7.5.6-1: INITIAL - HEALTH RISKS - LITERATURE SUMMARY

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

List of Abbreviations

4-NQO	4-nitroquinoline N-oxide
ACE	angiotensin-converting enzyme
ACS	American Cancer Society
AMI	acute myocardial infarction
ARIC	Atherosclerosis Risk in Communities
CA	California
CHD	coronary heart disease
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CPS-I	Cancer Prevention Study I
CPS-II	Cancer Prevention Study II
CSS	Cancer Surveillance System
CV	cardiovascular
CVD	cardiovascular disease
DCM	dichloromethane
DMBA	7,12-dimethylbenz(a)anthracene
DMSO	dimethyl sulfoxide
EBV	Epstein-Barr virus
ETOH	ethyl alcohol
FDA	Food and Drug Administration
HCPC	hamster cheek pouch cell
HCPC-1	hamster oral keratinocytes
HGEC	human gingival epithelial cells
HGF	human gingival fibroblasts
HOK	human oral keratinocyte cells
HR	hazard ratio
HSV	herpes simplex virus
HUVEC	human umbilical vein endothelial cells
IARC	International Agency for Research on Cancer
ICD-9	International Classification of Disease ninth revision
IFN	interferon
IHD	ischemic heart disease
IL	interleukin
LAK	lymphokine-activated killer activity
Lb	lameller bodies
LPS	lipopolysaccharide
LDH	lactate dehydrogenase
MC	20-methylcholanthrene
MI	myocardial infarction
mRNA	messenger RNA
MST	moist smokeless tobacco
MRTP	modified risk tobacco product
MRTPA	Modified Risk Tobacco Product Application
N/A	not applicable
NCSU	North Carolina State University
NEP	neutral endopeptidase
NHANES I	First National Health and Nutrition Examination Survey
NHEFS	NHANES I Epidemiologic Followup Study
NHIS	National Health Interview Survey
NHL	non-Hodgkin lymphoma

NK	natural killer
NLMS	National Longitudinal Mortality Study
NMFS	National Morality Followback Survey
NNK	4-(N- methyl-N-nitrosamine)-1-3-pyridinyl)-1-butanone
NNN	N-nitrosonornicotine
NO	nitric oxide
N/P	not presented
OR	odds ratio
PBMC	peripheral blood mononuclear cells
RCC	renal cell carcinoma
RE RR/OR	random effects relative risk/odds ratio
ROS	reactive oxygen species
RR	relative risk
SCE	sister chromatid exchange
ST	smokeless tobacco
STE	smokeless tobacco extract
STS	soft tissue sarcoma
TEM	transmission electron microscopy
TPA	12-O-tetradecanoyl phorbol-13-acetate;
TPM	total particulate matter;
TSNA	tobacco-specific nitrosamine
UK	University of Kentucky
U.S.	United States
USSTC	United States Smokeless Tobacco Company LLC
VIP	vasoactive intestinal peptide

7.5.6-1. LITERATURE SUMMARIZING THE HEALTH RISKS OF SMOKELESS TOBACCO

Section 7.5.6 summarizes the published scientific literature related to the health risks of using smokeless tobacco (ST) typically marketed in the United States (U.S.).

Various regulatory and scientific agencies have established that ST product use, including moist smokeless tobacco (MST), is not without potential risk to health. Authoritative bodies have previously prepared comprehensive summaries of the health risk profile of ST (International Agency on Research for Cancer (IARC), 2007; O'Berst & McIntyre, 1953; U.S. Dept. Health Human Services, 1986).

This review is not intended to be a recapitulation of all ST research that has been published (both international and domestic), nor is it intended to include or link all traditional elements of risk assessment (e.g., epidemiology observations/plausibility studies/mechanistic explanations). Rather, we provide published data that are sufficient to establish a baseline risk profile for ST use to facilitate a review of the comparative risk of the candidate modified risk tobacco product (MRTPs), Copenhagen® Snuff Fine Cut, relative to cigarette smoking.

We believe this summary of the published scientific literature addresses the following aspects of Section VI (A) (1) of the Food and Drug Administration (FDA) 2012 Draft Guidance for Modified Risk Tobacco Product Applications (MRTPA) as it relates to the candidate MRTP:

- the health risks associated with initiating use of the candidate MRTP as compared with never using tobacco products;
- the health risks associated with use of the candidate MRTP as compared with the use of other tobacco products on the market, including tobacco products within the same class of products;
- the changes in health risks to users who switch from using another tobacco product to using the candidate MRTP, including tobacco products within the same class of products;
- the health risks associated with switching to the candidate MRTP as compared with quitting the use of tobacco products;
- the health risks associated with using the candidate MRTP in conjunction with other tobacco products;
- the health risks associated with switching to the candidate MRTP as compared with using an FDA-approved tobacco-cessation medication.

Altria Client Services LLC conducted a comprehensive literature search to identify published information related to the health risks of ST products. A description of our literature search and review process is presented in Section 7.5.1 of this MRTPA. This review is limited to studies of ST products used in the U.S. that were published between through December 2014. From this search, a total of 6,742 publications were identified, and, after a comprehensive

and in depth critical review, 537 were determined to be in scope. These publications were further reviewed to assess which specific category(ies) in the MRTPA Draft Guidance each article addressed. Reports published after the date of our last search were included in this review when deemed to be significant contributions to this body of research. An updated literature review was conducted to bridge the original review to February 2017, and updated findings informing health risk of ST are presented in Section 7.5.6-2.

As stated by the Institute of Medicine Committee on Scientific Standards on Modified Risk Tobacco Products (Institute of Medicine, 2012), observational epidemiologic studies play a critical and central role in the evaluation of MRTPs. The large volume and quality of publications conducted with ST products manufactured in the U.S. lead us to conclude that the current literature characterizing the health risks of users is sufficiently robust to address the primary issues raised by the FDA in the MRTPA Draft Guidance. We are aware of the availability of many other scientific investigations using various international ST products are not included in our literature search. Although these studies are informative, we consider the subset of U.S.-specific data to be adequate to provide a realistic representation of health risks presented with U.S. products.

MST products comprise a significant proportion of the ST products in the U.S. market, and have done so for many years. The candidate MRTP was manufactured by United States Smokeless Tobacco Company, LLC (USSTC) and marketed during the period when major epidemiology studies were being conducted. The health risks of the candidate product can be sufficiently assessed using existing epidemiology data for U.S. smokeless tobacco products. Our reasoning, in brief, is as follows. First, MST products were the predominant form of ST used during the time period of the major U.S. epidemiology studies. Second, the candidate product and other USSTC MST products occupied sizeable market shares among the MST products used during the time period of these studies, which means that the epidemiological data reasonably reflects the health effects of the candidate product and other USSTC products. Third, the production process for USSTC MST products, including for the candidate product, was essentially unchanged over the time period of these studies, other than refinements, such as improved process control and reduced TSNA formation (Section 7.5.6-1.1.1).

We agree that the best way to reduce the harm from tobacco products is for consumers to quit using them entirely. However, for those who continue to use tobacco products, making available tobacco products that are acceptable to consumers and proven to be lower on the continuum of risk could reduce tobacco-related morbidity and mortality. For these tobacco products, providing accurate information on the health risks could complement cessation and prevention strategies for consumers. A growing body of evidence suggests that some tobacco products are lower risk than others (Zeller, 2009).

On the basis of the current published literature with U.S. ST products, we make the following observations:

- ST and MST are not without some risk for adverse health outcomes. ST products carry government-mandated health risk warnings informing consumers regarding these risks.

- There is compelling evidence that the potential for adverse health outcomes with ST use is substantially lower than that associated with cigarette smoking.

In the following sections, we address the topics identified in the MRTPA Draft Guidance and provide our perspective on the many scientific studies conducted with ST (Section 7.5.6-1.2 through Section 7.5.6-1.7) to substantiate these conclusions. We also include some considerations about the use of published U.S. literature when assessing the health risks of the candidate MRTP (Section 7.5.6-1.1), including issues related to product specificity, epidemiology associations, and limitation to nonclinical studies.

7.5.6-1.1. Some Considerations About the Use of Published U.S. Literature Results to Assess the Health Risks of the Candidate MRTP

The literature used to prepare our summary comprises many decades of studies and a vast array of investigative approaches. Given the diversity of scientific studies and methodologies used to produce data assessing the health risks associated with ST use, we describe in this section some of the strengths and limitations of using such data.

7.5.6-1.1.1. Product Specificity

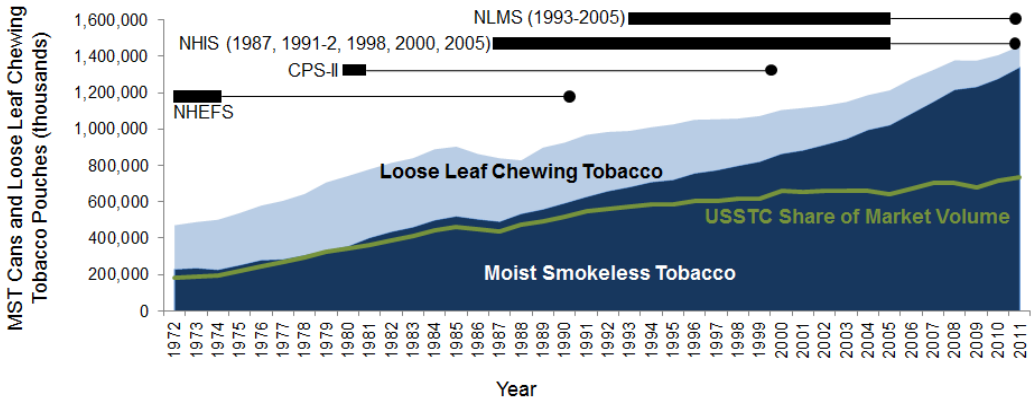
We noted from our review of the literature that many publications, particularly epidemiology and older nonclinical studies, generally lack reference to the specific ST products used. Additionally, some studies may refer to chewing tobacco, chew, snuff, moist snuff, or ST in their exposure assessments. Only a few studies included information about the specific type of ST product(s) used by the study population. Additionally, most studies fail to adequately characterize and report specific brand of the ST products.

While there are clearly some voids in the current scientific data regarding product specificity, we consider these uncertainties to be minor limitations in using published data regarding ST products in the U.S. As described below, USSTC products were well represented in the epidemiology data sets reviewed. USSTC products were approximately 50 percent of the ST market during the periods when many large U.S. epidemiology studies were conducted. USSTC products have been the predominant MST in the U.S. market for several years, and the manufacturing process has remained stable.

7.5.6-1.1.1.1. MST as the Predominant Form of ST Use

MST products are the predominant form of ST use. Figure 7.5.6-1-1 shows the estimated unit volume of MST and loose leaf chewing tobacco between 1972 and 2011. In 1972, MST products already accounted for nearly half of the ST category. Since then, the market share of MST products has steadily grown, accounting for half the category by the early 1980s, and 75% by the late 1980s. MST's rise to dominate the ST category coincides with the timing of major epidemiology studies of ST products conducted in the U.S., as shown by Figure 7.5.6-1-1. Collectively, these epidemiology studies span 1972 to 2011, including the Linked Mortality Analysis. Over the time period studied, therefore, the health effects of using smokeless tobacco products, as reported by U.S. epidemiological data, were increasingly associated with the use of MST.

Figure 7.5.6-1-1: USSTC Volume within MST and Chewing Tobacco Category (1972-2011) and Study Periods of Prospective Studies of the Health Effects of ST Products

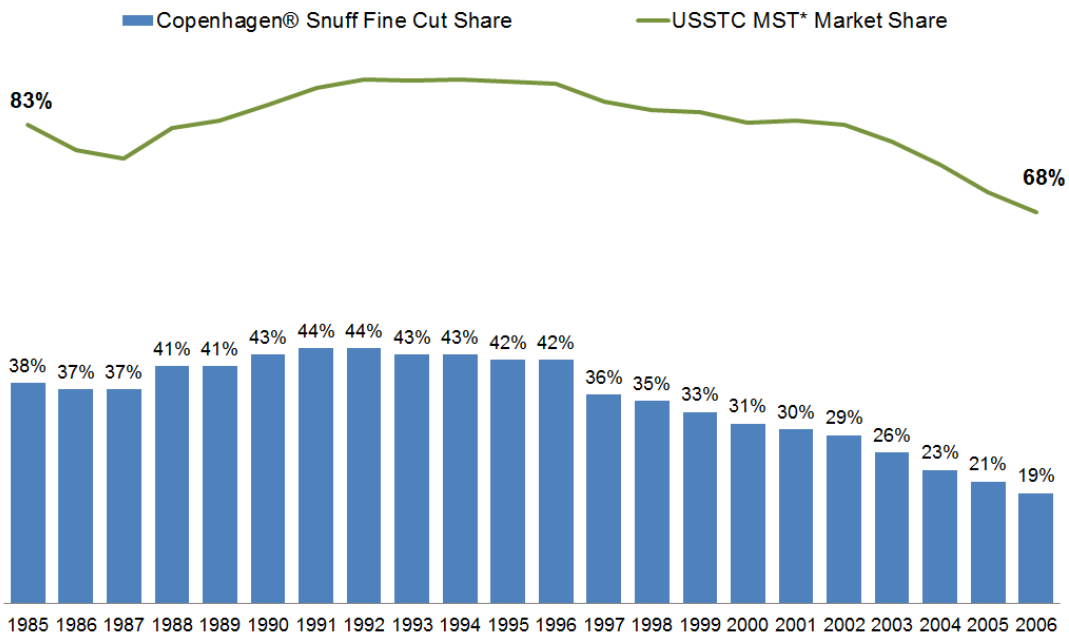


Source: Unit volume of moist smokeless tobacco and loose leaf chewing tobacco derived from Maxwell Reports 1972-2011 and study periods for prospective epidemiological studies of smokeless tobacco. USSTC volumes are based on USSTC historical shipment data and USSTC RAD SVT projected volume and share.
Black boxes represent the baseline periods for studies and black circles represent the end of studies follow-up period.

