ST users to polycyclic aromatic hydrocarbons (PAHs) from wood smoke condensate. Loose-leaf chewing tobacco and plug tobacco contain mostly air-cured tobacco and limited amounts of fire-cured tobacco (Johnson, 2007).

The process by which Swedish *snus* is made has been described (Foulds *et al.*, 2003). *Snus* is steamed for 24–36 hours, which kills bacteria in the product. Tobacco in US moist snuff products is fermented, whereas tobacco in Swedish *snus* is not. Fermentation results in higher nitrite levels in US snuff than in Swedish *snus*, which may contribute to higher TSNA levels in US traditional moist snuff products. Storage conditions for STs also differ. After the manufacturing process is completed, cans of Swedish *snus* are refrigerated until they are sold (Foulds *et al.*, 2003). Recently, US *snus* products have been developed. One US *snus* product (Camel\(^\circledR\) *snus*) is also refrigerated after production (Louis, 2006), whereas others (Marlboro\(^\circledR\) *snus*, Triumph\(^\circledR\) *snus*) are not (Swedish Match, 2008b). US traditional STs are not refrigerated until sold.

One US *snus* product, Marlboro\(^\circledR\) *snus*, differs from Swedish *snus* and US traditional moist snuff in ways that may be important to the risk of disease. It is similar to Swedish *snus* in that it is pasteurized, has lower TSNA levels than US traditional moist snuff products, and reduces salivation. However, this US *snus* product delivered less nicotine to users than that delivered to users of Swedish *snus* and traditional US moist snuff (Fisher, 2007; Foulds & Furberg, 2008). Some have proposed that this lower nicotine product may serve as a “graduation” product from which users will shift to another ST that delivers higher amounts of nicotine (Foulds & Furberg, 2008). Marlboro\(^\circledR\) *snus* also differs from traditional US moist snuff in that it is sold as a 0.23 g portion pouch (the approximate portion size for US traditional

\(^{10}\) In this report, “traditional US moist snuff products” refers to US moist snuff products other than US *snus* products that were recently developed.
products is 1.5 g), is pasteurized but not fermented, uses a flavor film technology, and contains about 12% moisture compared with 50% moisture for traditional US moist snuff products (Fisher, 2007; Foulds & Furberg, 2008).

Some researchers have proposed that some non-tobacco ingredients, such as flavorings, added to ST could increase the appeal of ST products to never users of tobacco and may cause greater tobacco use. Different STs contain various additives that may affect their overall toxicity. Some additives have known toxic effects (Dave et al., 1992; Merchant et al., 2000; Wary & Sharan, 1988). ST product packaging could also increase the appeal to youth and others who might otherwise not have become tobacco users.

Use of certain STs is also becoming more convenient. Some hard snuff products (Star Scientific, Inc., 2006) and recently developed US snus products do not require spitting (Fisher, 2007; Louis, 2006). ST users can thus use tobacco in situations in which smoking is not feasible, instead of abstaining from tobacco consumption. Some tobacco companies are developing snus products that appeal to women who wish to use snus without anyone realizing that they are using the product (Swedish Match, 2008a). The availability of an ST product that can be used more discreetly than traditional STs may also lead to greater tobacco consumption.

Users of portion-bag ST showed fewer oral and gingival soft tissue changes than did users of loose snuff, possibly because of the lower pH of the portion-bag version (Andersson et al., 1995).

10.1.2 Chemical Composition

The chemical composition of ST products varies considerably. Contributing to the variability of many international products is non-commercial production, although one product, gutkha, has been produced commercially since 1975 (Sinha, 2004). A study of the chemical composition of 34 ST products sold on the North American, Swedish, and British markets reported substantial variation in concentrations of certain constituents (Proctor & Sandi, 2007).

The GothiaTek® standard, a voluntary standard developed by Swedish Match Company, limits the level of undesired constituents—nitrite, tobacco-specific nitrosamines (TSNAs), N-nitrosodimethylamine, benzo[a]pyrene (B[a]P), pesticides, cadmium, lead, arsenic, nickel, and chromium—in ST. TSNAs are not found in freshly harvested green tobacco (Baker, 1999; Brunnemann & Hoffman, 1991) and are primarily formed from tobacco alkaloid nitrosation (Brunnemann et al., 1996). US and Swedish products contained lower TSNA levels than toombak, a Sudanese ST (Idris et al., 1998a; Stepanov et al.,
Comparative Risk Assessment of Smokeless Tobacco and Cigarettes

2006). TSNA levels differed for STs made from *Nicotiana tabacum*. A modified curing method has led to reduced TSNA levels in tobacco used for some STs (Star Scientific, Inc., 1999, 2006). Hard snuff contained only tobacco manufactured by using this microwave curing process (Star Scientific, Inc., 1999). One study found that hard snuff products had lower TSNA levels than did a US traditional moist ST product and Swedish *snus* (Stepanov et al., 2006). Another study reported that dry snuff had the highest total TSNA levels (*N*-nitrosonornicotine [NNN] + *N*-nitrosoanabasine [NAB] + 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK]) of US ST products (Jansson et al., 2003).

Hatsukami et al. (2004a) reported that Swedish *snus* contained considerably lower levels of TSNA and PAHs than ST products sold in the US, Africa, and India. Indian tobacco products had a wide range of TSNA levels. A study of ST products sold in India reported that *khaini* and *zarda* had some of the highest TSNA levels. *Toombak* contained up to 100 times the TSNA levels of US and Swedish STs (Idris et al., 1994). Other ST products such as tooth powder and *guthka* had lower TSNA levels (Stepanov et al., 2005).

Levels of the PAH B[a]P in different STs were also compared. Swedish *snus*, hard snuff, and the international product, *zarda*, had lower B[a]P levels than did a brand of US moist snuff; these levels were 1.99 ng/g for Swedish *snus*; 0.4 ng/g for hard snuff; 2.04 ng/g for *zarda* from the UK; 2.83 ng/g for *zarda* from India; and 19.3 ng/g for a US brand of moist snuff (McNeill et al., 2006).

ST products also differ with regard to heavy metals, radionuclides, and levels and types of flavoring and other substances also differ for ST products. *Zarda* had the highest levels of heavy metals, US moist snuff and Swedish *snus* had similar lower levels, and hard snuff had the lowest levels (McNeill et al., 2006).

Nicotine delivery is influenced by the pH of the ST product. A higher pH leads to higher proportion of free or unionized nicotine and a greater absorption of nicotine. McNeill et al. (2006) reported that pH values for Swedish *snus*, a hard snuff tobacco lozenge, and US moist snuff were 7.86, 7.57, and 7.39, respectively. Corresponding nicotine concentrations for the products (mg/g) were 15.2, 9.2, and 25.8. In contrast, *zarda*, and ST used in the UK and India, had significantly lower pH values of 5.32 and 4.88 and nicotine levels of 48.4 mg/g and 55 mg/g, respectively (McNeill et al., 2006).
10.1.3 Genotoxicity and Cytotoxicity Assays

Genotoxicity and cytotoxicity studies of ST products produced results of considerable variability, which makes it difficult to arrive at conclusions about comparative risk for different categories of STs via these assays. LSRO has noted that the reliability of data from such studies could be improved by including appropriate reference ST products in preclinical studies for STs (see Chapter 4 of this report). Although reference ST products are available for traditional US moist snuff, dry snuff, and chewing tobacco (North Carolina Agricultural Research Service, 2005), none are available for Swedish snus and international ST products.

One study showed that Swedish snus was not mutagenic when tested in the Salmonella mutagenicity assay, which used strains that detected point mutations but not frameshift mutations or base-pair substitutions (Jansson et al., 1991). However, evidence of mutagenicity is available for US flue-cured and fermented products, such as chewing tobacco (Guttenplan, 1987; Stamm et al., 1994; Whong et al., 1985), dry snuff, and moist snuff (Rickert et al., 2007). Many international ST products (e.g., betel quid with tobacco, gutkha, shammah, zarda, and masherī) were mutagenic in the Salmonella mutagenicity assay (Hannan et al., 1986; Jaju et al., 1992; Kulkarni et al., 1987; Niphadkar et al., 1996; Rickert et al., 2007; Shah et al., 1985; Shirname et al., 1983). Areca nut and lime, which are additives in some international products, are known as genotoxic (Agrawal et al., 1986; International Agency for Research on Cancer, 1985; Panigrahi & Rao, 1986; Tanaka et al., 1983).

Evidence exists for mammalian cell mutagenicity for Indian ST products. Mishri (Kulkarni et al., 1987), pan masala with tobacco, and betel quid extracts induced chromosomal aberrations and increased the frequency of micronucleated cells (Jaju et al., 1992; Patel et al., 1994). The international products (masherī/mishrī) caused chromosomal aberrations in bone marrow cells of Swiss mice (Kulkarni et al., 1987) and in Chinese hamster lung cells (Jaju et al., 1992; Patel et al., 1994; Shirname et al., 1984), induced sister chromatid exchange, and increased the frequency of micronucleated cells (Jaju et al., 1992; Patel et al., 1994). Khaini deregulated genes involved in cell growth, cell cycle regulation, and apoptosis (Rohatgi et al., 2005).