7.5.6-1.1.1.2. Candidate Product and USSTC Contribution to Total MST Volume

Figure 7.5.6-1-2 provides market share data for USSTC products and the candidate product through 2006, which encompasses the latest survey periods of the relevant epidemiological evidence. In 1985, for example, USSTC products comprised 83% of MST industry volume, and the candidate product accounted for 38% of the MST category. Over the time period of the epidemiology studies, the candidate product occupied a sizeable market share among the MST products. For this reason, and the reasons presented above, we conclude that the health risks of the candidate product can be sufficiently assessed using existing epidemiology data for U.S. smokeless tobacco products.

Figure 7.5.6-1-2: Contribution of Copenhagen Fine Cut Snuff to USSTC's Market Share, 1985-2016



Source: Copenhagen® Fine Cut Natural and USSTC Shipments 1985-2006 based on USSTC historical shipment data and USSTC RAD SVT projected volume and share. 1985-2000 share estimated using USSTC's growth rate during that period and total industry total volume as of 2002.
Yearly data shown until Feb. 2007 (grandfathered product date).

7.5.6-1.1.1.3. Tobacco and Manufacturing Considerations for the Candidate Product

From its earliest days, USSTC has sourced Dark Air-cured and Dark Fire-cured tobaccos for all of its MST products from growers in the same regions of Kentucky and Tennessee. USSTC has produced MST using a semi-solid fermentation process for almost 200 years. This trade secret process was invented in 1822 and can be documented based on historical product formulas for the candidate product to as early as 1905. In brief, this batch process includes addition of water, flavoring ingredients and salts, to a blend of dried, cut tobacco. A small portion of fermented tobacco, added to each batch, serves as a "starter" microbial culture. Fermentation is complete after ~42 days. A complete description of the manufacturing process for the candidate product appears in Section 3.1.

Across its product portfolio, USSTC MST products have contained the same tobacco types and have been manufactured using consistent processes over time, other than process improvements that lowered TSNA levels. The candidate product, in particular, used tobacco types, blends, and manufacturing processes comparable to all USSTC MST products, throughout the time period of the epidemiology studies. The candidate product, therefore, has a similar constituent profile, and health risks reasonably expected to be similar, compared to other USSTC MST products.

Although USSTC's moist snuff production process has remained almost unchanged, there have been process refinements. USSTC implemented these refinements to improve process control and reduce TSNA formation in its current MST products, relative to historical levels (Fisher et al., 2012) as shown in Figure 7.5.6-1-3. These efforts, which are described below, included: (1) improvements to farming practices; (2) manufacturing process enhancements; and (3) Vertically Integrated Process Management (VIPM).

First, manufacturers and academic researchers collaborated during the 2000s to develop low converter seed varieties to help reduce TSNA formation in tobacco leaf relative to earlier generations of seed varieties. Other efforts to reduce TSNAs at the farm level have focused on fertilizer application rates, barn structure, ventilation, and temperatures associated with the curing process. Since 2005, USSTC has included certain Good Agricultural Practices, including production, harvesting, and curing requirements in its contracts with farmers, to help reduce the formation of TSNAs on the farm.

Second, USSTC implemented procedures in the manufacturing process more than a decade ago that prevent TSNA formation from the time USSTC purchases tobacco leaf from farmers through the end of retail shelf life of the product. We focused on separating and characterizing bacteria essential for fermentation from other, nonessential bacteria which cause increases in levels of nitrite, pH, and moisture, which lead to increases in TSNA levels. To address this, we started to "select" fermentation seed with significantly reduced levels of nitrate reducing bacteria and saw further TSNA reductions in our products. And, we developed a cleaning and sanitation program that would further minimize levels of these nitrate-reducing bacteria on equipment between manufacturing runs.

Third, in 2001, we modified our manufacturing process through our VIPM program. Part of the VIPM program involved using production equipment that can be easily sanitized and systematically examining TSNA levels. By 2005, we had achieved our goal of preventing any increase in TSNA from the time we purchased the leaf from farmers through the end of the product's shelf life.

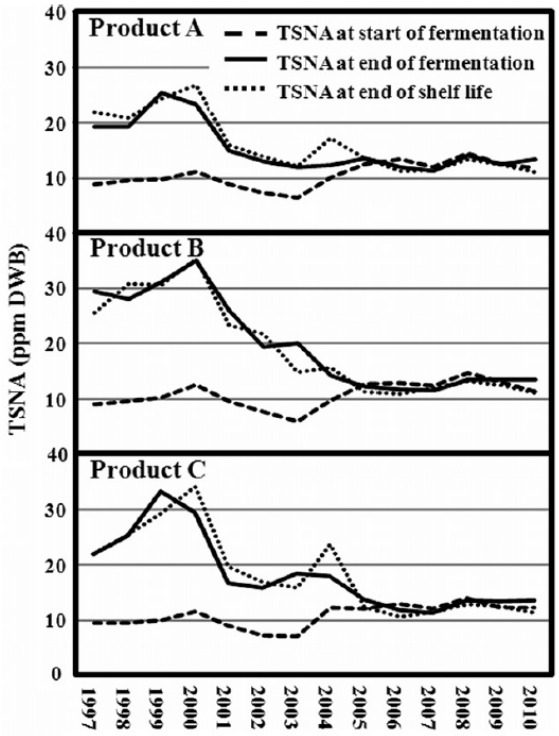
This comprehensive program from the mid-1970s until 2005 sought to address TSNA levels and formation in USSTC products. Through these efforts, a substantial reduction in TSNA levels in its products was observed, as USSTC increased its understanding and introduced mitigation efforts. While not completely eliminated, these efforts resulted in substantial TSNA reductions of up to 90% in USSTC's products by the late 1990s.

Djordjevic et al observed general TSNA reductions in the marketplace, reporting that, over the time period of 1980 to 1998, TSNA content was reduced by 70-90% for two "leading U.S. snuff brands."[1] We note that during this period the candidate product had approximately 40% of the MST market share. Since full implementation of process refinements by USSTC in 2005, TSNA levels have been consistently about 10 µg/g or lower (Fisher et al, 2012) and

[1]Values are drawn from the published literature for samples that could be reliably identified as USSTC products, (Borgerding et al., 2012; Brunnemann et al., 1982; Brunnemann et al., 2002; Djordjevic, V et al., 1989; Hoffmann et al., 1986; Hoffmann et al., 1987; Hoffmann et al., 1995; Hoffmann & Adams, 1981; Richter et al., 2008) as well as unpublished measurements of USSTC moist snuff products collected by ALCS between 2005 and 2008.

are consistently no higher at the end of product retail shelf life than those levels found in tobaccos purchased from farmers.

Figure 7.5.6-1-3: Average TSNA Levels (Ppm, Dry Weight Basis) in Commercial Moist Smokeless Tobacco Products a, B, and C 1997–2010



Source: (Fisher et al., 2012). (Figure 7).
The heavy dashed lines indicate TSNA levels in tobacco blend prior to fermentation with annual average levels ranging between 5.9 and 13.5 ppm across the three brands. The solid lines indicate annual average TSNA levels at the end of fermentation. The light dashed lines indicate TSNA levels after storage simulated shelf-life conditions. After 2005, fermentation process control was such that TSNA were not formed during the process and, therefore, TSNA levels are determined by levels in starting material.
Product A = Copenhagen Fine Cut; Product B = Copenhagen Long Cut; Product C = Skoal Fine Cut

Therefore, refinements to USSTC's production process since 2005 have not resulted in changes in product composition that challenge the relevance of the available epidemiology to current USSTC moist snuff products.

7.5.6-1.1.2. Epidemiology Associations

Observational epidemiologic studies, when available, will be one of the components in the sources of scientific evidence in the evaluation of MRTPs. As indicated in the Institute of Medicine report on Scientific Standards for Studies on Modified Risk Tobacco Products (Institute of Medicine, 2012), "Given the great diversity of health consequences of tobacco use (see Table 1-1 in Chapter 1), determining the contrasting potential effects of MRTPs on

disease outcomes and population health is a difficult matter. Long, intensive, and robust studies of actual health outcomes would be required to fully evaluate the net effects of MRTPs relative to conventional tobacco products."

Epidemiology studies measure characteristics of populations; however, such studies have practical constraints and can be subject to bias including selection bias and information (or recall bias) bias (Coggon, 2003; Craun, 2005). Selection bias occurs when the participants are not representative of the wider population. An example of this is when participants do not fully complete surveys or drop out of a study. Information bias occurs when participants misreport or misidentify exposure.

Recall bias is particularly relevant for epidemiology studies conducted with tobacco product exposure. The latency period for disease development generally associated with tobacco-related diseases requires that studies examine retrospective observations in the population over a long period, sometimes decades. In some cases, the investigators relied on participant self-reports or proxy reports by relatives or friends of study subjects. Such uncertainty about actual exposure conditions and recall bias can lead to considerable misclassification about the type and extent of tobacco product exposure.

Epidemiology studies conducted to measure the impact of tobacco product exposure require careful understanding of the strengths and limitations of the individual studies. Furthermore, the evidence from epidemiological studies should not only consider the temporal associations between exposure and disease, but should also consider the consistency across repeated observations, strength of association, dose response, and biological plausibility (Hill, 1965).

We noted in the literature that there is variation regarding the criteria used to define the unexposed comparator group used in risk estimate calculations. In some instances, surveys or researchers have used never-users of tobacco for comparison, while in other cases some researchers accept a previous low and/or non-routine level of tobacco use in the control group. Given the long-term health consequences of smoking, past smoking history, whatever the level, may confound some data.

With regard to our analysis of the ST health risk literature we present here, we have accepted the author's interpretation of the non-user comparative group and make no effort to eliminate studies which may be confounded. In our evidence tables, we generally report calculated risk estimates for the exposed group only. The risk estimate for the non-use group is, by default, 1.00, but is not always shown. The reported risk estimates are generally accompanied by some estimation of uncertainty (most often 95 percent confidence intervals). Wide confidence intervals indicate less certainty in the risk estimate, generally reflecting small numbers of cases within the dataset.

7.5.6-1.1.3. Nonclinical Endpoints

Nonclinical models can provide information about biological or chemical mechanisms and can be useful in addressing mode of action–related questions, including biological plausibility for a disease/exposure association. An array of *in vivo* and *in vitro* research techniques have been applied to study tobacco-related diseases, but not all results are directly relatable to human exposure conditions. Nonclinical research conducted to assess

mechanistic associations between MST and disease should be interpreted with caution. As discussed in Section 7.5.6-1.2.5, several reports exist regarding laboratory animal or cellular models in which MST exposure induces or aggravates a condition or disease; however, the relevance of exposure conditions to human exposure and disease should be considered in the context of MST product use behavior in consumers.

For example, MST users are exposed to the tobacco product with gaps in exposure between daily applications (dips) and overnight use. Furthermore, consumers move the product in the mouth and also prefer to change the placement of the product; consumers also expectorate most of the "juice" extracted from the product. Many nonclinical studies use continual MST exposure for days to years, or use acute MST exposures at very high concentrations. Additionally, some nonclinical studies use organic solvent-derived MST extracts that could potentially perturb the biological membranes, thereby, leading to misleading outcomes. Clearly, these are important and challenging issues that affect our interpretation of preclinical study findings and relevance to human data.

7.5.6-1.1.4. Summary

As we discuss in the following review of published scientific information, ST use is not without potential health risk (e.g., oral disease), but cigarette smoking remains the most prevalent form of tobacco use and based on current evidence presents the greatest health risk for the user. On the basis of the literature analysis, we believe that ST use is a viable alternative for cigarette smokers who want to use tobacco but also want to reduce their risk to major cigarette smoking–associated health risks.

In the following sections of this MTRPA we use the literature to address the specific health risk issues raised in the MRTP Draft Guidance document. Our synthesis of the diverse data set of epidemiology and nonclinical studies published over many years provides information on the potential risks of the candidate MRTP by itself as well as in comparison with other more risky products like cigarettes. Nonetheless, the inferences drawn from this literature should be considered in the context of the challenges described.