The variability of in vitro data makes it difficult to draw firm conclusions as to the degree of reduced toxicity of one ST product compared with another. Hard snuff was not mutagenic (Rickert et al., 2007). Swedish snus appears to be less mutagenic than ST products other than hard snuff. Many Indian products are mutagenic.
10.1.4 Animal Studies

Animal investigations have provided evidence of ST carcinogenicity. *Snus* accelerated gastric tumor development in transgenic mice (Stenström *et al.*, 2007). Moist snuff placed in the oral cavity increased tumors in the nasal and oral cavities of the rat (Johansson *et al.*, 1989). However, US moist snuff extract did not cause tumor formation when placed in the cheek pouch of Syrian golden hamsters (Barley *et al.*, 2004). An extract of betel quid with tobacco did produce lung tumors in mice (Shirname *et al.*, 1983) and squamous cell carcinoma in the hamster cheek pouch (Suri *et al.*, 1971). Indian chewing tobacco extract produced lung and liver tumors in Swiss mice (Shah *et al.*, 1985). Some ST additives may contribute to toxicity of tobacco products. For example, Indian chewing tobacco alone did not induce early malignant changes. When mixed with betel nut and applied to the skin, however, it induced skin papilloma and epidermoid carcinoma in the hamster cheek pouch and Swiss mice (Ranadive *et al.*, 1976). Studies showed that certain STs are carcinogenic, but data are not available for all international STs. The International Agency for Research on Cancer (IARC) has conducted an extensive review of ST products and their carcinogenicity and has categorized ST as carcinogenic to humans (International Agency for Research on Cancer, 2007b).

10.1.5 Clinical Studies

10.1.5.1 Biomarkers of exposure and effect

Data on biomarkers of exposure and effect for STs are limited. One study reported that Swedish *snus* users had higher maximal blood nicotine levels than did users of US moist snuff products (Lunell & Lunell, 2005). Another study reported that users of one US moist snuff product had higher blood nicotine concentrations than users of 3 newer tobacco products (Kotlyar *et al.*, 2007). Two of the products were hard snuff and one was a moist ST product that releases nicotine without chewing.

Tobacco chewers did not have significantly higher urine mutagenicity than moist snuff users and non-users of tobacco (Benowitz *et al.*, 1989). US moist snuff users had higher TSNA levels compared with Swedish *snus* users. ST users who switched from a moist ST product to a low-nitrosamine *snus*-like product had lower levels of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in urine (Hatsukami *et al.*, 2004b). Extremely high levels of total NNAL were measured in the urine of Sudanese male toombak users (Idris *et al.*, 1994; Murphy *et al.*, 1994).
The IARC concluded that chewing tobacco is more likely than snuff to have stronger cardiovascular effects (on blood pressure and heart rate) (International Agency for Research on Cancer, 2007b), but acknowledged that this conclusion was based on limited data from a study by Benowitz et al. (1989). No firm conclusions can be reached about risk for different ST users from biomarker studies.

10.1.6 Health Outcomes Assessment

10.1.6.1 Lung cancer and chronic obstructive pulmonary disease

Because all ST products reduce the risk of lung cancer and chronic obstructive pulmonary disease (COPD) compared with cigarettes, and data are insufficient to determine the extent to which they reduce risk, the effects of STs on the risk of LC and COPD cannot be used to discriminate between the health risks of different ST products. Reviews of data for other diseases are required for this purpose.

10.1.6.2 Cardiovascular disease

Most studies have reported that Swedish snus users did not have an increased risk of cardiovascular disease (CVD) (Asplund et al., 2003; Hergens et al., 2005; Huhtasaari et al., 1992, 1999; Wennberg et al., 2007). One study reported a slightly higher risk of CVD; however, the increase in risk was lower than that for smokers (Bolinder et al., 1994).

Heavy use of snus increased risk of CVD mortality for Swedish construction workers (Hergens et al., 2007). One study reported that users of US ST products did not have an increased risk of death from CVD compared with non-users of tobacco (Accortt et al., 2002), but another problematic study reported increased risk (Henley et al., 2005). Female betel quid users had an increased incidence of CVD (OR=1.37; 95% CI: 1.1–1.6), but men had no increased risk (OR=0.5; 95% CI: 0.14–1.9) (Guh et al., 2007). Chewing tobacco used in 52 countries increased the risk of non-fatal myocardial infarction (OR=2.23; 95% CI: 1.41–3.52), but because of the small sample size, the authors did not draw any conclusions (Teo et al., 2006). Some evidence exists that ST users had an increased risk of CVD compared with non-users; however, the available data did not allow for a distinction of risks for different ST products to be made.
### 10.1.6.3 Oral cancer

A review of 5 large studies concluded that Swedish *snus* did not cause oral cancer (Fagerström & Schildt, 2003; Foulds et al., 2003). A number of studies have shown that users of Swedish *snus* did not have an elevated risk of oral cancer compared with non-users of tobacco (Boffetta et al., 2005; Lewin et al., 1998; Luo et al., 2007; Rosenquist et al., 2005; Schildt et al., 1998). Some studies reported that US snuff was associated with an increased risk of oral cancer (Blot et al., 1988; Institute of Medicine, 2001; Kabat et al., 1994a, 1994b; Kabat & Hebert, 1994; Stockwell & Lyman, 1986; Winn et al., 1981), but others found no effect (Accortt et al., 2005a; Mashberg et al., 1993; Sterling et al., 1992).

International ST products appear to pose a significantly greater risk of oral cancer than do US and Swedish STs. Users of betel quid with tobacco had an increased risk of oral cancer compared with non-tobacco users (Balaram et al., 2002; Dikshit & Kanhere, 2000; Jayant et al., 1977; Rao et al., 1994; Sankaranarayanan et al., 1989a; Wasnik et al., 1998). Some Indian, Pakistani, and Sudanese studies of betel quid with tobacco, chewing tobacco, *toombak*, and *shammah* reported that these STs increased risk of oral cancer (Balaram et al., 2002; Elbeshir et al., 1989; Gupta et al., 1980; Idris et al., 1995a; Jayant et al., 1977; Rao et al., 1994; Sankaranarayanan et al., 1989a; Wasnik et al., 1998).

Users of cigarettes plus ST products had an increased risk of cancers of the oral cavity, oropharynx, hypopharynx, and larynx compared with non-users of cigarettes or ST (Jayant et al., 1977). Pharyngeal cancer risk was increased for betel quid with tobacco users (Lee et al., 2005). Betel quid with tobacco did not increase the risk of base tongue and laryngeal cancers (Sankaranarayanan et al., 1990; 1990a, 1990b).

*Toombak* increased the risk of squamous cell carcinoma of the lip, buccal cavity, and mouth floor compared with controls (OR=3.9; 95% CI: 2.9–5.3) (Elbeshir et al., 1989; Gupta et al., 1980; Idris et al., 1995a). *Toombak* also increased the risk of tongue, palate, and maxillary sinus cancers compared to a control group (Idris et al., 1995a, 1995b).

Critchley and Unal (2003) reported that using betel quid and tobacco resulted in a “substantial risk of oral cancer,” and the IARC also reported an increased risk of oral cancer for betel quid and tobacco (International Agency for Research on Cancer, 2004a). Epidemiological data demonstrated that *snus* appeared to have significantly lower risks of oral cancer than some international products. US ST products may confer an intermediate level of risk.
10.1.6.4 All-cause mortality

The only published analysis of Swedish snus and all-cause mortality reported that snus users had increased all-cause mortality risk (RR=1.4; 95% CI: 1.3–1.8) compared with non-tobacco users (Bolinder et al., 1994). Exclusive use of US ST did not increase all-cause mortality (HR=1.1; 95% CI: 0.9–1.3) when compared with no tobacco use (Accortt et al., 2002). This study was limited by inclusion of pipe and/or cigar smokers in the no-tobacco use group and of one-time ST users in the ever users of tobacco group. US men who used moist snuff and chewing tobacco had higher all-cause mortality (CPS I: HR=1.17; 95% CI: 1.18–1.30; CPS II: 1.18; 95% CI: 1.08–1.29) compared with non-tobacco users, but this study had significant confounders (see Section 7.2.1) (Henley et al., 2005). Indian tobacco chewers had an increased risk of all-cause mortality (Gupta et al., 1984; Gupta & Mehta, 2000). Chewing betel quid with tobacco increased all-cause mortality risk (RR=1.3 for females) compared with non-tobacco users (no confidence intervals reported) (Gupta et al., 1984).

10.1.7 Population Risk

In 1986, ST was characterized by the US Surgeon General as an addictive drug (U.S. Department of Health and Human Services, 1986a). Nicotine is one of the primary substances in tobacco that leads to addiction. ST provides doses of nicotine that are psychoactive, and users eventually develop tolerance to nicotine. The development of tolerance leads to more frequent tobacco use and graduation to stronger products (Tomar et al., 1995). ST users also experience withdrawal effects similar to those of cigarette smokers who attempt to quit using cigarettes (American Psychiatric Association, 2000; Hatsukami et al., 1987).

There are concerns that ST could be a gateway product to tobacco. Investigations from Sweden indicated that ST was useful as a smoking cessation aid and that ST did not appear to serve as a gateway to smoking (Furberg et al., 2005, 2006; Gilljam & Galanti, 2003; Ramström & Foulds, 2006). Few data are available to assess whether easy access to some types of ST products increases tobacco product use.
10.2 PROPOSED FACTORS FOR ASSESSING COMPARATIVE RISK OF SMOKELESS TOBACCO PRODUCTS

ST includes a wide spectrum of products that contain tobacco from diverse geographic regions that is cured and processed via different methods. ST products have different pHs and additives and contain various levels of nicotine, carcinogens, and other toxins. The products are stored, produced, and used differently by various cultures; use starts at different ages; and products expose users to different levels of product constituents.

Distinctions have been made among STs according to the following factors:

- **Geographic origin of tobacco**: *Nicotiana tabacum* has lower TSNA levels than *Nicotiana rustica*. The tobacco leaf may contain different levels of metals because soil metal content varies.

- **Chemical composition of the product**: A limited number of tobacco constituents and additives have been measured. The inclusion of a substance that is not GRAS (Generally Recognized as Safe) in an ST product may contribute to increased risk. Additives such as areca nut, betel leaf, and powdered lime that are used in certain international STs may increase the toxicity and neurological effects of tobacco (Rodu & Godshall, 2006). A minority opinion expressed in the LSRO Expert Panel was that GothiaTek® and expanded standards may be used to rank the ST products. However, the Expert Panel did not reach a unanimous decision about the usefulness of chemistry studies as a ranking method for ST products. Lower levels of a specific carcinogen do not ensure lower risk.

- **Manufacturing conditions for the product**: Some ST products are manufactured via non-standardized methods which increases the uncertainty about the toxicity of the products.

- **Variability of product composition**: Although variability among ST products is expected, the significant variability in ST composition results in differences in associated risk. Reduction of such variation is essential for improving understanding of ST product-related risk. A standardized ST manufacturing process would help assessment of the comparative risk of STs.

- **Refrigeration of the ST product**: Refrigeration of Swedish *snus* and some US *snus* products slows bacterial growth. Certain US *snus* products are not refrigerated but do undergo pasteurization (Williams, 2008). Other STs are not refrigerated until used.
Differentiating the Health Risks of Categories of Tobacco Products

- Fermentation of tobacco: Loose-leaf and plug tobacco products are likely to have more bacterial activity than Swedish snus because they contain fermented tobacco. Fermented tobacco tends to have higher amounts of TSNAs than tobacco that is not fermented.
- Curing: Fire-cured tobacco may increase exposure to PAHs.
- Performance of the product in genotoxicity and cytotoxicity assays: Mutagenicity should be a strong sorting factor. Use of a standard snus-like product in standard genotoxicity and cytotoxicity assays is required so that ST products can be compared. Air-cured tobacco is more mutagenic than flue-cured tobacco.
- Animal toxicity studies: Neither animal studies nor current toxicity studies offer a satisfactory approach to ranking products.
- Biomarker of exposure and effect: Biomarker studies provide data about differences in exposure to product constituents and effects of exposure.
- Health effects assessment (epidemiological studies): Extensive, high-quality data are available for Swedish snus, but the amount and quality of data are significantly lower for other STs.
- Behavior related to the use of STs: Product use behavior analyses can address concerns about how the products affect use. Additional studies are needed to assess the impact of different STs on disease risk.

The quality of data in studies of US and international products is poor compared with that for Swedish snus, and information is not available for all products. Missing are well-conducted epidemiological studies showing that CVD, cancer, and all-cause mortality are reduced for ST users compared with cigarette smokers. Population studies that indicate that ST products do not increase addiction, do not increase the rate of initiation of ST and tobacco use, and do not reduce cessation of tobacco use are also lacking. Data are insufficient to allow determination of critical factors influencing risk.

10.3 STRATIFICATION OF CATEGORIES OF SMOKELESS TOBACCO PRODUCTS ACCORDING TO RISK

Data on the health effects of ST products are lacking. LSRO’s stratification of STs is based on available data and could change with additional studies and modifications of product formulations. In addition to utilizing the weight of evidence approach described previously, LSRO stratifies products
according to whether the product is manufactured commercially. Because of the extensive data on the health effects of Swedish *snus* that were generated from well-conducted epidemiological studies and what these data indicate in term of disease risk reduction compared with cigarette smoking, LSRO considers Swedish *snus* to be the least harmful ST product. In time, data may indicate that other STs reduce risks to a similar or greater extent than Swedish *snus*, but present data are insufficient for this purpose. Less information is available for US ST products; however, on the basis of data from the studies reviewed, LSRO classifies US ST products as having intermediate risk. Data are sparse for international ST products, with results from epidemiological studies missing.

Another consideration is that tobacco products are changing. Manufacturers of moist snuff products sold in the US are adopting practices similar to manufacturing practices used for Swedish *snus*. Determination of whether other Swedish products and US products present a risk reduction magnitude similar to the risk reduction associated with Swedish *snus* must await future research.

Information about hard snuff is also insufficient, partly because these ST products were developed in the last decade, in contrast to other ST products, which have been used for 100 years or more. No long-term epidemiological studies of hard snuff products have been conducted. However, biomarker studies indicate lower TSNA levels, less mutagenicity, and lower exposure to TSNAs in hard snuff products than in other ST products (Mendoza-Baumgart *et al.*, 2007).

Some international products appear to confer a significantly greater risk than Swedish *snus*. Limited data about certain international products, *e.g.*, betel quid with tobacco, suggest that these international products are significantly more harmful than are Swedish *snus* and US ST products. These products appear to be the most mutagenic and to present the highest cancer risk compared with Swedish *snus* and US traditional moist STs. This result may be a function of the type of tobacco in the product and, more importantly, ingredients added to the product. However, the factors and compounds contributing to health risks are not completely known. Although it is reasonable to suppose that a product with characteristics more like Swedish *snus* than other ST products will carry risks similar to Swedish *snus*, such a conclusion is by no means a secure one, based on detailed scientific knowledge.

The following reflects LSRO's stratification of ST products. As indicated by epidemiological studies, Swedish *snus* is likely to be the least harmful ST.
LSRO considers US traditional moist snuff and chewing tobacco to confer an intermediate level of risk to users. In general, LSRO considers international ST products to be the most harmful STs. Few comparisons have been made among US products, but dry snuff has been suggested to present higher risk than moist snuff and chewing tobacco. Rodu and Jansson (2004) summarized oral cancer relative risk estimates for different types of tobacco and found similar risk estimates for moist snuff and chewing tobacco but significantly higher risk estimates for dry snuff. However, available data do not allow LSRO to make clear distinctions among US ST products. Although no epidemiological studies of hard snuff have been published, information on product characteristics, chemistry, and biomarker of exposure studies suggests reduced exposure to some product constituents.
In the course of developing this report, the Life Sciences Research Office (LSRO) identified gaps in the scientific literature that would benefit from further investigation. LSRO’s recommendations for use of standardized methods in research on smokeless tobacco (ST) and for future research on ST are described in the following.

11.1 STANDARDIZED METHODS

11.1.1 Preclinical Studies

Studies of the chemical composition of ST products have primarily focused on a relatively small number of tobacco constituents (e.g., nicotine, tobacco-specific nitrosamines [TSNAs], polycyclic aromatic hydrocarbons [PAHs], heavy metals, and radionuclides) (Brunnemann & Hoffman, 1991; Hoffmann et al., 1987; McNeill et al., 2006). At this time, the chemical components of ST products responsible for increased health risks remain incompletely characterized. A more comprehensive investigation of the levels and uptake of toxicants in ST products is necessary for evaluation of the potential harm associated with these products (Hatsukami et al., 2007).

- LSRO recommends that all commercially available ST products be rigorously characterized for their chemical composition and that analytical chemistry methods that are used to detect chemicals in ST products be standardized and be state-of-the-art.

Most in vitro and in vivo studies use smokeless tobacco extract (STE) for testing the effects of ST products in biological assays. ST is commonly extracted with one solvent or a combination of solvents such as alcohol, chloroform, dimethyl sulfoxide, and water. Methods for extracting ST should be standardized.

- LSRO recommends that investigators consult the guidelines of the International Organization for Standardization (ISO) for preparation of STE to be used in in vitro and in vivo studies. The ISO has well-established and accepted extraction methods for preparing test
substances for both genotoxicity and cytotoxicity studies (International Organization for Standardization, 2007).

The genotoxicity and cytotoxicity literature on STs is confusing and inconsistent because of the failure to adequately address issues related to exposures, controls, and testing methodologies (Organization for Economic Cooperation and Development, 2004; U.S. Food and Drug Administration et al., 2001).

- LSRO recommends the testing of ST in genotoxicity and cytotoxicity assays recommended previously for evaluating potential reduced-risk tobacco products (Life Sciences Research Office, 2007a). The analyses should be performed according to the guidelines of the International Conference on Harmonisation (2004) and the US Food and Drug Administration (2008).
- LSRO recommends, for long-term carcinogenicity studies, that animals receive a daily dose of the test substance for a minimum of 1 year (Organization for Economic Cooperation and Development, 1981).

Reference ST products for research purposes are available from the North Carolina Agricultural Research Service (2005). Reference STs provide researchers with useful information on tobacco blending, added ingredients, distribution, and storage conditions.

- LSRO recommends the use of reference STs to monitor consistency of laboratory instruments and to allow comparison of results among independent laboratories.

Although reference products for loose-leaf chewing tobacco, dry snuff, and moist snuff are available, no reference *snus*—i.e., pasteurized reference ST produced entirely from air- or sun-cured tobacco—is currently available for *snus* products, which are rapidly being introduced into the US market.