7.5.6-1.2. Health Risks Associated with Initiating Use of the MST Product as Compared with Never Using Tobacco Products

7.5.6-1.2.1. Epidemiology Studies

In the following sections, we provide a disease-specific summary of published epidemiology literature developed with users of U.S. ST products. We include evidence tables that briefly capture some of the main elements of the individual studies reviewed. While we have tried to present a consistent summary of the literature to present the major methods and findings of the studies, we encourage the reviewer to review the entire publication for additional information which we may not have captured.

We believe the best way to interpret the existing epidemiology on ST is to look at the aggregate dataset, including trends, magnitude of effect and consistency across multiple studies, rather than to concentrate on any one particular study because of size, type of study, sample, or outcome.

The studies we review in this section vary in any number of parameters; for example size, quality of exposure assessment, age of participants. Many of the epidemiological studies we identified provided both unadjusted and adjusted risk estimates. In these cases, our summary includes only the adjusted estimates (e.g., alcohol, age, race, etc.) that account for potential confounders. We include mortality and disease incidence risk estimates for both males and females when available. However, in many cases the number of female ST users is small or not reported. We did not attempt to assign a weight of evidence score to the data due to lack of consensus about appropriate objective scoring system and methods to minimize potential unintended biases. We have referenced meta-analyses, when available, that include many of the specific studies included in our review. As appropriate, we have included major strengths and limitations often described by the authors of the studies.

To provide a quantitative estimation of comparative risk, epidemiology data are often used to mathematically compute a relative risk (RR), hazard ratio (HR), or odds ratio (OR) number comparing the disease incidence among the exposed sample to the disease incidence among a control, or unexposed, sample. Further, meta-analyses of multiple individual epidemiology studies often subsume multiple estimates of various types and provide RR or OR calculations on a fixed effects or random effects basis. While all of these statistical estimates are technically different in terms of specific statistical interpretation, for our purposes we wish to only use the estimates to interrogate the direction and magnitude of the risk estimate calculations and identify general trends across multiple studies. Statistical calculation of a risk estimate can help develop evidence of an association between exposure and disease risk (Craun, 2005); however, the strength of the evidence based on calculated risk estimates should be interpreted with caution. For instance, Monson (1990) has suggested the following:

- Increased risk between 1.0 and 1.2, or decreased risk between 0.9-1.0, have no strength of association.
- Increased risk between 1.2 and 1.5 or decreased risk between 0.7-0.9, suggests only a weak association.
- Increased risk of > 1.5 indicates that the association is moderate or strong.

In addition to the review of published literature described in the following sections, this MRTPA provides our unpublished analysis of epidemiologic data, derived from respondents in various national health interview surveys with prospective mortality follow-up provided through linkage to the National Death Index (Section 7.4.1: Linked Mortality Analysis), to further support our conclusions.

7.5.6-1.2.2. Relationship Between ST Use and All-Cause Mortality

All-cause mortality incorporates outcomes related to the various diseases associated with tobacco use into a single measure. It is the most all-inclusive endpoint for assessing the impact of ST use on overall individual health. Two publications assessed the relationship between use of ST products and mortality from all causes.

Accortt et al. (2002) evaluated all-cause mortality risk among ST users using data from the First National Health and Nutrition Examination Survey (NHANES I) and the NHANES I Epidemiologic Follow-up Study (NHEFS) and concluded that there was “no association

between ST use and all-cause mortality." This study included 817 males and 251 females (1,068 total) 45 years of age or older at baseline (1971-1975) who reported ever use of ST. Participants were followed up 20 years later in 1992. The adjusted HR for all-cause mortality for male ever ST users was 1.0 (95 percent CI: 0.8-1.3), and the adjusted HR for all-cause mortality for females ever ST users was 1.3 (95 percent CI: 0.9-1.7).[2]

Henley et al. (2005) estimated the relationship between current ST use and all-cause mortality among males in the CPS-I and the CPS-II and concluded that, in both studies, men who currently used snuff or chewing tobacco had "higher rates of death from all causes" than men who did not use tobacco products. The CPS-I included 7,745 men who reported current exclusive use of snuff or chewing tobacco among 456,487 total men enrolled in the study. These men were enrolled in 1959 and were followed up 12 years later in 1971. The adjusted HR for death from all causes among current ST users was 1.17 (95 percent CI: 1.11-1.23).[3] The CPS-II included 3,327 men who reported exclusive use of snuff or chewing tobacco among 508,351 total males enrolled in the study. Respondents for the CPS-II were enrolled in 1982 and were followed up 18 years later in 2000. The adjusted HR for all-cause mortality among current ST users was 1.18 (95 percent CI: 1.08-1.29).[4]

Table 7.5.6-1-1 summarizes these two large studies assessing the association between ST use and all-cause mortality.

[2] Cox proportional hazard model adjusted for age, race and poverty index ratio.
[3] Cox proportional hazard model adjusted for age, race, education level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use.
[4] Cox proportional hazard model adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use.

Table 7.5.6-1-1: Literature Evaluating the Relationship Between ST and All-Cause Mortality

Reference	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Henley, 2005)	Two large prospective studies of mortality among men who use snuff or chewing tobacco (United States).	Cohort study 1959 CPS-I and 1982 CPS-II: males CPS-I Exclusive snuff or chewing tobacco use: N = 7,745 No previous use of any tobacco product N = 69,662 12-year follow-up: N = 11,871 deaths CPS-II Exclusive snuff or chewing tobacco use: N = 3,327 No previous use of any tobacco product N = 111,482 18-year follow-up: N = 19,588 deaths	"Men who currently used snuff or chewing tobacco at baseline had higher death rates from all causes than men who did not in both CPS-I…and CPS-II."	CPS-I: Current ST users HR = 1.17 (1.11-1.23) CPS-II: Current ST users HR = 1.18 (1.08-1.29)	CPS-I Age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use CPS-II Age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use	Strengths Sample size and prospective design Limitations Exposure assessment conducted only at baseline Participants more likely to be more educated, married, middle-class, and white than the general U.S. population "In both cohorts, ACS volunteers invited families of their friends, neighbors, and acquaintances to participate."

Table 7.5.6–1-1: Literature Evaluating the Relationship Between ST Use and All-Cause Mortality (continued)

Reference	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Accortt, 2002)	Chronic disease mortality in a cohort of smokeless tobacco users	Cohort study First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study (1971 to 1975) Oversampling of the elderly, the poor, and women of childbearing age 20-year mortality follow-up ST users: N = 1,068 Non-ST users: N = 5,737	"After adjustment for confounders, no association between smokeless tobacco use and all-cause mortality"	Ever ST users (male) HR = 1.0 (0.8-1.3) Ever ST users (female) HR = 1.3 (0.9-1.7)	Age, race, and poverty index ratio	Strengths Based on a national probability sample Limitations Potential residual confounding Used proxies for exposure assessment Exposure category based on ever use of ST rather than current use

Overall, the published epidemiologic data relating ST use in the U.S. and all-cause mortality risk are mixed (Table 7.5.6-1-2). The two publications reviewed reached contradictory conclusions. Both the CPS-I and the CPS-II found excess risk for all-cause mortality among current male ST users. Although the strength of association is determined to be statistically significant, the increased risk was low, and there is a possibility of some misclassification. In contrast to data from the CPS-I and CPS-II, data from NHANES I and NHEFS showed no elevated risk.

Table 7.5.6-1-2: Summary of Published All-Cause Mortality Risk Estimates for ST Users

Study	Group	ST Exposure	Risk Estimate	95% Confidence Interval
(Accortt, 2002) NHANES I and NHEFS	Males	Ever	HR = 1.0	0.8-1.3
	Females	Ever	HR = 1.3	0.9-1.7
(Henley, 2005) CPS-I and CPS-II	Males: CPS-I	Current	HR = 1.17	1.11-1.23
	Males: CPS-	Current	HR = 1.18	1.08-1.29

7.5.6-1.2.3. Relationship Between ST Use and Risks of All Cancers

The broadest measure of the carcinogenic risk associated with ST use is from the measure of all-cancer mortality or incidence in ST users. Epidemiology studies and meta-analyses provide data to evaluate the association between the use of ST products in the U.S. and the risk of mortality or incidence from any cancer.

Henley et al. (2005) estimated the relationship between current ST use and all-cancer mortality among males who currently use ST and never used other tobacco products in the CPS-I and the CPS-II. In the CPS-I, the adjusted HR for death from all cancers among current ST users was 1.07 (95 percent CI: 0.95-1.20).[5] In the CPS-II, the adjusted HR for all-cancer mortality was 1.19 (95 percent CI: 1.02-1.40).[6] The authors concluded that in "CPS-II, but not CPS-I, men who used chewing tobacco or snuff had higher death rates from all cancers combined." Different time periods and different sample populations may account for the inconsistencies between studies.

Accortt et al. (2002) evaluated all cancer mortality risk among male and female ever ST users in the NHANES I and the NHEFS. The adjusted HRs for all cancer mortality were 0.9 (95 percent CI: 0.3-2.3) for male ever ST users and 1.7 (95 percent CI: 1.0-2.8) for females

[5] Adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use.

[6] Adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use.

ever ST users.[7] The authors concluded that the data for males suggested that there is "ST use did not substantially increase the risk for cancer incidence above that of non-tobacco users, particularly among males." However, they concluded female ST users experienced some increased cancer risk compared with nontobacco users.

Sterling et al. (1992) evaluated the relationship between lifetime use of ST products and all-cancer mortality using combined data from the 1986 National Mortality Followback Survey and the 1987 National Health Interview Survey. This sample included 32,453 respondents who reported using ST between 100 to 9,999 times and 90,325 respondents who reported using ST 10,000 times or more. These authors reported that the RR of all-cancer mortality (International Classification of Disease ninth revision [ICD-9] codes 140-208) for those reporting ST use between 100 to 9,999 times was 0.37 (95% CI: 0.26-0.54) and for those reporting use of ST 10,000+ times was 0.88 (95% CI: 0.69-1.12).[8] Neither estimate was statistically significant from those who used ST less than 99 times. A limitation of this study design was the reliance on proxy informants for the presence of risk factors that could result in misclassification. Lee and Hamling (2009b) conducted a systematic review and meta-analysis evaluating all-cancer mortality among ST users. On the basis of risk estimates from five U.S. studies, these authors calculated the RR of all-cancer mortality for ST users to be 0.95 (95% CI: 0.74-1.22). When the analysis was limited to never-smoking ST users, however, the RR for all-cancer mortality was calculated to be 1.10 (95% CI: 1.01-1.20). The authors noted that studies included in the meta-analysis generally do not fully characterize exposure (frequency or duration of use) or ST product type.

There was one publication investigating the incidence of all cancers associated with ST use. Accortt et al. (2005), in a later publication using data from the CPS-I and the CPS-II, evaluated cancer incidence among ever users of ST using the same data set as used in the mortality risk analysis. The HR for all-cancer incidence among males was 0.8 (95 percent CI: 0.4-1.6),[9] and the HR for all-cancer incidence among females was 1.2 (95 percent CI: 0.7-2.1).[10]

Table 7.5.6-1-3 summarizes published literature assessing the association between ST use and all-cancer mortality.

[7] Adjusted for age, race and poverty index ratio.
[8] Adjusted for active smoking, alcohol consumption and occupational exposure.
[9] Adjusted for age, race and poverty index ratio.
[10] Adjusted for age, race and poverty index ratio.