- LSRO recommends development of a reference ST for US *snus*-like products.
11.2 RESEARCH NEEDS

11.2.1 Preclinical Studies

Evaluations of the oral carcinogenicity of ST products have used 3 experimental animal models: a surgically created oral test canal in rodents, hamster cheek pouch, and oral cavity swabbing in rodents (Barley et al., 2004; Hecht et al., 1986; Johansson et al., 1989). These models have been criticized and may not appropriately represent human ST product use.

- LSRO recommends that future research develop new, less invasive, validated animal models of diseases that are relevant to ST use. Furthermore, future research should address how to expose animals in a way that better approximates human ST use.

11.2.2 Clinical Studies

11.2.2.1 Biomarkers of exposure

Nicotine is the main constituent in tobacco responsible for maintenance of a tobacco use habit. The dose of nicotine delivered is not totally characterized. Few nicotine pharmacokinetic studies have been conducted with different categories or among different brands of US and Swedish ST products (Benowitz et al., 1988; Fant et al., 1999).

- LSRO recommends that nicotine pharmacokinetic studies of all US ST products be conducted. Pharmacokinetic information, especially for the newer STs such as hard snuff products, is needed.

Cotinine, the major metabolite of nicotine, is a specific biomarker of nicotine exposure in cigarette smokers (Benowitz & Jacob, III, 1994). Unlike cigarette smokers, ST users may absorb additional nicotine through the gastrointestinal tract by means of swallowing the salivary extract of ST products (Benowitz et al., 1989). When the amount of tobacco juice that is swallowed varies for ST users, cotinine may be an imprecise measure of nicotine exposure in ST users (Ebbert et al., 2004). Few studies have quantified absorption of ST products from the gastrointestinal tract. Furthermore, the interaction between saliva and ST constituents is not well characterized.

- LSRO recommends that future research identify relevant biomarker(s) of nicotine exposure in ST users and investigate interaction between saliva and ST constituents.
11.2.2.2 Biomarkers of effect

The degree to which ST use increases the risk of cardiovascular disease (CVD) compared with never use of tobacco needs quantification.

- LSRO recommends that investigations of ST use evaluate the following biological processes that are relevant (but not necessarily directly causal) to development of CVD: lipid metabolism, inflammation, thrombosis and coagulation, oxidative stress, endothelial function, atherosclerosis, myocardial function, and electrical cardiac activity (Life Sciences Research Office, 2007a).

1.2.3 Health Outcomes

The Swedish National Health Service has extensive population data and excellent recording services (Swedish National Board of Health, 2005) for evaluation of the health outcomes of Swedish snus use. In contrast, information gaps on health effects of US and international ST products exist, which makes it difficult to compare the relative health risks of different categories of STs.

- LSRO recommends the following:
  - More well-designed studies of the health effects associated with commercially available ST categories that reflect the current marketplace of US and international ST products should be conducted. Potential health risks, especially those related to CVD, cancer, and all-cause mortality, should be evaluated.
  - Health risks of the dual use of cigarettes and ST products, which appears to be a more common practice in the US than in Sweden, need analysis.
  - The degree to which US ST products pose a higher risk of oral cancer than Swedish snus needs to be ascertained. Inclusion of more oral biological specimens, such as oral mucosa tissue swab cells, from actual ST users is warranted for biopathology studies. Future research on oral cancer should carefully control for confounders, such as socioeconomic status, smoking history, and alcohol use, and should reflect the current marketplace of all commercially available US ST products.

The International Agency for Research on Cancer (2007a) reviewed the scientific literature to characterize the changes in the risk of cancer, CVD, and chronic obstructive lung diseases after smoking cessation. Few cohort
studies of risk reduction among smokers who switch to using moist snuff have been published (Tomar, 2007).

- LSRO recommends that future research evaluate changes in the risk of cancer, CVD, and mortality when different ST products are substituted for cigarettes.

11.2.4 Behavior

To date, no published randomized clinical trials analyzing ST as a smoking cessation method are available (Tomar, 2007). The effectiveness of snuff use as a smoking cessation method was evaluated in a pilot study (Tilashalski et al., 1998). However, the study had certain limitations such as small sample size and no control group.

- LSRO recommends evaluation of the use of ST as a smoking cessation method in a larger, randomized controlled trial.

Changes in marketing ST products in the US—i.e., introducing new packaging and flavors that appeal to youth and making available ST products that can be used more discreetly than traditional ST products—may lead individuals to consume more of these products. The impacts of these changes are unknown.

- LSRO recommends that future research address the following questions:
  o What is the pattern of ST use among subgroups of the population?
  o How do product design and flavorings affect tobacco use initiation rates for youth?
  o How does use of one tobacco product affect use of another tobacco product (Hatsukami et al., 1991a)? Does a change in ST composition or an increase in nicotine levels cause individuals to hold the product in the mouth for longer periods?
  o What are the effects of ST use on subsequent smoking onset? How are these related to age and other aspects of the initiation of tobacco use, including social, environmental, and motivation variables (Peterson et al., 1989)?

Currently, ST use prevalence is higher for men (4.5%) than for women (0.2%) (Centers for Disease Control and Prevention, 2006). Therefore, data on ST consumption pertain mostly to men. LSRO recommends that future research
investigate whether behavioral differences in ST consumption exist between men and women. For example, do women, like men, move from flavored lower nicotine ST products to higher nicotine brands?

11.3 CONCLUSIONS

ST use is increasing in the US, and the health risks of different categories of ST products are incompletely understood. In this report, LSRO identified areas that would benefit from further research. Research is needed to completely characterize the chemistry of ST products. Validated and standardized techniques for biological assays are also needed. Information about the chemistry and biological effects of ST products would serve as a basis for determining which intrinsic components of ST are responsible for increasing health risks to humans. Improved understanding of the physiological mechanisms of absorption of ST constituents from the gastrointestinal tract and interactions of ST constituents with saliva would aid in development of additional relevant biomarkers of exposure. Larger, randomized controlled trials are necessary for better evaluation of the use of ST as a smoking cessation method. Sex differences and differences in subgroups of the population related to the pattern of ST use also require further investigation. In sum, well-designed studies of health effects, especially CVD, cancer, and all-cause mortality, are key to making decisions about the relative risk of adverse health outcomes from different categories of ST products.
LITERATURE CITATIONS


Differentiating the Health Risks of Categories of Tobacco Products


Life Sciences Research Office. (2007b) Scientific Methods to Evaluate Potential Reduced-Risk Tobacco Products. (St. Hilaire, C. L., ed.) Bethesda, MD: LSRO.


Differentiating the Health Risks of Categories of Tobacco Products


A cohort study of smoking, alcohol consumption, and dietary factors for pancreatic cancer (United States). Cancer Causes Control 4: 477-482.

Stereoselective metabolism and tissue retention in rats of the individual enantiomers of 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), metabolites of the tobacco-specific nitrosamine, 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanolone (NNK). Carcinogenesis 25: 1237-1242.
APPENDICES

APPENDIX A.LIFE SCIENCES RESEARCH OFFICE

Differentiating Tobacco Risks Expert Panel

Alwynelle (Nell) Ahl, Ph.D., D.V.M.
Nell Ahl is Principal Scientist at Highland Rim Consulting, Inc. (HRC). 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 HRC, Dr. Ahl had a distinguished career at the US Department of Agriculture (USDA) and served 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 of the Society for Risk Analysis and has served on several panels at the National Academy of Sciences. Her research interests include public policy related to 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.

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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. He 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
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Shayne Cox Gad is the Principal at Gad Consulting Services. After majoring in chemistry and biology at Whittier College, he obtained his doctorate in pharmacology/toxicology from the University of Texas. As a fellow at Bushy Run Research Center, Union Carbide Toxicology Laboratory, he developed a system for assessment of toxicity of polymer thermal decomposition products. Later, he worked at the Shell Development Laboratory and established a new inhalation toxicology research facility. As Manager of Mammalian Toxicology at Allied Corporation, Dr. Gad was responsible for all mammalian toxicity testing including the operation of the entire Department of Toxicology laboratory and all external contract testing. Dr. Gad has also worked at G.D. Searle & Co., as Director of Toxicology and Senior Director of Product Safety and Metabolism, where he used an interdisciplinary approach to oversee safety and toxicity research programs, assisted with the prioritization of research and development efforts, interacted with foreign firms and regulatory agencies, and developed worldwide occupational airborne control objectives. He also served as the Director of Medical Affairs Product Support Services at Becton Dickinson and Director of Toxicology at Synergen. Dr. Gad has directed the design, conduct, writing, and filing of investigational new drug applications, new drug applications, drug disposition studies, and medical device petitions. Dr. Gad’s presentations and publications total more than 300 and include 27 books and 35 book chapters.

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, Oregon. 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, where he concentrated on physiological processes and mathematical models; as Associate Professor at Brown University; and as a
Robert Orth, Ph.D.

Robert Orth is a physical chemist at Apis Discoveries, LLC. He is also a consultant to the Monsanto Company and an 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 on 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 in food and agricultural science. His current work at Apis Discoveries includes establishing business units for ultratrace analysis, consulting for companies submitting direct and indirect food additives to the US Food and Drug Administration, and studying the analysis and remediation of organic pollutants. He has more than 100 publications and presentations on analytical and physical chemistry and holds two patents.

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 his 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 an Advanced Clinical Fellow of the American Cancer Society, both at Mount Sinai Hospital in New York. After his fellowship, Dr. Rubin spent 14 years at Mount Sinai Hospital's Pathology Service, with increasing responsibilities, which culminated in his appointment as Pathologist-in-Chief. Dr. Rubin then became the Director of Laboratories at the Hahnemann
University Hospital. His many academic appointments include the Irene Heinz and John LaPorte 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, which include his current position. Among 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 by the US-Canadian Academy of Pathology and the National Institutes of Health MERIT Award. The American Society of Investigative Pathology presented him with the Gold Cane Award. He has held many editorial positions, including Editor-in-Chief of Laboratory Investigations for 14 years, and has served as a consultant to many organizations. He has more than 300 publications, including 13 textbooks and one CD-ROM.

Life Sciences Research Office Staff

MaryBeth Bernhard, B.S., is an Associate Staff Scientist at the Life Sciences Research Office (LSRO). She graduated summa cum laude from Towson University, where she obtained her B.S. in both Psychology and Mass Communication and Communication Studies. Ms. Bernhard completed internships in the Towson University Adult Psychology Research Laboratory, where she coordinated an ongoing study that contributed to a symposium presentation at the 57th Annual Scientific Meeting of the Gerontological Society of America. As Project Coordinator/Lab Manager, Ms. Bernhard collected, recorded, and analyzed data for the study and presented her research findings at the Sixth Annual Research and Scholarship Expo. Before joining LSRO, Ms. Bernhard worked in the Marketing Department of Crist Instrument Company, a research and development facility for biomedical research equipment. Ms. Bernhard has also participated in community outreach efforts through the Dowell Health Center HIV Counseling and Testing Program. Ms. Bernhard is currently working toward completing her Master of Public Health degree at the George Washington University Medical Center School of Public Health and Health Services, with a concentration in epidemiology.

Amy M. Brownawell, Ph.D., is an LSRO Senior Staff Scientist. Dr. Brownawell obtained her Ph.D. in cellular and molecular pharmacology at the University of Virginia and holds both a B.S. and M.S. in chemistry from Georgetown University. She completed her postdoctoral training at the Center for Cell Signaling at the University of Virginia, where she conducted research on nuclear transport mechanisms of proteins and RNA. She has coauthored 13 peer-reviewed journal articles and 2 book chapters, served as editor for LSRO's
Dental Amalgam Review and Biological Effects Assessment in the Evaluation of Potential Reduced-Risk Tobacco Product reports, and is a member of the American Society for Cell Biology, Sigma Xi, and the Society of Toxicology.

Daniel M. Byrd III, Ph.D., D.A.B.T., retired as Deputy Director of LSRO in October 2007. He received B.A. and Ph.D. degrees from Yale University. He first received certification from the American Board of Toxicology in 1982. Previously, Dr. Byrd taught pharmacology and conducted independent research on mechanisms and dosimetry of chemotherapeutic drugs at Roswell Park Memorial Institute and at the University of Oklahoma. At the US Environmental Protection Agency, he subsequently held positions in the Office of Chemical Control, Office of Pesticide Programs, Carcinogen Assessment Group, and Science Advisory Board, for which he was awarded the Silver Medal for Management and Leadership. He also managed committees for three trade associations and was the president of Consultants in Toxicology, Risk Assessment and Product Safety (CTRAPS), a scientific support firm that helped clients acquire, interpret, and use biomedical information. He is the author of more than 100 regulatory documents and 40 scientific articles and coauthored the textbook Introduction to Risk Analysis: A Systematic Approach to Science-Based Decision Making (Byrd & Cothern, 2000). Dr. Byrd is currently enjoying his retirement in Holiday, Florida.

Fabiana F. De Moura, Ph.D., is an LSRO Staff Scientist. She received her Ph.D. in nutrition from the University of Maryland, College Park, and completed her postdoctoral training in the Nutrition Department at the University of California, Davis. She holds an M.S. degree in food science from the State University of Campinas and a B.S. degree in food engineering from the Federal University of Viçosa, both institutions in Brazil. During her doctoral and postdoctoral training, Dr. De Moura’s research focused on elucidation of human metabolism of vitamins and carotenoids. In collaboration with researchers at the National Eye Institute, she investigated the possible beneficial effects of lutein in prevention of age-related macular degeneration. Dr. De Moura is the primary author of a book chapter and the author or coauthor of eight peer-reviewed journal articles. She is a member of the American Society for Nutritional Sciences and the International Carotenoid Society.

Michael C. Falk, Ph.D., is Executive Director of 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 Principal Investigator, he was a key member of the Scientific Advisory Board and the Acting Director of the Institute. He was
also Director of the Wound Repair Program and pioneered a new position as Director of Biochemistry and Cell Biology. Also, as 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 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 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, D.C., 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 the implementation of the HHMI Predoctoral Fellowship in Biological Sciences program. While serving as Science Information Specialist at the Distilled Spirits Council of the United States, she managed the installation of a local area network and participated in the development and maintenance of an electronic research database for the beverage alcohol industry. As a Report Coordinator at Microbiological Associates, Inc., she conducted statistical analyses and prepared technical reports about toxicology studies using animal models. She served as data management administrator for the National Toxicology Program’s sponsored studies. Ms. Feldman currently 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.
Heather Gorby, Ph.D., is an LSRO Staff Scientist. She received her Ph.D. and M.A. in biopsychology from Stony Brook University and her B.S. in biopsychology from the University of California, Davis. She completed her postdoctoral training at the National Institute of Mental Health in Bethesda, Maryland. Her research has examined the feasibility of stress-related hormones as potential therapeutics against bacterial pathogens, and the effects of elevated stress-related hormones on brain anatomy and behavior in rodent models. Dr. Gorby is the coauthor of a peer-reviewed journal article and three book chapters. She is a member of the Society for Neuroscience, the Endocrine Society, and the American Association for the Advancement of Science.

Rebecca Johnson, Ph.D., is 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 1000 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. Dr. Johnson currently assists in maintaining the LSRO library, responding to requests for reports, and organization the scientific literature required by staff scientists for sponsored projects. 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. She 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 completed her postdoctoral research at Yale University. 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 and served as editor for LSRO’s Review of Ingredients Added to Cigarettes Phase Two: Scientific Criteria for the Evaluation of Ingredients Added to Cigarettes and Exposure Assessment in the Evaluation of Potential Reduced-Risk Tobacco Product reports. She is a member of the Association for Chemoreception Sciences.

James L. Seale, Ph.D., is a Scientific Consultant at LSRO. Dr. Seale earned his B.S. in mechanical engineering from the University of Arizona and his M.S. and Ph.D. in bioengineering from Texas A & M University. He served as Research Biomedical Engineer in the Diet and Human Performance
Laboratory of the USDA Agricultural Research Service, Beltsville Human Nutrition Research Center, where he investigated factors affecting human energy metabolism. Dr. Seale also served as Consulting Engineer in the Custom Applications Branch of the National Institutes of Health Center for Information Technology, where he developed plans for quality assurance, change control, security, testing, and validation processes. Dr. Seale currently teaches physics in the Howard County, Maryland, public school system. Dr. Seale has authored or coauthored more than 25 publications.

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APPENDIX B. PUBLIC AND INVITED COMMENTS

Differentiating the Health Risks of Categories of Tobacco Products Open Meeting Speakers, June 5, 2007

Evidence from the Swedish Experience: Nicotine, Prevalence, Gateway, Cessation, and Epidemiology
Karl Olav Fagerström, Ph.D.
Fagerström Consulting AB and Smokers Information Center
Helsingborg, Sweden

Health Risks of Smoking Compared to Swedish Snus
Niel Roth, Ph.D.
Roth Associates
Rockville, MD

Differentiating the Health Risks of Categories of Tobacco Products Open Meeting Speakers, October 3, 2007

Presentation: Differences Between Swedish and American Smokeless Tobacco Products
David M. Johnson, Ph.D., Director of Scientific and Regulatory Affairs
Swedish Match N. America, Inc.
Owensboro, KY

Presentation: Potential Marketing and Postmarketing Evaluation Tools to Assess Tobacco Use Attitudes and Behaviors While Minimizing Population Effects as Part of a Tobacco Harm Reduction Strategy
James Dillard III, Vice President
U.S. Smokeless Tobacco Company
Greenwich, CT

Presentation: Marketing and Consumer Data
Chris Proctor, Head of Science and Regulation and Delcio Sandi
Group R&D, British American Tobacco
London, UK

Presentation: Snus Smokeless Tobacco Products
Michael T. Fisher, Ph.D., Senior Research Scientist
Philip Morris USA

14 Speaker presentations can be found at www.lsro.org.
APPENDIX C. PRECLINICAL STUDIES


APPENDIX D. SUMMARY OF HEALTH EFFECTS FOR CIGARETTE SMOKERS AND SMOKELESS TOBACCO USERS
## APPENDIX D. Summary of Health Effects for Cigarette Smokers and Smokeless Tobacco Users