Table 7.5.6-1-3: Literature Evaluating the Relationship Between ST Use and All-Cancer Mortality

Reference	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Sterling, 1992)	Analysis of the relationship between smokeless tobacco and cancer based on data from the National Mortality Followback Survey.	Cross sectional survey NMFS N = 18,733	"For all cancers.. no significant increased risk for heavy use of ST."	100-9,999 times users RR = 0.37 (0.26-0.54) 10,000+ times users RR = 0.88 (0.69-1.12)	Active smoking, alcohol consumption and occupational exposure	Strengths Based on a national probability sample Limitations Exposure assessment based on proxy interviews
(Accortt, 2002)	Chronic disease mortality in a cohort of smokeless tobacco users	Cohort study First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study (1971 to 1975) 20-year mortality follow-up ST users: N = 1,068 Non-ST users: N = 5,737	"After adjustment for confounders, no association between smokeless tobacco use and all-cancer mortality"	Ever ST users (male) HR = 0.9 (0.3-2.3) Ever ST users (female) HR = 1.7 (1.0-2.8)	Age, race, and poverty index ratio	Strengths Based on a national probability sample Limitations Oversampling of the elderly, the poor, and women of childbearing age Potential residual confounding Use of proxies for exposure assessment Exposure category based on ever use of ST rather than current use

Table 7.5.6–1-3: Literature Evaluating the Relationship Between ST Use and All-Cancer Mortality (continued)

Reference	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Accortt, 2005)	Cancer incidence among a cohort of smokeless tobacco users (United States).	Cohort study First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study (1971 to 1975) 20-year mortality follow-up ST users: N = 414 Non-ST users: N = 2,979	"Exclusive ST use was not associated with increased incidence of all cancer in males…or females."	Ever ST users (male) HR = 0.8 (0.4-1.6) Ever ST users (female) HR = 1.2 (0.7-2.1)	Race and poverty index ratio	Strengths Based on a national probability sample Limitations Reliance on self-reporting for exposure assessment, potential confounders Ever use of ST as exposure category Oversampling of the elderly, the poor, and women of childbearing age

Table 7.5.6–1-3: Literature Evaluating the Relationship Between ST Use and All-Cancer Mortality (continued)

Reference	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Henley, 2005)	Two large prospective studies of mortality among men who use snuff or chewing tobacco (United States).	Cohort study: males CPS-I (1959) Exclusive snuff or chewing tobacco use: N = 7,745 No previous use of any tobacco product N = 69,662 12-year follow-up: N = 11,871 deaths CPS-II (1982) Exclusive snuff or chewing tobacco use: N = 3,327 No previous use of any tobacco product N = 111,482 18-year follow-up: N = 19,588 deaths	"In CPS-II, but not CPS-I, men who used chewing tobacco or snuff had higher death rates from all cancers combined."	CPS-I Current ST user (male) HR = 1.07 (0.95-1.20) CPS-II Current ST user (male) HR = 1.19 (1.02-1.40)	CPS-I: Age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use CPS-II: Age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use	Strengths Sample size and prospective design Limitations "In both cohorts, ACS volunteers invited families of their friends, neighbors, and acquaintances to participate." Exposure assessment conducted only at baseline Participants more likely to be more educated, married, middle-class, and white than the general U.S. population

Table 7.5.6–1-3: Literature Evaluating the Relationship Between ST Use and All-Cancer Mortality (continued)

Reference	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Lee, 2009b)	Systematic review of the relation between smokeless tobacco and cancer in Europe and North America.	Meta-analysis Available published epidemiological cohort and case-control studies relating any form of cancer to ST use. Overall U.S. data: 5 estimates Smoking-adjusted U.S. data: 5 estimates Never smokers: 4 estimates	"The combined estimate for all the smoking-adjusted data is not elevated..." "The data are consistent with any excess risk of cancer in ST users being small."	U.S. data Overall data: RE RR/OR = 0.95 (0.74-1.22) Smoking-adjusted data: RE RR/OR = 0.95 (0.74-1.22) Never smokers: RE RR/OR = 1.10 (1.01-1.20)	N/A	Limitations [Limitations of studies included in meta-analysis] Small numbers of cases in may studies Unclear description of inclusion and exclusion criteria, Lack of clear description of ST type used Failure to adjust for confounders, especially smoking

Overall, the published epidemiologic data in the U.S. relating to an association between ST use and all-cancer mortality risk are mixed (Table 7.5.6-1-4). Most published risk estimates show no excess risk of mortality from all-cancers in ST users compared to those who do not use tobacco. A small excess all-cancer risk, however, was indicated in the CPS-II dataset (Henley, 2005), and was also indicated in the meta-analysis (Lee, 2009b). The large size of CPS-II potentially skews the finding of excess mortality risk due to all-cancers in ST users in the meta-analysis, which incorporates consideration of study size.

Table 7.5.6-1-4: Summary of Published All-cancer Mortality Risk Estimates for ST Users

Study	Group	ST Exposure	Risk Estimate	95% Confidence Interval
(Sterling, 1992)	All	100-9,999 uses	RR = 0.37	0.26-0.54
		10,000+ uses	RR = 0.88	0.69-1.12
(Accortt, 2002)	Males	Ever	HR = 0.9	0.3-2.3
	Females	Ever	HR = 1.7	1.0-2.8
(Accortt, 2005)	Males (incidence)	Ever	HR = 0.8	0.4-1.6
	Females (incidence)	Ever	HR = 1.2	0.7-2.1
(Henley, 2005)	Males: CPS-I	Current	HR = 1.07	0.95-1.20
	Males: CPS-II	Current	HR = 1.19	1.02-1.40
(Lee, 2009b)	Meta-analysis	Overall data	RE RR/OR = 1.22	0.82-1.83
		Smoking-adjusted data	RE RR/OR = 1.38	0.72-2.64
		Never smokers	RE RR/OR = 1.79	0.91-3.51

7.5.6-1.2.4. Relationship Between ST Use and Lung Cancer

Five publications, including one meta-analysis, have evaluated epidemiological data relating to lung cancer risk among ST users in the U.S.

Henley et al. (2005) estimated the relationship between current ST use and lung cancer mortality among males who currently use ST and never used other tobacco products from data collected in the CPS-I and the CPS-II. The analysis by Henley et al. of the CPS-I data indicated an adjusted HR for death from lung cancer among current ST users of 1.08 (95 percent CI: 0.64-1.83), after adjustment for age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin

use. However, the subsequent analysis using the CPS-II data set indicated an adjusted HR for lung cancer mortality of 2.00 (95 percent CI: 1.23-3.24).[11]

Accortt et al. (2005) evaluated lung cancer incidence among male and female ever ST users in the NHEFS. Among male exclusive ST users, no lung cancer cases were reported. Among 189 female exclusive ST users, however 4 cases of lung cancer were found yielding an adjusted HR for lung cancer, compared with that for female never tobacco users, of 6.8 (95 percent CI: 1.6-28.5).[12] The authors called this association between exclusive ST use and lung cancer "unexpected," and suggested that the finding may reflect some undefined specific product type or may be due to uncontrolled confounding or misclassification.

Wynder and Stellman (1977) conducted a retrospective case-control study of 3,716 lung, mouth, larynx, esophagus, or bladder cancer patients with over 18,000 controls. The sample included ever chewing tobacco users (291 cases with 233 controls) and ever snuff users (79 cases with 69 controls). The RR of lung cancer (Kreyberg Types I and II) were not elevated for either ever chewing tobacco users or ever snuff users. The primary limitation of this study is the small number of ST users among the cases and controls.

Zahm et al. (1992) included lung cancer incidence as one outcome of a population-based, case-control study of soft tissue sarcoma (STS). This study included 28 STS cases and 127 controls who reported ever using ST (chewing tobacco or snuff). On the basis of five cases, these authors calculated the OR for STS of the lung, pleura, or thorax among ever ST users to be 3.1 (95 percent CI: 0.9-10.5). The small number of subjects who reported ever using ST limits this study. In addition, the nonspecificity of the cancer site (lung, pleura, or thorax) limits its utility for assessing specific lung cancer risk associated with ST use.

Lee and Hamling (2009b) conducted a systematic review and meta-analysis evaluating lung cancer risk among ST users. On the basis of six U.S. risk estimates, these authors calculated the RR of lung cancer mortality for ST users to be 1.22 (95 percent CI: 0.82-1.83). Limiting the analyses to smoking-adjusted data or to never smokers resulted in slightly greater RR estimates of 1.38 (95 percent CI: 0.72-2.64) and 1.79 (95 percent CI: 0.91-3.51), respectively. Neither of these risk estimates were statistically significant, and both estimates reflected greater uncertainty. The authors noted that the studies included in the meta-analysis generally do not fully characterize exposure (frequency or duration of use) or ST product type.

Table 7.5.6-1-5 summarizes published literature assessing the association between ST use and lung cancer.

[11] Adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use.
[12] Adjusted for age, race and poverty index ratio.

Table 7.5.6-1-5: Literature Evaluating the Relationship Between ST Use and Lung Cancer

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Wynder, 1977)	Comparative epidemiology of tobacco-related cancers	Case-control study Retrospective study, interviews of patients in 20 hospitals in 8 American cities N = 3,716 lung, mouth, larynx, esophagus, or bladder cancer patients N = 18,000 controls.	"All relative risks computed from this table [of smokeless tobacco users] include 1.0 within 99% confidence limits"	Not shown	N/A	Limitations Small numbers of ST users among cases and controls
(Zahm, 1989)	A case-control study of soft-tissue sarcoma.	Case-control study Kansas, white males n = 133 sarcoma cases n = 948 controls	"greater risks were observed for tumors of the...lung, pleura, and thorax"	Ever ST user OR = 3.1 (0.9-10.5)	None	Limitations Result based on five cases

Table 7.5.6–1-5: Literature Evaluating the Relationship Between ST Use and Lung Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Accortt, 2005)	Cancer incidence among a cohort of smokeless tobacco users (United States).	Cohort study First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study (1971 to 1975) 20-year mortality follow-up ST users: N = 414 Non-ST users: N = 2,979	No cases of lung cancer were observed among exclusive male ST users "The association between ST use and lung cancer among female ST users 65 years of age and older was unexpected"	Males Exclusive ST users no cases Females Exclusive ST users HR = 6.8 (1.6-28.5)	Age, race, and poverty index ratio	Strengths Based on a national probability sample Limitations Oversampling of the elderly, the poor, and women of childbearing age Reliance on self-reporting for exposure assessment, potential confounders Ever use of ST as exposure category

Table 7.5.6–1-5: Literature Evaluating the Relationship Between ST Use and Lung Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Henley, 2005)	Two large prospective studies of mortality among men who use snuff or chewing tobacco (United States).	Cohort study: Males 1959 CPS-I or 1982 CPS-II <u>CPS-I</u> Exclusive snuff or chewing tobacco use: N = 7,745 No previous use of any tobacco product N = 69,662 12-year follow-up: N = 11,871 deaths <u>CPS-II</u> Exclusive snuff or chewing tobacco use: N = 3,327 No previous use of any tobacco product N = 111,482 18-year follow-up: N = 19,588 deaths "In both cohorts, ACS volunteers invited families of their friends, neighbors, and acquaintances to participate."	CPS-II: "Current users of any type of spit tobacco had statistically significantly higher death rates than never users from …lung cancer"	CPS-I Current ST users (male) HR = 1.08 (0.64-1.83) CPS-II Current ST users (male) HR = 2.00 (1.23-3.24)	CPS-I: age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use CPS-II: age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use	<u>Strengths</u> Large sample size and prospective design <u>Limitations</u> Exposure assessment conducted only at baseline Participants more likely to be more educated, married, middle-class, and white than the general U.S. population

Table 7.5.6–1-5: Literature Evaluating the Relationship Between ST Use and Lung Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Lee, 2009b)	Systematic review of the relation between smokeless tobacco and cancer in Europe and North America.	Meta-analysis Available published epidemiological cohort and case-control studies relating any form of cancer to ST use. Overall data: 6 estimates Smoking-adjusted data: 4 estimates Never-smoking data: 3 estimates	"The meta-analyses show no evidence that ST use increases risk of lung cancer"	Overall data (6 studies): RE OR/OR = 1.22 (0.82-1.83) Smoking-adjusted data: RE RR/OR = 1.38 (0.72-2.64) Never smokers: RE RR/OR = 1.79 (0.91-3.51)	Not applicable	Limitations [Limitations of studies included in meta-analysis] Small numbers of cases in may studies Unclear description of inclusion and exclusion criteria, Lack of clear description of ST type used Failure to adjust for confounders, especially smoking

Overall, the published epidemiological data relating ST use in the U.S. and lung cancer risk are mixed (Table 7.5.6-1-6). Four lung cancer risk estimates for ST users are not statistically different from those of never tobacco users. However, three lung cancer risk estimates in ST users are elevated, with two from either the Zahm study (1989) and Accortt study (2005) considered statistically significantly different from those for never tobacco users. Both of these estimates were based on relatively few cases. We note the wide CIs reported in for females, indicating some uncertainty in the result.

From the mixed results of the lung cancer risk in ST users compared with that in never tobacco users, additional information was investigated. Given the strong association between cigarette smoking and lung cancer, both the potential misclassification of ST users or unaccounted for cigarette smoke exposure among ST users, could explain the seeming elevated lung cancer risks among ST users from some published studies. Alternatively, Hecht and Hoffmann (1988) hypothesized that exposure to the TSNAs, N'-nitrosonornicotine (NNN) or 4'-(4' (nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), found in ST could promote cancer development. NNK particularly seems to be organ specific for the lung in rat, mouse, and hamster animal models. Exposures via subcutaneous injection, oral swabbing, or topical application have all produced increased numbers of animals with lung tumors, with DNA adduct formation providing a potential mechanistic explanation for this phenomena (Hecht, 1988). Nilsson (2006) questioned the relevance of the animal data in terms of NNN and NNK risk assessment, indicating that the rat lung is overly sensitive to lung tumor induction and may not be the best model for human risk assessment. At this time, there continues be uncertainty and contradictory information about the relationship between the TSNAs identified in ST and lung cancer development. Considerably more work needs to be done in this area to establish a mechanistic link.

Table 7.5.6-1-6: Summary of Published Lung Cancer Risk Estimates for ST Users

Study	Group	ST Exposure	Risk Estimate	95% Confidence Interval
(Wynder, 1977)	Males + females	Ever	Not significant	-
(Zahm, 1989)	Males	Ever	OR = 3.1	0.9-10.5
(Henley, 2005)	Males: CPS-I	Current	HR = 1.08	0.64-1.83
	Males: CPS-II	Current	HR = 2.00	1.23-3.24
(Accortt, 2005)	Males (incidence)	Ever	No cases	-
	Females (incidence)	Ever	HR = 6.8	1.6-28.5
(Lee, 2009b)	Meta-analysis	Overall data	RE RR/OR = 1.22	0.82-1.83
		Smoking adjusted	RE RR/OR = 1.38	0.72-2.64
		Never smokers	RE RR/OR = 1.79	0.91-3.51

7.5.6-1.2.4.1. Relationship Between ST Use and Oropharyngeal Cancer

Both the U.S. Surgeon General and the International Agency for Research on Cancer have concluded that a causal relationship exists between ST use and oropharyngeal cancers (O'Berst, 1953; U.S. Dept. Health Human Services, 1986). However, both evaluations included studies conducted outside the U.S.

Table 7.5.6-1-7 shows oropharyngeal cancer risk estimates from 26 studies conducted in the U.S. (2 using prospective design and 24 using case control design) for ST users compared with never tobacco users. These studies represented a range of regional and national studies covering an extensive time period. Together these estimates present a fairly inconsistent picture of an association between ST use and oral cancer. While many estimates suggest no association, several others suggest a clear association. Perhaps most notable in this table is the indication that while many early studies found an association between ST use and oral cancer, many of the later studies do not support an association. Additionally, limitations in the data, including such things as minimal data from cohort studies; failure to include histopathological confirmation of diagnosis; reliance on medical records in some studies; poor definition of exposure to ST; small numbers of ST exposed cases of oropharyngeal cancer; lack of adjustment for smoking and alcohol consumption, etc. need to be considered when assessing the relevance of the published estimates.

Table 7.5.6-1-7: Published Oropharyngeal Cancer Risk Estimates among ST Users

Study	Region	ST Type	Sex	Cases	RR/OR Estimate	95% Confidence Interval
(Broders, 1920)	Minnesota	ST, Chew	Male and Female	130	2.05	1.48-2.83
(Moore, 1953)	Minnesota	ST	Male	65	3.00	1.37-6.54
(Wynder, 1957)	New York	Chew	Male	91	2.00	1.16-3.47
(Peacock, Jr., 1960)	North Carolina	ST	Male	14	3.06	1.08-8.63
			Female	11	2.00	0.66-6.01
(Vogler, 1962)	Georgia	Chew	Male	46	7.38	4.31-12.62
			Female	54	38.28	21.49-68.15
(Vincent, 1963)	New York	Snuff	Male	12	4.22	1.41-12.63
(Martinez, 1969)	Puerto Rico	Chew	Male	4	2.29	0.62-8.48
			Female	1	0.34	0.04-2.79
(Keller, 1970)	National	ST	Male	11	3.63	1.02-12.95
(Williams, 1977)	National	ST	Male	16	0.91	0.53-1.56
			Female	2	1.54	0.37-6.42
(Wynder, 1977)	6 States	ST	Male	71	1.02	0.78-1.34

Study	Region	ST Type	Sex	Cases	RR/OR Estimate	95% Confidence Interval
(Westbrook, 1980)	Arkansas	Snuff	Female	50	540.00[13]	60.97-4782.82
(Winn, 1981)	North Carolina	Snuff	Female	107	2.67	1.83-3.90
(Wynder, 1983)	5 states	ST	Male	49	0.90	0.57-1.41
(Stockwell, 1986)	Florida	ST	Male and Female	11	2.02	1.01-4.02
(Blot, 1988)	4 States	ST	Male	46	0.85	0.57-1.26
			Female	11	3.44	1.09-10.91
(Spitz, 1988)	Texas	ST	Male and Female	25	1.05	0.57-1.91
(Zahm, 1992)	National	ST	Male	129	4.11	2.90-5.84
(Maden, 1992)	Washington	ST	Male	19	4.50	1.50-14.30
(Sterling, 1992)	National	ST	Male and Female	28	1.04	0.41-2.68
(Mashberg, 1993)	New Jersey	ST	Male	52	0.96	0.70-1.33
(Perry, 1993)	Michigan	ST	Male and Female	10	1.43	0.64-3.21
(Kabat, 1994)	4 states	Chew	Male	67	1.11	0.81-1.53
(Muscat, 1998)	4 states	ST	Male and Female	4	1.19	0.26-5.45
(Schwartz, 1998)	Washington	ST	Male	-	1.00	0.40-2.30
(Henley, 2005)	National	ST	Male	1	Not reported	Not reported

7.5.6-1.2.4.2. Relationship Between ST Use and Esophageal Cancer

Seven publications assessed the relationship between ST use and cancer of the esophagus among ST users in the U.S., including a meta-analysis.

Brown et al. (1988) conducted a case-control study of esophageal cancer patients drawn from four hospitals in Charleston, South Carolina. There was one ST user among the 207

[13] Both IARC (International Agency for Research on Cancer. *Tobacco habits other than smoking; betel-quid and areca-nut chewing; and some related nitrosamines*, Volume 37. Lyon, France: IARC; 1985. and the US Surgeon-General (US Surgeon General. *The health consequences of smoking - 50 years of progress: a report of the Surgeon General*. Atlanta, Georgia: US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2014. have pointed out that this estimate is very unreliable as the probability of snuff use being mentioned in medical records, had it occurred, seems likely to be much greater for the cases than controls.

esophageal cancer cases and 12 ST users among the control group (n=422). The adjusted OR for esophageal cancer for ST users was 1.2 (95 percent CI: 0.1-13.3).

Pottern et al. (1981) explored risk factors for esophageal cancer among African American males in Washington, D.C., in an effort to understand the high rate of disease in this population. The study included 120 cases and 250 controls. However, only 3.3 percent of cases and controls used ST (snuff or chewing tobacco), and the authors did not detect an excess risk of esophageal cancer in ST users.

Williams and Horm (1977) reported results from the Third National Cancer Study, including assessment of the relationship between ST use and esophageal cancer. This study appeared to measure some dose-response as level 1 ST use was defined as 1-50 snuff or chewing tobacco years, and level 2 greater than level 1 (>50 years). Snuff or chewing tobacco years was not further explained. There were only three esophageal cancer patients among exposed cases: two for chewing or snuff tobacco level 1 and one for chewing tobacco or snuff level 2. The ORs for these exposure groups were 0.82 and 0.73, respectively.

Wynder and Stellman (1977) conducted a case-control study among 3716 patients with tobacco-related cancers and over 18,000 controls. Among esophageal cancer patients, 20 reported ever chewing tobacco, and 11 reported ever using snuff. The RR for esophageal cancer among snuff users was 1.7, although the CIs overlapped 1.0 (as stated by the authors). The RR for esophageal cancer among chewing tobacco users was also not different from that among never tobacco users.

Martinez (1969) conducted a case-control study of esophageal, mouth, and pharynx cancer patients in Puerto Rico. Each case was matched to three controls by age and sex, one control drawn from the same hospital and two other controls drawn from the local community. The results were 8.5% of male esophageal cancer patients used ST, as compared with 3.6% for controls. However, for female patients, 11.9% of esophageal cancer patients used ST, as compared with 7.3% of controls. The author did not provide OR calculations; however, subsequent authors calculated ORs using data presented in the paper (Lee, 2009a). For male ST users, the ORs of esophageal cancer were 1.18 (95% CI: 0.28-4.90) based on three cases; for female ST users, the odds were 2.69 (95% CI: 0.92-7.87) based on seven cases.

Wynder and Bross (1961) evaluated risk factors for esophageal cancer among 150 esophageal cancer patients and 150 hospital controls at two New York hospitals. A total of 20% of cases and 10% controls reported ever use of chewing tobacco, and the authors concluded that esophageal cancer was more common among chewing tobacco users than among controls. However, the authors noted that all chewing tobacco users also smoked cigarettes.

Lee and Hamling (2009b) conducted a systematic review and meta-analysis evaluating esophageal cancer risk among ST users. On the basis of risk estimates from six U.S. studies, these authors calculated the RR of esophageal cancer for ST users to be 1.56 (95 percent CI: 1.11-2.19). Limiting the analyses to smoking-adjusted data or to never smokers increases the RE RR/OR point estimates for esophageal cancer risk to 1.89 (95 percent CI: 0.84-4.25). However, as seen with lung cancer estimates, the risk estimate was not statistically significant, and substantial uncertainty was indicated by the wide CIs. The authors again

noted that studies included in the meta-analysis generally do not fully characterize exposure (frequency or duration of use) or ST product type.

Table 7.5.6-1-8 summarizes published literature assessing the association between ST use and esophageal cancer.

Table 7.5.6-1-8: Literature Evaluating the Relationship Between ST Use and Esophageal Cancer

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Lee, 2009b)	Systematic review of the relation between smokeless tobacco and cancer in Europe and North America	Meta-analysis Available published epidemiological cohort and case-control studies relating any form of cancer to ST use. Overall data: 6 estimates Smoking-adjusted data: 3 estimates Never-smoking data: 3 estimates	"Overall, the data must be regarded as providing suggestive evidence of a possible weak relationship between ST use and oesophageal cancer."	Overall data (6 studies): RE RR/OR = 1.56 (1.11-2.19) Smoking-adjusted data: RE RR/OR = 1.89 (0.84-4.25) Never smokers: RE RR/OR = 1.89 (0.84-4.25)	N/A	Limitations [Limitations of studies included in meta-analysis] Small numbers of cases in many studies Unclear description of inclusion and exclusion criteria, Lack of clear description of ST type used Failure to adjust for confounders, especially smoking
(Brown, 1988)	Environmental factors and high risk of esophageal cancer among men in coastal South Carolina	Case-control study Cases identified through 4 Charleston, SC, hospitals 1 ST using case, 12 controls	"There were large significant increases in risk for all forms of [tobacco] use except exclusive smokeless tobacco use"	Ever ST users OR = 1.2 (0.1 - 13.3)	Study series, alcohol	Limitations Small number of exposed cases/controls

Table 7.5.6–1-8: Literature Evaluating the Relationship Between ST Use and Esophageal Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Pottern, 1981)	Esophageal cancer among black men in Washington, D.C. I, Alcohol, tobacco, and other risk factors	Case-control study Recent black male cancer decedents 120 cases, 250 similarly aged controls 3.3% ever chewed tobacco	"Other forms of tobacco [including ST] revealed no increases in risk"	Not calculated	N/A	Limitations Very small number of exposed cases/controls Reliance on next of kin for exposure assessment
(Williams, 1977)	Association of cancer sites with tobacco and alcohol consumption and socioeconomic status of patients: interview study from the Third National Cancer Survey	Case-control study Snuff or chew level 1 (1-50 snuff-years): 2 male exposed cases Snuff or chew level 2 (>50 snuff-years): 1 male exposed case 1,788 total controls Controls were patients with cancer at other sites	No association reported	Snuff or chew level 1 OR = 0.82 Snuff or chew level 2 OR = 0.73	Age, race	Strengths Exposure assessed for both duration and intensity of use Limitations Very small number of exposed cases No adjustment for potential confounders beyond age and race

Table 7.5.6–1-8: Literature Evaluating the Relationship Between ST Use and Esophageal Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Wynder, 1977)	Comparative epidemiology of tobacco-related cancers	Case-control study Cases/controls drawn from 20 hospitals in eight U.S. cities 20 ever chewing tobacco using cases, 163 controls 8 ever snuff using cases, 175 controls	"Data contain insufficient cases to demonstrate and increased risk due to chewing tobacco and snuff use alone"	Ever ST user RR = 1.7 (CI included 1.0)	None	Limitations Small number of exposed cases
(Martinez, 1969)	Factors associated with cancer of the esophagus, mouth and pharynx in Puerto Rico	Case-control study Community-based study of cancer patients (1966) 3 male chewing tobacco using cases, 13 age/sex matched controls 7 female chewing tobacco using cases, 13 age/sex matched controls	No specific conclusions stated	Not calculated As calculated by Lee and Hamling (2009b): Male RR = 1.18 (95 percent CI: 0.28-4.90) Female RR = 2.69 (95 percent CI: 0.92-7.87)	None	Limitations Small numbers of exposed cases and controls

Table 7.5.6–1-8: Literature Evaluating the Relationship Between ST Use and Esophageal Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Wynder, 1961)	A study of etiological factors in cancer of the esophagus	Case-control study Sample drawn from hospitals in New York City (1956-1959)	"Twenty one per cent of the patients with cancer of the esophagus were chewers as compared to 10% of the controls. The data showed no difference in the duration of tobacco chewing. All of the tobacco chewers were also tobacco smokers."	Not calculated	None	Limitations Small numbers of exposed cases/controls

Overall, the published literature provides mixed evidence for an association between ST use and esophageal cancer (Table 7.5.6-1-9). Four U.S. case-control studies and one meta-analysis provide quantitative risk estimates for esophageal cancer among ST users. Although point estimates are elevated for some cohorts, the estimates were not statistically different from values for never tobacco users. Two additional studies, Pottern et al. (1981) and Wynder and Bross (1961), provide qualitative information comparing esophageal cancer risk among ST users, and neither support an association.