<table>
<thead>
<tr>
<th>Enrollment Dates; Follow-up Dates</th>
<th>Tobacco Category</th>
<th>Cigarette Smokers</th>
<th>Smokeless Tobacco Users</th>
<th>Controlled Factors</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>HR, OR, or RR 95% CI</td>
<td>No. of Cigarette Smokers</td>
<td>HR, OR, or RR 95% CI</td>
<td>No. of Smokeless Tobacco Users</td>
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<tr>
<td>Lung Cancer</td>
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</tr>
<tr>
<td>1960, 1969–1971</td>
<td>Snuff or chewing tobacco</td>
<td>2.74 NS 63</td>
<td>0.65 NS 26</td>
<td>Age, race, alcohol, SES, CS</td>
<td>Williams &amp; Horm, 1977</td>
</tr>
<tr>
<td>1959; 1982</td>
<td>Snuff or chewing tobacco</td>
<td>1.08 0.64–1.83 18</td>
<td>2.00 1.23–3.24 18</td>
<td>Age, race, education, BMI, alcohol, exercise, aspirin, CS</td>
<td>Henley et al., 2005 (CPS I)</td>
</tr>
<tr>
<td>1982; 2000</td>
<td>Snuff or chewing tobacco</td>
<td>2.00 1.23–3.24 18</td>
<td>2.00 1.23–3.24 18</td>
<td>Age, race, education, BMI, alcohol, exercise, aspirin, CS</td>
<td>Henley et al., 2005 (CPS II)</td>
</tr>
<tr>
<td>1964–1967; 2001</td>
<td>Swedish snus</td>
<td>0.80 0.58–1.11 1,999</td>
<td>2.00 1.23–3.24 18</td>
<td>Age, race, poverty index ratio, CS, females</td>
<td>Boffetta et al., 2005</td>
</tr>
<tr>
<td>1978–1992; 2004</td>
<td>Swedish snus</td>
<td>7.20 6.00–8.50 2,062</td>
<td>0.80 0.40–1.30 34,818</td>
<td>Age, race, poverty index ratio, CS, females</td>
<td>Luo et al., 2007</td>
</tr>
</tbody>
</table>
### Appendix D. Summary of Health Effects for Cigarette Smokers and Smokeless Tobacco Users (continued)

<table>
<thead>
<tr>
<th>Enrollment Dates; Follow-up Dates</th>
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<th>Controlled Factors</th>
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<td>HR, OR, or RR 95% CI</td>
<td>No. of Cigarette Smokers</td>
<td>HR, OR, or RR 95% CI</td>
<td>No. of Smokeless Tobacco Users</td>
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<td>Lung Cancer (cont.)</td>
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<tr>
<td>1959; 1965</td>
<td>Cigarette</td>
<td>11.9</td>
<td>9.5–14.9</td>
<td>1.035</td>
<td>Age, CS, males</td>
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<tr>
<td>1959; 1965</td>
<td>Cigarette</td>
<td>2.7</td>
<td>2.1–3.5</td>
<td>1.57</td>
<td>Age, CS, females</td>
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<tr>
<td>1982; 1988</td>
<td>Cigarette</td>
<td>23.2</td>
<td>19.3–27.9</td>
<td>1.781</td>
<td>Age, CS, males</td>
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<tr>
<td>1982; 1988</td>
<td>Cigarette</td>
<td>12.8</td>
<td>11.3–14.7</td>
<td>1.014</td>
<td>Age, CS, females</td>
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<tr>
<td>COPD</td>
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<td>1959; 1965</td>
<td>Snuff or chewing tobacco</td>
<td>1.86</td>
<td>1.12–3.06</td>
<td>25</td>
<td>Age, race, education, BMI, alcohol, exercise, aspirin, CS</td>
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<td>1982; 2000</td>
<td>Snuff or chewing tobacco</td>
<td>1.28</td>
<td>0.71–2.32</td>
<td>12</td>
<td>Age, race, education, BMI, alcohol, exercise, aspirin, CS</td>
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<td>1959; 1965</td>
<td>Cigarette</td>
<td>9.3</td>
<td>6.6–12.9</td>
<td>284</td>
<td>Age, CS, males</td>
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<tr>
<td>1959; 1965</td>
<td>Cigarette</td>
<td>6.7</td>
<td>4.4–10.2</td>
<td>56</td>
<td>Age, CS, females</td>
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<tr>
<td>1982; 1988</td>
<td>Cigarette</td>
<td>11.7</td>
<td>9.1–15.0</td>
<td>422</td>
<td>Age, CS, males</td>
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<tr>
<td>1982; 1988</td>
<td>Cigarette</td>
<td>12.8</td>
<td>10.4–15.9</td>
<td>303</td>
<td>Age, CS, females</td>
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Appendix D. Summary of Health Effects for Cigarette Smokers and Smokeless Tobacco Users (continued)

<table>
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<tr>
<th>Enrollment Dates; Follow-up Dates</th>
<th>Tobacco Category</th>
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<th>Smokeless Tobacco Users</th>
<th>Controlled Factors</th>
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<tr>
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<tr>
<td>All Cardiovascular Disease</td>
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</tr>
<tr>
<td>1959; 1982</td>
<td>Snuff or chewing tobacco</td>
<td>1.18</td>
<td>1.11–1.26</td>
<td>1,399</td>
<td>Age, race, education, BMI, alcohol, exercise, aspirin, CS</td>
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<tr>
<td>1982; 2000</td>
<td>Snuff or chewing tobacco</td>
<td>1.23</td>
<td>1.09–1.39</td>
<td>278</td>
<td>Age, race, education, BMI, alcohol, exercise, aspirin, CS</td>
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<tr>
<td>1971–1974; 1988</td>
<td>Swedish snus</td>
<td>1.90</td>
<td>1.7–2.2</td>
<td>13,518</td>
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<td>1971–1974; 1988</td>
<td>Swedish snus</td>
<td>3.20</td>
<td>2.6–3.9</td>
<td>5,785</td>
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<td>1985–2000</td>
<td>Swedish snus</td>
<td>1.86</td>
<td>1.13–3.05</td>
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<td>1.16</td>
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<td>1992–1994</td>
<td>Swedish snus</td>
<td>2.8</td>
<td>2.3–3.4</td>
<td>616</td>
<td>0.73</td>
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<td>1987; 2004</td>
<td>Swedish snus</td>
<td>0.99</td>
<td>0.90–1.10</td>
<td>453</td>
<td>Age, BMI, place of residence, CS</td>
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<td>1989–1991</td>
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<td>1.87</td>
<td>1.40–2.48</td>
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<td>1994</td>
<td>Swedish snus</td>
<td>3.65</td>
<td>2.67–4.99</td>
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<tr>
<td>1985–1999</td>
<td>Swedish snus</td>
<td>2.6</td>
<td>1.91–3.54</td>
<td>396</td>
<td>0.82</td>
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## Differentiating the Health Risks of Categories of Tobacco Products

### Enrollment Dates; Follow-up Dates

<table>
<thead>
<tr>
<th>Tobacco Category</th>
<th>Cigarette Smokers</th>
<th>Smokeless Tobacco Users</th>
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<tbody>
<tr>
<td></td>
<td>HR, OR, or RR</td>
<td>95% CI</td>
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</tbody>
</table>

### Controlled Factors

- Age, race
- Education
- BMI
- Alcohol
- Exercise
- Aspirin
- Occupation

### Reference

- Winn et al., 1981
- Winn et al., 1984
- Mashberg et al., 1993
- Henley et al., 2005 (CPS I)
- Henley et al., 2005 (CPS II)
- Sterling et al., 1992
- Schildt et al., 1998
- Rosenquist et al., 2005
- Boffetta et al., 2005
- Luo et al., 2007
- Brinton et al., 1984
- Brinton et al., 1984
### Oral and Pharyngeal Cancer

<table>
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<tr>
<th>Enrollment Dates; Follow-up Dates</th>
<th>Smokeless Tobacco Category</th>
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<th>Smokeless Tobacco Users</th>
<th>Controlled Factors</th>
<th>Reference</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>HR, OR, or RR, 95% CI</td>
<td>No. of Cigarette Smokers</td>
<td>HR, OR, or RR, 95% CI</td>
<td>No. of Smokeless Tobacco Users</td>
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<tr>
<td>1984–1985</td>
<td>Snuff</td>
<td>3.0, 2.0–4.5</td>
<td>527</td>
<td>6.2, 1.9–19.8</td>
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<td>1969–1971</td>
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<td>486</td>
<td>3.88, NS</td>
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<td>1953–1957; 1980</td>
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<td>3.0, 2.0–4.5</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>1953–1957; 1980</td>
<td>Snuff or chewing tobacco</td>
<td>8.7, 4.1–18.3</td>
<td>NS</td>
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<tr>
<td>1977–1990</td>
<td>Chewing tobacco</td>
<td>3.25, 2.44–4.33</td>
<td>1,343</td>
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<td>1977–1990</td>
<td>Snuff</td>
<td>4.34, 3.22–5.85</td>
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<td>34.5, 8.49–140.1</td>
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<td>1985–1987</td>
<td>Snuff or chewing tobacco</td>
<td>3.4, 1.0–10.9</td>
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## Appendix D. Summary of Health Effects for Cigarette Smokers and Smokeless Tobacco Users (continued)