Table 7.5.6-1-9: Summary of Published Esophageal Cancer Risk Estimates for ST Users

Study	Group	ST Exposure	Risk Estimate	95% Confidence Interval
(Martinez, 1969)[14]	Males	Ever	RR = 1.18	0.28-4.90
	Females	Ever	RR = 2.69	0.92-7.87
(Williams, 1977)	Males	Ever, level 1	OR = 0.82	Not calculated
		Ever, level 2	OR = 0.73	Not calculated
(Wynder, 1977)	Males	Ever	RR = 1.7	Not significant
(Brown, 1988)	Males	Ever	OR = 1.2	0.1-13.3
(Lee, 2009b)	Meta-analysis	Overall data	RE RR/OR = 1.56	1.11-2.19
		Smoking adjusted	RE RR/OR = 1.89	0.84-4.25
		Never smokers	RE RR/OR = 1.89	0.84-4.25

7.5.6-1.2.4.3. Relationship Between ST Use and Bladder Cancer (Including Urinary Tract)

Seven publications (including six case-control studies and a meta-analysis) have evaluated epidemiological data specifically relating to bladder cancer risk among ST users in the U.S.

Burch et al. (1989) conducted a population-based, case-control study with 826 bladder cancer cases and 792 controls. This Canadian study is relevant to the U.S. because the ST products used in the two countries are largely similar. In the study, the OR for bladder cancer among ever snuff users was 0.47 (95 percent CI: 0.21-1.07), as compared with never snuff users (9 cases/18 controls).[15] For ever chewing tobacco users (26 cases/34 controls), the OR was 0.60 (95 percent CI: 0.34-1.06).) The authors concluded that the use of chewing tobacco or snuff "was not associated with increased risk of bladder cancer."

[14] Risk estimates shown are extracted from Lee and Hamling, 2009 using data from Martinez, 1969.
[15] Adjusted for lifetime cigarette consumption.

Hartge et al. (1985) evaluated bladder cancer risk among snuff and chewing tobacco users as part of the National Bladder Cancer Study, a population-based, case-control study. Among snuff users there were 11 cases/50 controls, while among chewing tobacco users, there were 40 cases/133 controls. The RR for bladder cancer among snuff users, compared with non-users, was 0.77 (95 percent CI: 0.38-1.56); among chewing tobacco users, compared with those not using tobacco, it was 1.02 (95 percent CI: 0.67-1.54).[16] The authors concluded that the finding of no increased risk among ST users is consistent with the published literature, but the "estimates are too unstable to permit any firm conclusions to be drawn."

Kabat et al. (1986) conducted a case-control study among bladder cancer patients. The authors noted that statistically significantly more female bladder cancer patients used snuff than controls, but there is no quantitative risk estimate provided. The authors concluded "the finding of the current study that cases were more likely to use snuff than controls must be interpreted with caution in view of the extremely small number of snuff users."

Slattery et al. (1988) assessed bladder cancer risks among snuff and chewing tobacco users in a population-based, case-control study. The ORs for bladder cancer risk among never-smoking snuff users and chewing tobacco users were 2.73 (95% CI: 0.48-15.57) and 2.78 (95% CI: 0.38-20.20), respectively. There were two cases with three controls for never-smoking snuff users, and one case with 11 controls for never-smoking chewing tobacco users. Among snuff and chewing tobacco users who smoked, the odds of bladder cancer were 0.70 (95% CI: 0.36-1.35) and 1.22 (95% CI: 0.68-2.19). These groups had 14 cases with 29 controls and 19 cases with 34 controls, respectively.

Wynder and Stellman (1977) conducted a case-control study that evaluated bladder cancer risk among snuff (11 cases with 69 controls) and chewing tobacco users (47 cases with 233 controls). Risk estimates were not reported, but the authors stated that all RRs for chewing tobacco and snuff users included 1.0 in the 99 percent CIs.

Castelao et al. (2001) conducted a population-based case-control study of smoking and bladder cancer risk. In the study the authors reported bladder cancer risk for a subset of subjects (1 case with 6 controls) that used chewing tobacco or snuff and did not smoke. The authors found no association between ST use and bladder cancer, reporting an OR for the group of 0.4 (95 percent CI: 0.05-3.3).

Lee and Hamling (2009b) conducted a systematic review and meta-analysis evaluating bladder cancer risk among ST users. On the basis of risk estimates from nine U.S. studies, these authors calculated the RR of bladder cancer for ST users to be 1.11 (95 percent CI: 0.85-1.45). When the authors limited the analysis to studies that adjusted for smoking, there was a risk estimate of 1.24 (95 percent CI: 0.83-1.85). Five studies included bladder cancer risk estimates for never-smoking ST users; a meta-analysis of these results yielded an estimate of 1.25 (95 percent CI: 0.69-2.26). The authors conclude that these data provide "no real evidence of an association between ST use and bladder cancer."

[16] Adjusted for age, race, location, pipes, cigars and chewing tobacco or snuff.

In other urinary-tract related studies, Henley et al. (2005) assessed genitourinary cancer risk in the CPS-I and the CPS-II cohorts. For current ST users in the CPS-I, the HR of genitourinary cancer was 0.97 (95 percent CI: 0.77-1.22) and for the CPS-II the HR was 1.15 (95 percent CI: 0.85-1.56).[17] The authors concluded that "no association was observed between spit [smokeless tobacco] and genitourinary system cancers." Cole et al. (1971) conducted a case-control study with 468 lower urinary tract cancer patients and 498 controls matched for age and sex. On the basis of analyses restricted to men only, there were no differences in the observed versus expected incidence of lower urinary tract cancers for snuff users or chewing tobacco users.

Table 7.5.6-1-10 summarizes published literature assessing the association between ST use and bladder cancer.

[17] CPS-I adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use. CPS-II adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use

Table 7.5.6-1-10: Literature Evaluating the Relationship Between ST Use and Bladder Cancer (Including Urinary Tract)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Cole, 1971)	Smoking and cancer of the lower urinary tract	Case-control study 468 cases, 360 male, 108 female 498 controls age and sex matched controls, 381 male, 117 female	"No differences between observed and expected numbers of cases who had used snuff…or chewing tobacco."	None reported	Age	Limitations Likely small number of ST exposed cases/controls, no adjustment for potential confounders
(Wynder, 1977)	Comparative epidemiology of tobacco-related cancers.	Case-control study N = 47 ever chewing tobacco using cases, 233 controls N = 11 ever snuff using cases, 69 controls Essentially all ST users were male.	All relative risks…included 1.0 within the 99% CIs	None reported	None	Limitations Lack of adjustment for potential confounders Ever ST use as exposure category

Table 7.5.6–1-10: Literature Evaluating the Relationship Between ST Use and Bladder Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Hartge, 1985)	Bladder cancer risk and pipes, cigars, and smokeless tobacco.	Case-control study National Bladder Cancer study N= 11 snuff using cases, 50 controls N= 40 chewing tobacco using cases, 133 controls ST analyses limited to males	"Our observation that snuff dippers and tobacco chewers were not at increased risk of bladder cancer is consistent with the few published data but all of the existing estimates are too unstable to permit any firm conclusions to be drawn."	Snuff RR = 0.77 (0.38-1.56) Chewing tobacco RR = 1.02 (0.67-1.54)	Age, race, residence, pipes, cigars	Limitations Small number of exposed cases
(Kabat, 1986)	Bladder cancer in nonsmokers.	Case-control study Hospital-based study, 18 sites in 6 U.S. cities (1976-1983) N= 2 chewing tobacco using cases (1 male, 1 female) N= 3 snuff using cases, 1 control (all female)	"Snuff use among women, however, was positively associated with bladder cancer"	None reported	None	Limitations Small number of ST exposed cases/controls No adjustment for potential confounders

Table 7.5.6–1-10: Literature Evaluating the Relationship Between ST Use and Bladder Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Slattery, 1988)	Smoking and bladder cancer. The modifying effect of cigarettes on other factors.	Case-control study White men between the ages of 21 and 84 N= 16 ever snuff using cases, 32 controls N= 21 ever chewing tobacco using cases, 45 controls	"An increased but not significant risk was also seen for pipe, snuff, and chewing tobacco use in noncigarette smokers"	Snuff Never smoked: OR = 2.73 (0.48-15.57) Smokers: OR = 0.70 (0.36-1.35) Chewing tobacco Never smoked: OR = 2.78 (0.38-20.20) Smokers: OR = 1.22 (0.68-2.19)	None	Limitations Small number of exposed cases/controls No adjustment for potential confounders
(Burch, 1989)	Risk of bladder cancer by source and type of tobacco exposure: a case-control study.	Case-control study Alberta and south-central Ontario, Canada between (1979-1982) N= 9 ever snuff using cases, 18 controls N= 26 ever chewing tobacco using cases, 34 controls	"Other forms of tobacco use (pipes, cigars, chewing tobacco and snuff) were not associated with increased risks of bladder cancer"	Snuff OR = 0.47 (0.21-1.07) Chewing tobacco OR = 0.60 (0.34-1.06)	Smoking	Limitations Small number of exposed cases/controls

Table 7.5.6–1-10: Literature Evaluating the Relationship Between ST Use and Bladder Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Castelao, 2001)	Gender- and smoking-related bladder cancer risk.	Case-control study Population-based study in Los Angeles, CA. N= 1 chewing tobacco/snuff using case, 6 controls	"No associations were found between bladder cancer risk… chewing tobacco/snuff"	Ever ST user (male and female) OR = 0.4 (0.05-3.3)	Age, sex	Limitations One case

Table 7.5.6–1-10: Literature Evaluating the Relationship Between ST Use and Bladder Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Henley, 2005)	Two large prospective studies of mortality among men who use snuff or chewing tobacco (United States).	Cohort study: males 1959 CPS-I or 1982 CPS-II <u>CPS-I</u> Exclusive snuff or chewing tobacco use: N = 7,745 No previous use of any tobacco product N = 69,662 12-year follow-up: N = 11,871 deaths <u>CPS-II</u> Exclusive snuff or chewing tobacco use: N = 3,327 No previous use of any tobacco product N = 111,482 18-year follow-up: N = 19,588 deaths	"No association was observed between spit tobacco use and genitourinary system cancers"	CPS-I Current ST users (male) HR = 0.97 (0.77-1.22) CPS-II Current ST users (male) HR = 1.15 (0.85-1.56)	CPS-I: age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use CPS-II: age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use	<u>Strengths</u> Studies size and prospective design <u>Limitations</u> "In both cohorts, ACS volunteers invited families of their friends, neighbors, and acquaintances to participate." Exposure assessment conducted only at baseline Participants more likely to be more educated, married, middle-class, and white than the general U.S. population

Table 7.5.6–1-10: Literature Evaluating the Relationship Between ST Use and Bladder Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Lee, 2009b)	Systematic review of the relation between smokeless tobacco and cancer in Europe and North America.	Meta-analysis Available published epidemiological cohort and case-control studies relating any form of cancer to ST use. Overall data: 9 estimates Smoking-adjusted data: 6 estimates Never-smoking data: 5 estimates	"Considered together, the data provide no real evidence of an association between ST and bladder cancer."	U.S. data Overall data: RE RR/OR = 1.11 (0.85-1.45) Smoking-adjusted data: RE RR/OR =1.24 (0.83-1.85) Never smokers: RE RR/OR =1.25 (0.69-2.26)	N/A	Limitations [Limitations of studies included in meta-analysis] Small numbers of cases in may studies Unclear description of inclusion and exclusion criteria, Lack of clear description of ST type used Failure to adjust for confounders, especially smoking

Overall, the published data with U.S. ST indicate no evidence of an association between ST use and bladder cancer (Table 7.5.6-1-11).

Table 7.5.6-1-11: Summary of Published Bladder Cancer Risk Estimates for U.S. ST Users

Study	Group	ST Exposure	Risk Estimate	95% Confidence Interval
(Hartge, 1985)	Males	Ever snuff	RR = 0.77	0.38-1.56
		Ever chew	RR = 1.02	0.67-1.54
(Slattery, 1988)	Males	Snuff, never smoked	OR = 2.73	0.48-15.57
		Chew, never smoked	OR = 2.78	0.38-20.20
(Castelao, 2001)	Males + females	Ever ST	OR = 0.4	0.05-3.3
(Henley, 2005)	Males: CPS-I	Current ST	HR = 0.97	0.77-1.22
	Males: CPS-II	Current ST	HR = 1.15	0.85-1.56
(Lee, 2009b)	Meta-analysis	Overall data	RE RR/OR = 1.11	0.85-1.45
		Smoking adjusted	RE RR/OR = 1.24	0.83-1.85
		Never smokers	RE RR/OR =1.25	0.69-2.26

7.5.6-1.2.4.4. Relationship Between ST Use and Digestive Organ Cancers

Public health authorities have not concluded that there is a link between ST use and an elevated risk of digestive cancers. Five publications, including one meta-analysis, have evaluated epidemiological data relating to digestive cancer risk among ST users in the U.S.

Henley et al. (2005) estimated the relationship between current ST use and digestive cancer mortality among males who currently use ST and never used other tobacco products in the CPS-I and the CPS-II. In the CPS-I, the adjusted HR for death from digestive cancer among current ST users was 1.26 (95 percent CI: 1.05-1.52).[18] In the CPS-II, the adjusted HR for digestive cancer mortality was 1.04 (95 percent CI: 0.77-1.38).[19] These authors concluded that in the CPS-I, but not the CPS-II, “men who reported current use of spit tobacco had

[18] Adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use.
[19] Adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use.

statistically significantly higher death rates than never users from cancers of the digestive system."

Kneller et al. (1991) evaluated stomach cancer risk among a cohort of 17,633 white men. Based on 18 cases, the RR for stomach cancer among current and former ST users (chewing tobacco or snuff) was 2.3 (95 percent CI: 0.98-5.22). However, when the analysis was limited to ST users who never smoked cigarettes, the RR for stomach cancer, as compared with that for never tobacco users, was 3.8 (95 percent CI: 1.00-14.32). The authors concluded that "elevated risks [of stomach cancer] were also found …for smokeless tobacco use." This result was based on only three cases, and the study did not adjust for potential confounding factors.

Sterling et al. (1992) found no evidence of excess risk for cancers of the digestive organs[20] among ever ST users included in the 1986 National Mortality Followback Survey and the 1987 National Health Interview Survey. For respondents using ST 100 to 9,999 times in their life, compared with those using ST 0 to 99 times in their life, the RR for cancer of the digestive organs was 0.15 (95 percent CI: 0.04-0.52).[21] For respondents using ST 10,000 or more times, the RR for digestive organ cancer was 0.61 (95 percent CI: 0.34-1.10). In some cases in the 1986 National Mortality Followback Survey, the next of kin was used for identification of risk factors that could lead to misclassification. However, these authors provided several analyses to test the robustness of these data and concluded that any effect of misclassification is likely small and would not alter the overall conclusions. This study included a relatively large sample of lifetime ST users; estimating that out of greater than 109,000 digestive organ cancer deaths, there were 1,296 digestive organ cancer deaths among respondents using ST 100 to 9,999 times, and there were 4,254 digestive organ cancer deaths among respondents using ST 10,000 or more times in their lives.

Heineman et al. (1994) evaluated the association between ST use and colorectal cancer in the U.S. Veterans Cohort, comprising 248,046 veterans, including 41,124 ST users, followed prospectively for 26 years. Compared with never tobacco users, the RR of colon cancer among ST users was 1.2 (95% CI: 0.9-1.7), and the RR of rectal cancer among ST users was 1.9 (95 percent CI: 1.2-3.1).[22] These findings were based on 39 and 17 deaths, respectively. There was no evidence of a dose response. For both colon and rectal cancer, respondents who reported never heavy ST use had higher RRs than those reporting ever heavy ST use. The RR of colon cancer for never-heavy ST users was 2.0 (95% CI: 1.4-3.0), with 0.6 (95% CI: 0.4-1.1) calculated for ever-heavy ST users. For rectal cancer, the RR for never-heavy ST users was 2.5 (95% CI: 1.3-5.0), and 1.5 (95% CI: 0.7-3.0) for ever-heavy ST users. The authors noted that this study is limited by the absence of dietary information, which diet is a known risk factor for colorectal cancers.

Lee and Hamling (2009b) conducted a systematic review and meta-analysis evaluating digestive cancer risk among ST users. On the basis of risk estimates from five U.S. studies,

[20] ICD 9 codes 150-159.
[21] Adjusted for active smoking, alcohol consumption and occupational exposure.
[22] All comparisons adjusted for age, calendar time, year of questionnaire response, SES, and sedentary job.

these authors calculated the RR of digestive cancer mortality for ST users to be 0.86 (95 percent CI: 0.59-1.25). When the authors limited the analyses to never smokers, they found an estimate of 1.14 (95 percent CI: 0.99-1.33). The authors noted some evidence of heterogeneity among the various digestive cancer risk estimates included in the meta-analysis and suggested that the overall data were insufficient to "draw firm conclusions." These authors also conducted meta-analyses for stomach cancer specifically and found similar results as those for digestive organ cancers combined.

Table 7.5.6-1-12 summarizes published literature assessing the association between ST use and digestive organ cancers.

Table 7.5.6-1-12: Literature Evaluating the Relationship Between ST Use and Digestive Organ Cancers

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Kneller, 1991)	A cohort study of stomach cancer in a high-risk American population.	Cohort study White American men, largely of Scandinavian and German descent (1966) N= 18 ST using cases, 3 ST using cases who did not smoke	"Elevated risks were found for…ST [for stomach cancer mortality]"	Current and former ST users: RR = 2.3 (0.98-5.22) ST users/never smokers: RR = 3.8 (1.00-14.32)	None	Strengths Prospective design Limitations Lack of adjustment for potential confounders Small number of exposed cases, result for never-smoking ST users based on 3 cases
(Sterling, 1992)	Analysis of the relationship between smokeless tobacco and cancer based on data from the National Mortality Followback Survey.	Cohort study National Morality Followback Survey National Health Interview Survey Number of exposed participants not reported	"Heavy use of smokeless tobacco [is] not associated with increased risk of digestive cancer, moderate use exhibits a significant negative association."	Use 100-9999 times: RR = 0.15 (0.04-0.52) 10,000+ times: RR = 0.61 (0.34-1.10)	Active smoking, alcohol consumption, and occupational exposure	Strengths Based on national probability sample Limitations Reliance on informants for information on the presence of risk factors
(Heineman, 1994)	Increased risk of colorectal cancer among smokers: results of a 26-year follow-up of U.S. veterans and review	Cohort study 248, 046 U.S. veterans enrolled between 1954-1957 41,124 ST (snuff or chewing tobacco) users 99.5% male, almost all white	"Colon cancer was no higher among users of snuff or chewing tobacco" "Risk of rectal cancer [among ST users] was nearly twice that of non-tobacco users"	Colon cancer: RR = 1.2 (0.9-1.7) Rectal cancer: RR = 1.9 (1.2-3.1)	Age, calendar time, year of questionnaire response, socioeconomic status and sedentary job	Strengths Large sample size, prospective design Limitations No information on potential dietary confounders, exposure assessment at baseline only

Table 7.5.6–1-12: Literature Evaluating the Relationship Between ST Use and Digestive Cancers (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Henley, 2005)	Two large prospective studies of mortality among men who use snuff or chewing tobacco (United States).	Cohort study 1959 CPS-I or 1982 CPS-II Males <u>CPS-I</u> Exclusive snuff or chewing tobacco use: N = 7,745 No previous use of any tobacco product N = 69,662 12-year follow-up: N = 11,871 deaths <u>CPS-II</u> Exclusive snuff or chewing tobacco use: N = 3,327 No previous use of any tobacco product N = 111,482 18-year follow-up: N = 19,588 deaths "In both cohorts, ACS volunteers invited families of their friends, neighbors, and acquaintances to participate."	Men [in CPS-I] who reported current use of spit tobacco had statistically significantly higher death rates than never users from...cancers of the digestive system. [In CPS-II] No association was observed.	CPS-I Current ST user (male) HR = 1.26 (1.05-1.52) CPS-II Current ST user (male) HR = 1.04 (0.77-1.38)	CPS-I age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use CPS-II age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use	<u>Strengths</u> Studies size and prospective design <u>Limitations</u> Exposure assessment conducted only at baseline Participants more likely to be more educated, married, middle-class, and white than the general U.S. population demographic

Table 7.5.6–1-12: Literature Evaluating the Relationship Between ST Use and Digestive Cancers (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Lee, 2009b)	Systematic review of the relation between smokeless tobacco and cancer in Europe and North America.	Meta-analysis Available published epidemiological cohort and case-control studies relating any form of cancer to ST use. Overall data: 5 estimates Smoking-adjusted data: 5 estimates Never-smoking data: 4 estimates	The meta-analyses conducted…are nonsignificant	Overall: RE RR/OR = 0.86 (0.59-1.25) Smoking adjusted: RE RR/OR = 0.86 (0.59-1.25) Never smokers: RE RR/OR = 1.14 (0.99-1.33)	N/A	Limitations [Limitations of studies included in meta-analysis] Small numbers of cases in may studies Unclear description of inclusion and exclusion criteria Lack of clear description of ST type used Failure to adjust for confounders, especially smoking

Overall, evidence of an association between ST use and digestive cancers are mixed (Table 7.5.6-1-13). Most reported risk estimates point to no difference in risk between ST users and never tobacco users. The only statistically significant elevated risk estimated was a weak association (RR = 1.26) derived from the CPS-I. The results of the meta-analysis from Lee and Hamling (2009b), which includes the individual studies reviewed here; do not show evidence of an excess risk of digestive cancers associated with ST use.

Table 7.5.6-1-13: Summary of Published Digestive Organ Cancer Risk Estimates for ST Users

Study	Group	Smokeless Tobacco Exposure	Risk Estimate	95% Confidence Interval
(Kneller, 1991)	Males	Current and former	RR = 2.3	0.98-5.22
		Never smokers	RR = 3.8	1.00-14.32
(Sterling, 1992)	Males + females	100-9,999 times	RR = 0.15	0.04-0.52
		10,000+ times	RR = 0.61	0.34-1.10
(Henley, 2005)	Males: CPS-I	Current	HR = 1.26	1.05-1.52
	Males: CPS-II	Current	HR = 1.04	0.77-1.38
(Lee, 2009b)	Meta-analysis	Overall data	RE RR/OR = 0.86	0.59-1.25
		Smoking adjusted	RE RR/OR =0.86	0.59-1.25
		Never smokers	RE RR/OR =1.14	0.99-1.33

7.5.6-1.2.4.5. Relationship between ST Use and Pancreatic Cancer

Ten studies, including three meta-analyses[23] and one systematic review, evaluated ST use as a risk factor for pancreatic cancer.

Alguacil and Silverman (2004) conducted a case-control study among tobacco users, including ST users, who had never smoked cigarettes (7 cases with 44 controls that had ever used ST and 5 cases with 28 controls that had only used ST). The OR for pancreatic cancer among ever ST users, compared with that among never tobacco users, was 1.4 (95% CI: 0.5-3.6); among individuals who had only used ST, the OR for pancreatic cancer was 1.1 (95% CI: 0.4-3.1).[24] Use of less than or equal to 2.5 oz of ST per week was not associated with increased pancreatic cancer risk (OR: 0.3, 95% CI: 0.04-2.5) while use of greater than 2.5 oz of ST per week was associated with increased pancreatic cancer risk (OR: 3.5, 95% CI: 1.1-10.6). There was a suggestion of a time effect, with those using ST up to 20 years having directionally lower risk than those using ST for more than 20 years (OR: 1.1 and 1.5, respectively; neither estimate statistically different from never tobacco users). This study is

[23] Two of the meta-analyses Lee and Hamling (Lee, 2009b) and Sponsiello-Wang et al. are based on similar data sets.
[24] Adjusted for race, gender, geographic site, cigar smoking and age.

unique in that participants were all non–cigarette smokers and in that exposure information was obtained using patient interviews.

Falk et al. (1988) evaluated risk factors associated with pancreatic cancer in a hospital-based case-control study comprising 363 cases and 1,234 matched controls based on age (±5 years), sex, and race. Twelve percent of this population reported use of chewing tobacco and less than 3 percent reported use of snuff. No excess risks were detected among ST users.

Farrow and Davis (1990) conducted a case-control study in which 6.9 percent of subjects had ever used chewing tobacco. No evidence of excess pancreatic cancer risk was found among ever chewing tobacco users (OR: 0.8, CIs included 1.0 but not reported).[25] The small number of subjects who used chewing tobacco limits the power of this study.

Hassan et al. (2007) reported the results of a hospital-based, case-control study that included 808 pancreatic adenocarcinoma patients with 808 controls. A total of 34 cases with 54 controls reported ever use of chewing tobacco and 18 cases with 34 controls reported ever use of snuff. The adjusted OR for these groups was 0.7 (95 percent CI: 0.4-1.1) and 0.6 (95 percent CI: 0.3-1.1), respectively, when compared with the adjusted OR for never users of chewing tobacco or snuff.[26] No dose response was evident for high consumption compared with low or moderate consumption. The small number of exposed cases and controls limits this study.

Muscat et al. (1997) conducted a hospital-based study of 484 male and female pancreatic cancer patients with 954 controls. This sample included six cases and five controls that chewed tobacco and did not currently smoke. The OR for pancreatic cancer risk for this group, compared with never tobacco users/long-term quitters, was 3.6 (95 percent CI: 1.0-12.8). The authors concluded that "tobacco juice may also cause pancreatic cancer when ingested or absorbed through the oral cavity." The sample size for this study is very small and the risk estimate is not adjusted for any potential confounders.