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<th>Smokeless Tobacco Users</th>
<th>Controlled Factors</th>
<th>Reference</th>
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<td></td>
<td>HR, OR, or RR</td>
<td>No. of Cigarette Smokers</td>
<td>HR, OR, or RR</td>
<td>No. of Smokeless Tobacco Users</td>
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<td>1959–1972; 1982</td>
<td>Cigarette</td>
<td>1.7</td>
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<td>1.11–1.23</td>
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<tr>
<td>1982–2000</td>
<td>Snuff or chewing tobacco</td>
<td>1.18</td>
<td>1.08–1.29</td>
<td>567</td>
<td>Age, race, education, BMI, alcohol, exercise, aspirin, CS</td>
</tr>
<tr>
<td>1971–1974; 1988</td>
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<td><strong>2.20</strong></td>
<td>2.0–2.4</td>
<td><strong>1.40</strong></td>
<td>1.3–1.8</td>
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<td>4.4–9.5</td>
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<td>0.5–1.9</td>
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<td>1982</td>
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<td>11.6</td>
<td>8.8–15.4</td>
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<td>7.3</td>
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## Appendix D. Summary of Health Effects for Cigarette Smokers and Smokeless Tobacco Users (continued)

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### Appendix D. Summary of Health Effects for Cigarette Smokers and Smokeless Tobacco Users (continued)

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<th>Enrollment Dates; Follow-up Dates</th>
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## Appendix D. Summary of Health Effects for Cigarette Smokers and Smokeless Tobacco Users (continued)

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<th>Enrollment Dates; Follow-up Dates</th>
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### Appendix D. Summary of Health Effects for Cigarette Smokers and Smokeless Tobacco Users (continued)

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## Appendix D. Summary of Health Effects for Cigarette Smokers and Smokeless Tobacco Users (continued)

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BMI: body mass index; CI: confidence interval; CPS: Cancer Prevention Study; COPD: chronic obstructive pulmonary disease; CS: cigarette smoking; HR: hazard ratio; NS: not stated; OR: odds ratio; RR: risk ratio; SES: socioeconomic status.
APPENDIX E. GLOSSARY, ACRONYMS, AND ABBREVIATIONS

GLOSSARY

Animal model of disease
A test in animals developed to mimic human disease pathogenesis after exposure to toxins.

Areca nut
The seed of the fruit of the Oriental palm Areca catechu. It is a basic ingredient in various widely used chewed products. Thin slices of the nut, either natural or processed, may be mixed with different substances including slaked lime (calcium hydroxide) and spices and flavorings such as cardamom, coconut, and saffron. Most commonly, slices are mixed with tobacco products or wrapped in the leaf of the Piper betel plant.

BALB/c mouse
McDowell started inbreeding the BALB/c mouse in 1923. It is a popular strain and is used in many different research disciplines, but it is used most often in the production of monoclonal antibodies. Classified as an inbred strain from the production of 20 or more successive brother-sister matings, the BALB/c mouse is albino and small.

Betel quid
Consists of a betel leaf wrapped around areca nut and slaked lime; added spices and sweeteners (e.g., menthol, camphor, sugar, rosewater, aniseed, mint, and other spices) depend on regional differences. Tobacco may or may not be added to the quid (National Cancer Institute & Centers for Disease Control and Prevention, 2002).

Bidi cigarette
An unfiltered cigarette wrapped in temburni tree leaf (Mudur, 2001).

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

Biomarker of effect
A measured effect including an early subclinical biological effect; alteration in morphology, structure, or function; or clinical symptom 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)**
Successful smoking cessation is defined as abstinence from smoking for at least 6 months (Centers for Disease Control and Prevention, 2005b).

**Chewing tobacco**
A form of smokeless tobacco (ST) that is chewed, not smoked. Chewing tobacco is one of two main forms of ST used in the US.

**Chronic disease**
In humans, a disease that develops during a period of years to decades. In animals (rodents), a disease that develops for a number of months to 1–2 years.

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

**Cigarette**
A rod of tobacco wrapped in paper.

**Clinical studies**
Studies conducted with human subjects.

**Comparative risk assessment**
A risk assessment in which risk associated with one set of exposures (e.g., from an ST product) is compared with risk associated with a different set of exposures (e.g., from conventional cigarettes).
Continuing smokers
Individuals who cannot or will not stop smoking.

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 plasma, saliva, and urine of smokers and used as a biomarker of exposure to cigarette smoke.

Crohn’s disease
A chronic inflammatory bowel disease that can affect any part of the gastrointestinal tract, from the mouth to the anus.

Cytotoxicity tests
Assays that measure the ability of a chemical or chemical mixture to damage or kill cells.

Dose
The amount of substance absorbed by an organism.

Dose-response relationship
A relationship between the amount of a substance in environmental media that comes in contact with an organism (human, animal) 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.

Dry snuff
Finely cut ground or powdered ST that is intended to be inhaled through the nose; it does not include moist snuff.

Dual users
Individuals who routinely use both cigarettes and ST products.

Environmental tobacco smoke
Smoke consisting of aged, diluted sidestream smoke and exhaled mainstream smoke.
**Epidemiological studies**
Investigations conducted with human populations to evaluate whether a causal relationship exists between exposure to a substance and adverse health effects. These studies measure the risk of illness or death in an exposed population compared with that risk in an identical (e.g., same age, sex, race, social status) unexposed population.

**Exposure assessment**
One of two critical components of a risk assessment. Its purpose is to evaluate all relevant data on the exposure of interest.

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

**Frameshift mutation**
A genetic mutation caused by insertion or deletion of nucleotides, the result being a different DNA sequence. The insertion or deletion can disrupt the reading frame (grouping of codons), which leads to a completely different translation from the original DNA sequence.

**Genotoxicity tests**
Assays of the propensity of a substance to damage the genetic material (DNA) of test cells; a more general term that encompasses mutagenicity tests.

**Gutkha**
ST product made with areca nut, tobacco, slaked lime, saffron, and flavorings.

**Hard snuff**
A US ST product that is a compressed tobacco lozenge.

**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.
Hazard ratio (HR)
The ratio between the predicted hazard for a member of one group and that for a member of the other group, with everything else held constant. Useful when risk is not constant with respect to time. Typically used in the context of survival over time.

International smokeless tobacco products
ST products other than those used in the US and Sweden.

In vitro test
An assay that is performed with single-cell organisms, such as bacteria and yeast, or single cells or organs derived from animals or humans.

In vivo test
An assay that is performed with a whole, living animal or human.

Lung cancer
Cancer that forms in tissues of the lung, usually in the cells lining the air passages. The are four main histological types: squamous cell carcinomas, adenocarcinomas, small cell carcinomas, and large cell carcinomas.

Maillard reaction
A non-enzymatic browning reaction caused by the condensation of an amino group and a reducing carbohydrate.

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

Mishri
ST product made with toasted powdered tobacco and used as a dentifrice.

Moist snuff
Finely cut, ground or powdered ST that is designed to be placed or dipped in the mouth; it does not include dry snuff.

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

Mortality
Death.
Nicotine
A cyclic tertiary amine composed of a pyridine ring and a pyrrolidine ring. It is a colorless water-soluble fluid alkaloid derived from plants of the genus *Nicotiana*. The substance acts as a stimulant in mammals and is one of the main factors responsible for dependence-inducing properties of tobacco smoke.

Nicotine dependence
A dependence characterized by both tolerance and withdrawal symptoms in relation to nicotine use.

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

Odds ratio (OR)
Odds of an event happening in the experimental group expressed as a proportion of the odds of an event happening in the control group.

Pack-year
Unit of measure of smoking exposure. One pack-year represents use of 20 cigarettes/day (one pack) for 1 year by 1 person.

Pan masala
A commercially manufactured preparation of betel quid. *Pan* refers to freshly prepared versions of betel quid (National Cancer Institute & Centers for Disease Control and Prevention, 2002).

Pharmacokinetics
The pattern of absorption, distribution, and excretion of a drug over time.

Plug tobacco
A compressed form of loose-leaf chewing tobacco that is molded and pressed into a flat bar.

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

Power
The probability that a statistical test will reject the null hypothesis. Statistical power depends on the effect size and sensitivity of the data.
Preclinical studies
Studies generally conducted before studies in humans. For PRRTPs, preclinical studies include product characterization, chemistry (smoke or product), genotoxicity and cytogenicity assays, and animal studies.

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

Prospective studies
Studies designed to assess outcomes in human subjects, such as development of a disease, during the study period.

Reference smokeless tobacco product
ST product developed for research purposes. The 3 types of reference ST product are dry snuff, moist snuff, and loose-leaf chewing tobacco. Reference ST products have defined compositions and are stored under specific conditions (North Carolina Agricultural Research Service, 2005).

Relative risk (RR)
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
Probability that harm (e.g., smoking-related disease) will occur.

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

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

Shammah
Mixture of powdered tobacco, lime, ash, black pepper, oils, and flavorings.

Small for gestational age
Having a birth weight greater than 2 standard deviations below the mean birth weight for gestational age according to sex.
Smoke
Usually, a suspension of fine particles in air that scatters light and is physically visible. Cigarette smoke contains many chemical substances in both gas and liquid states. These substances are suspended in a dynamic aerosol that is created by incomplete combustion and that changes both physically and chemically over time.

Smoke chemistry studies
Investigations of the general composition of smoke or the yield of specific smoke constituents.

Smokeless tobacco products
Products that do not require combustion or production of tobacco aerosol (smoke) by other means at the time of use (World Health Organization Scientific Advisory Committee on Tobacco Products Regulation, 2003).

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

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).

Snuff
Several types of ST, including dry snuff and moist snuff. Traditionally, snuff referred to a finely ground tobacco that was to be inhaled through the nose, not smoked.

Snus
A moist to semi-moist, ground, oral tobacco product that is placed between the upper lip and gum.

Tobacco lozenge
See Hard snuff.

Tobacco-specific nitrosamines
N-Nitroso compounds formed by nitrosation of the major tobacco alkaloid nicotine and a suspected human carcinogen.
Total particulate matter
Particles in smoke, larger than 1 µm in diameter, that are trapped on a Cambridge filter as smoke passes through the filter; usually obtained from mainstream smoke.

Toxicant
A poisonous substance.

Traditional US smokeless tobacco products
US moist snuff products other than US “snus” products that have recently been developed.

Twist tobacco
Loose-leaf tobacco that is twisted into pliable, rope-like strands that are dried.

Ulcerative colitis
A disease that causes inflammation and sores (ulcers) in the lining of the colon and rectum.

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

Zarda
Mixture of tobacco, lime, spices, vegetable dyes, and areca nut.
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS</td>
<td>American Cancer Society</td>
</tr>
<tr>
<td>ALS</td>
<td>Amyotrophic lateral sclerosis</td>
</tr>
<tr>
<td>B[a]P</td>
<td>Benzo[a]pyrene</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
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<td>CORESTA</td>
<td>Centre de Coopération pour les Recherches</td>
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<tr>
<td>COX-2</td>
<td>Cyclooxygenase-2</td>
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<tr>
<td>CPS I and II</td>
<td>Cancer Prevention Study I and II</td>
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<tr>
<td>CPS-TUS</td>
<td>Current Population Survey-Tobacco Use Supplements</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>DRR</td>
<td>Death rate ratio</td>
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<tr>
<td>DTR</td>
<td>Differentiating Tobacco Risks</td>
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<tr>
<td>FTC</td>
<td>Federal Trade Commission</td>
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<tr>
<td>HDL-C</td>
<td>High-density lipoprotein cholesterol</td>
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<td>HPB</td>
<td>4-Hydroxy-1-(3-pyridyl)-1-butanone</td>
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<td>Hazard ratio</td>
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<tr>
<td>HS9</td>
<td>Human-derived liver enzymes</td>
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<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
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<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
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<tr>
<td>IFN-γ</td>
<td>Interferon-γ</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>IMT</td>
<td>Intima-media thickness</td>
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<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
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<tr>
<td>LBW</td>
<td>Low birth weight</td>
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<tr>
<td>LC</td>
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<td>LDH</td>
<td>Lactate dehydrogenase</td>
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<td>LPS</td>
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<td>LSRO</td>
<td>Life Sciences Research Office</td>
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<tr>
<td>NAB</td>
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<td>NAHSIT</td>
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<tr>
<td>NAP</td>
<td>Nitrosation assay procedure</td>
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<td>NAT</td>
<td>N'-Nitrosoanatabine</td>
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<tr>
<td>NDMA</td>
<td>N-Nitrosodimethylamine</td>
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<td>NF-κB</td>
<td>Nuclear factor-κB</td>
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<td>National Health Interview Survey</td>
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<td>Full Form</td>
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<td>NNAL</td>
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<tr>
<td>NNN</td>
<td>$N'$-Nitrosonornicotine</td>
</tr>
<tr>
<td>NRT</td>
<td>Nicotine replacement therapy</td>
</tr>
<tr>
<td>OOSCC</td>
<td>Oropharyngeal squamous cell carcinoma</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PAHs</td>
<td>Polycyclic aromatic hydrocarbons</td>
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<tr>
<td>PRRTP</td>
<td>Potential reduced-risk tobacco product</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>RRRP</td>
<td>Reduced-Risk Review Project</td>
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<tr>
<td>RS9</td>
<td>Rat or hamster liver enzymes</td>
</tr>
<tr>
<td>SALT</td>
<td>Screening across lifespan twin study (Sweden)</td>
</tr>
<tr>
<td>SIDS</td>
<td>Sudden infant death syndrome</td>
</tr>
<tr>
<td>ST</td>
<td>Smokeless tobacco</td>
</tr>
<tr>
<td>STE</td>
<td>Smokeless tobacco extract</td>
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<tr>
<td>TAPS</td>
<td>Teenage Attitudes and Practices Survey</td>
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<tr>
<td>TNF-(\alpha)</td>
<td>Tumor necrosis factor-(\alpha)</td>
</tr>
<tr>
<td>TSNA</td>
<td>Tobacco-specific nitrosamine</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<td>YCYL</td>
<td>Your Country and Your Life</td>
</tr>
</tbody>
</table>
INDEX

Animal model of disease, 48, 124, 261
   BALB/c mouse, 47, 51, 260

Biomarker, 4-5, 53-54, 61, 138, 154-155, 179, 261
   of effect, 5, 53-54, 61, 64, 148, 154, 157, 169, 173-174, 180, 260
   of exposure, 4-5, 53-54, 61, 138, 143, 154, 169, 173-174, 179, 261

Cancer, 2, 5-6, 15, 20, 24-25, 39, 50-51, 100
   Bladder, 6, 106-108
   Esophageal, 6, 101, 131
   Gastric, 6, 50, 100, 102
   Lung, 5, 15, 21, 84, 86-87, 265
   Pancreatic, 6, 104, 108
   Ulcerative colitis, 110, 268

Diseases
   Cancer, 2, 6-7, 15, 20, 24-25, 39, 50-51, 100
   Bladder, 6, 106, 108
   Esophageal, 101, 131
   Gastric, 50, 100, 102
   Lung, 5, 15, 21, 84, 86-87, 264
   Pancreatic, 104, 108
   Cardiovascular disease, 61, 90-91, 127, 148, 157, 261
   Chronic obstructive pulmonary disease, 2, 84, 88, 90, 138, 141, 146, 156, 160, 170, 261
   Crohn's disease, 110, 262
   Ulcerative colitis, 110, 268

Cessation (smoking), 75, 77, 81, 100, 139, 141, 162, 172, 180-181, 261

Cigarette, 2, 15, 21, 75, 77, 82, 86-87, 90-91, 95, 99, 101-102, 105, 108, 135, 137-163, 249, 261
   Bidi cigarette, 125, 127, 131, 260

Cytotoxicity tests, 11, 24, 41, 43, 45, 121, 153, 168, 173, 177, 262

Dose, 4, 24, 44-45, 49, 55, 57, 68, 70, 111, 127, 133-161, 172, 177, 179, 262
   Dose-response, 41, 86, 90, 94, 100, 104, 127

Dual users, 15, 82, 140, 143, 262

Environmental tobacco smoke, 5, 15, 55, 113, 138, 155, 162

Exposure assessment, 21, 24-25, 143, 146, 148, 151, 263

Ex-smokers, 84, 90, 157, 263

Frameshift mutation, 41, 121, 143, 168, 263

Genotoxicity tests, 3, 24, 41, 47, 121, 142, 168, 177

Harm, 1, 14, 21-25, 138-139, 173, 177, 263
   Harm reduction, 1-2, 139, 152, 263

International ST products
   Gutkha, 11, 117-122, 166, 168, 263
   Mishri, 11, 117, 121-123, 133, 160, 168, 264
Pan masala, 117, 123, 125, 168, 265
Shammah, 11, 117, 121, 127, 168, 171, 266
Zarda, 11, 117, 121-122, 166, 168, 268

In vitro test, 11, 24, 41-52, 123, 138, 153, 164, 168, 177, 264

In vivo test, 41, 49-51, 123-124, 177, 264

Morbidity, 139-140, 264

Nicotine, 4, 32, 55, 70, 73, 112, 134, 265

Nicotine Replacement Therapy, 81, 112, 265

Pack-year, 86, 265
Pharmacokinetics, 70, 132, 265
Power, 265

Product characteristics, 3, 9, 121, 153, 177, 179, 266

Reference smokeless tobacco product, 31-33, 266

Risk
Comparative risk assessment, 7-9, 22, 137-174, 261
Risk assessment, 7-9, 21-27, 138-139, 163-174, 266
Risk reduction, 7-9, 15, 137-138, 140, 148, 155, 157, 159-160, 164, 174, 180, 266

Smokeless tobacco products, 1, 23, 30, 33-34, 36, 70, 84, 86, 94, 98, 100, 104, 116-122, 173-174, 267

Snuff
Dry, 9, 17-19, 22, 26, 29-30, 33, 36, 41, 47, 94, 123, 139, 164, 166, 168, 174, 176, 267
Hard, 9-11, 17, 30, 34, 36, 57, 59, 139, 154, 165-166, 169, 179, 263
Tobacco lozenge, 18, 30, 73, 81, 139, 154, 166, 267
Moist, 9-10, 17, 19, 22, 26, 29-31, 33, 36, 41, 47, 168, 177


Studies
Clinical, 4-5, 53, 61, 154, 169, 179, 261
Preclinical, 3-4, 41-52, 119, 152, 177, 179, 266

Tobacco
Plug, 9, 18, 30, 61, 165, 173
Twist, 9, 18, 30-31, 268

Tobacco-specific nitrosamines, 32, 34-35, 53, 56, 120, 138, 166, 177, 267

Weight of evidence, 2, 21, 25, 84, 98, 138-139, 152, 164, 174, 268
LSRO Report on

DIFFERENTIATING THE HEALTH RISKS OF CATEGORIES OF TOBACCO PRODUCTS

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