Zheng et al. (1993) evaluated pancreatic cancer risk factors among 17,633 white male participants in the Lutheran Brotherhood Insurance Society cohort study. The pancreatic cancer risk for ever users of ST, based on 16 deaths, was 1.7 (95 percent CI: 0.9-3.1).[27]

Burkey et al. (2014) conducted a systematic literature review based on 11 studies pertaining to ST use and pancreatic cancer risk. These authors determined that the data were insufficient to conduct a meta-analysis and concluded that "the association between ST use and pancreatic adenocarcinoma is inconclusive."

Bertuccio et al. (2011) examined data from the International Pancreatic Cancer Case-Control Consortium to evaluate pancreatic cancer risk among ST users through a meta-analysis. Although this is an assemblage of international studies, the studies, which included ST exposure assessment, were all U.S. studies except one. Therefore, these results are applicable

[25] Adjusted for race and education.
[26] Adjusted for age, sex, race/ethnicity, cigarette smoking, history of diabetes, alcohol consumption, educational level, state of residency, and marital status.
[27] Adjusted for age, alcohol and smoking.

to U.S. ST products. Among ever ST users, there were 130 pancreatic cancer cases with 267 controls. The OR for ever ST users, compared with never tobacco users, was 0.98 (95 percent CI: 0.75-1.27).[28] For patients who only ever used ST, the OR for pancreatic cancer risk was 0.62 (95 percent CI: 0.37-1.04). The authors noted that risk estimates could be biased due to underreporting of tobacco consumption.

Lee and Hamling (2009b) conducted a systematic review and meta-analysis evaluating pancreatic cancer risk among ST users. On the basis of risk estimates from five U.S. studies, these authors calculated the RR of pancreatic cancer for ST users to be 0.86 (95% CI: 0.47-1.57). Limiting the analysis to studies that adjusted for smoking produced a risk estimate of 0.99 (95% CI: 0.51-1.91). Three studies included pancreatic cancer risk estimates for never-smoking ST users; a meta-analysis of these results yielded an estimate of 1.09 (95% CI: 0.44-2.67). Another meta-analysis from the same group and using much of the same published data calculated the risk for pancreatic cancer among U.S. and Canadian ST users to be 0.92 (95% CI: 0.65-1.29) when using a fixed-effect model and to be 0.89 (95% CI: 0.50-1.60) when using a random-effect model (Sponsiello-Wang, 2008). The authors noted that "…no increased risk is demonstrated in studies in North America or in case-control studies (all of which were in North America), there is some evidence of an increased risk in studies in Sweden or Norway and in the cohort studies." Additionally, for both meta-analyses, the authors noted various issues with the underlying study data, including small sample sizes, limited adjustment for potential confounders, and use of surrogates to identify potential pancreatic cancer risk factors.

Table 7.5.6-1-14 summarizes published literature assessing the association between ST use and pancreatic cancer.

[28] Adjusted for center, race, sex, age, education, history of diabetes, body mass index and total alcohol consumption.

Table 7.5.6-1-14: Literature Evaluating the Relationship Between ST Use and Pancreatic Cancer

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Falk, 1988)	Life-style risk factors for pancreatic cancer in Louisiana: a case-control study	Case-control study 363 cases 1,234 age, sex, and race-matched hospital controls Less than 3% of study participants used snuff	"No excess risks"	None reported	Age, smoking, coffee consumption, income, diet, alcohol consumption, education, ethnicity, family history of cancer, residence	Limitations Small number of exposed cases/controls
(Farrow, 1990)	Risk of pancreatic cancer in relation to medical history and the use of tobacco, alcohol and coffee.	Case-control study Cancer Surveillance System (CSS) of the Fred Hutchinson Cancer Research Center Cases Married men ages 20-74 148 cases/188 controls 6.9% of subjects ever chewed tobacco	"The use of cigar, pipe and chewing tobacco do not affect the risk of disease"	Ever ST use OR = 0.8 (CI not reported)	None	Limitations Small number of exposed cases/controls
(Zheng, 1993)	A cohort study of smoking, alcohol consumption, and dietary factors for pancreatic cancer (United States).	Cohort study White men in the United States (1966 with follow-up in 1986) White males N= 16 deaths among ST users .	"Among ever-users of smokeless tobacco, the age-, alcohol-, and smoking adjusted risk was increased, although not statistically significant"	Ever ST use RR = 1.7 (0.9-3.1)	Age, alcohol and smoking	Limitations Small number of pancreatic cancer deaths among exposed group

Table 7.5.6–1-14: Literature Evaluating the Relationship Between ST Use and Pancreatic Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Muscat, 1997)	Smoking and pancreatic cancer in men and women.	Case-control study Hospital subjects (1985-1993) N = 484 patients with pancreatic carcinoma N = 954 controls	"Tobacco juice may also cause pancreatic cancer when ingested or absorbed through the oral cavity."	Regular ST user OR = 3.6 (1.0-12.8)	None	Limitations Small number of exposed cases/controls, Use of proxies for identification of exposure Lack of adjustment for potential confounders
(Alguacil, 2004)	Smokeless and other noncigarette tobacco use and pancreatic cancer: a case-control study based on direct interviews.	Case-control study Atlanta, Georgia, Detroit, Michigan, and 10 counties in New Jersey (1986-1989) 7 cases/44 controls ever used ST 5 cases/28 controls only used ST	"Subjects who ever used smokeless tobacco and never smoked cigarettes had a 40% increased risk of pancreatic cancer..."	Ever used ST OR = 1.4 (0.5-3.6) Only used ST OR = 1.1 (0.4-3.1) Ounces per wk ≤2.5, OR = 0.3 (0.04-2.5) >2.5, OR = 3.5 (1.1-10.6) No. of years used ≤20, OR = 1.1 (0.1-11.0) >20, OR = 1.5 (0.6-4.0)	Race, gender, geographic site, cigar smoking and age	Strengths Analyses based solely on nonsmokers of cigarettes Population-based study design Exposure assessment based on direct interview Limitations Small number of exposed cases

Table 7.5.6–1-14: Literature Evaluating the Relationship Between ST Use and Pancreatic Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Hassan, 2007)	Passive smoking and the use of noncigarette tobacco products in association with risk for pancreatic cancer: a case-control study.	Case-control study The University of Texas M.D. Anderson Cancer Center (2000-2006) 34 ever chewing tobacco using cases, 54 controls 18 ever snuff using cases, 34 controls	"The current observations did not support a role for passive smoking or the use of noncigarette tobacco products in the etiology of pancreatic cancer"	Snuff - ever use OR = 0.6 (0.3-1.1) Chewing tobacco - ever use OR = 0.7 (0.4-1.1)	Age, sex, race/ethnicity, cigarette smoking, history of diabetes, alcohol consumption, educational level, state of residency, and marital status	Limitations Small number of exposed patients Strengths Adjustment for many potential confounders Analysis of nonsmoking ST users
(Sponsiello-Wang, 2008)	Systematic review of the relation between smokeless tobacco and cancer of the pancreas in Europe and North America.	Meta-analysis Four U.S. studies, one Canadian study included in "USA or Canada" meta-analysis	No increased risk is demonstrated in studies in North America or in case-control studies (all of which were in North America)	FE RR/OR = 0.92 (0.65-1.29) RE RR/OR = 0.89 (0.50-1.60)	N/A	Limitations [Of studies included in meta-analysis] Small numbers of exposed cases Tobacco use assessed at baseline only Limited reporting of data specific to ST

Table 7.5.6–1-14: Literature Evaluating the Relationship Between ST Use and Pancreatic Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Lee, 2009b)	Systematic review of the relation between smokeless tobacco and cancer in Europe and North America.	Meta-analysis Available published epidemiological cohort and case-control studies relating any form of cancer to ST use. Overall data: 5 estimates Smoking-adjusted data: 5 estimates Never-smoking data: 3 estimates	No significant associations are seen in the separate meta-analyses for the United States	U.S. data Overall data: RE RR/OR = 0.86 (0.47-1.57) Smoking-adjusted data: RE RR/OR = 0.99 (0.51-1.91) Never smokers: RE RR/OR = 1.09 (0.44-2.67)	N/A	Limitations [Of studies included in meta-analysis] Small numbers of cases in may studies Unclear description of inclusion and exclusion criteria, Lack of clear description of ST type used Failure to adjust for confounders, especially smoking
(Bertuccio, 2011)	Cigar and pipe smoking, smokeless tobacco use and pancreatic cancer: an analysis from the International Pancreatic Cancer Case-Control Consortium (PanC4).	Meta-analysis 11 case-control studies of pancreatic cancer Eight studies were conducted in North America	"Our results on smokeless tobacco use are in broad agreement with a recently published meta-analysis of all published data on the issue, which reported no excess risk of pancreatic cancer in case-control studies"	Ever ST users OR = 0.98 (0.75-1.27) ST-only user OR = 0.62 (0.37-1.04)	Center, race, sex, age, education, history of diabetes, body mass index, and total alcohol consumption.	Limitations (Of studies included in meta-analysis) Potential underreporting of tobacco consumption, especially between cases and controls.

Table 7.5.6–1-14: Literature Evaluating the Relationship Between ST Use and Pancreatic Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Burkey, 2014)	The association between smokeless tobacco use and pancreatic adenocarcinoma: A systematic review.	Systematic review 11 studies (three cohort studies, seven case-control studies, and one study that pooled data from multiple case-control studies)	"The association between smokeless tobacco use and pancreatic adenocarcinoma is inconclusive. More definitive conclusions regarding this relationship await the results of more methodologically rigorous epidemiologic studies."	N/A	N/A	Limitations [Of studies included in review] Heterogeneous exposure assessment. Results limited population subgroups studied (e.g., males)

Overall, the U.S epidemiology data regarding a possible association between ST use and pancreatic cancer are mixed (Table 7.5.6-1-15). The majority of studies found no association, with only two cohorts showing a weak association. The Alguacil and Silverman study (2004) does provide some evidence of a potential dose response since the heaviest users of ST had a statistically significant elevated risk for pancreatic cancer. The three meta-analyses, which consider the data included here, do not provide evidence of an excess risk of pancreatic cancer among ST users in the U.S.

Table 7.5.6-1-15: Summary of Published Pancreatic Cancer Risk Estimates for ST Users

Study	Group	ST Exposure	Risk Estimate	95% Confidence Interval
(Farrow, 1990)	Males	Ever	OR = 0.8	not significant
(Zheng, 1993)	Males	Ever	RR = 1.7	0.9-3.1
(Muscat, 1997)	Males	Regular chew	OR = 3.6	1.0-12.8
(Alguacil, 2004)	Males + females	Ever	OR = 1.4	0.5-3.6
		Only exclusive	OR = 1.1	0.4-3.1
		≤2.5 oz ST/wk	OR = 0.3	0.04-2.5
		>2.5 oz ST/wk	OR = 3.5	1.1-10.6
		Used ≤20 years	OR = 1.1	0.1-11.0
		Used >20 years	OR = 1.5	0.6-4.0
(Hassan, 2007)	Males + females	Ever - snuff	OR = 0.6	0.3-1.1
		Ever - chew	OR = 0.7	0.4-1.1
(Sponsiello-Wang, 2008)	Meta-analysis	Fixed effect	RR/OR = 0.92	0.65-1.29
		Random effect	RR/OR = 0.89	0.50-1.60
(Lee, 2009b)	Meta-analysis	Overall data	RE RR/OR =0.86	0.47-1.57
	-	Smoking adjusted	RE RR/OR =0.99	0.51-1.91
	-	Never smokers	RE RR/OR =1.09	0.44-2.67
(Bertuccio, 2011)	Meta-analysis	Ever	OR = 0.98	0.75-1.27
	-	Only ever	OR = 0.62	0.37-1.04

7.5.6-1.2.4.6. Relationship Between ST Use and Hematopoietic and Lymphoid Cancer

We identified four case-control studies, two cohort studies, and one meta-analysis with data assessing the association between ST use and hematopoietic or lymphoid cancers.

Bracci and Holly (2005) reported a significant increased risk of non-Hodgkin lymphoma among men who used ST. The study was a population-based, case-control study and included seven cases with six controls who reported current use of snuff or chewing tobacco. The OR for current ST users compared with non-users was 4.0 (95 percent CI: 1.3-12.0).[29] Exposure was assessed through in-home interviews.

Brown et al. (1992a) conducted two population-based, case-control studies that evaluated risk of non-Hodgkin lymphoma and multiple myeloma among ever ST users. The OR for non-Hodgkin lymphoma among ever ST users was 1.3 (95 percent CI: 0.7-2.5) and for multiple myeloma was 1.9 (95 percent CI: 0.5-6.6).[30] For the non-Hodgkin lymphoma study, 19 cases with 23 controls reported ever ST use. The result for multiple myeloma was based on 5 cases with 8 controls. Exposure was assessed through interviews with patients or proxies.

Brown et al. (1992b) also assessed the risk of leukemia among ever ST users in a population-based, case-control study that included 24 cases with 23 controls. The OR for leukemia among ever ST users compared with never tobacco users was 1.8 (95 percent CI: 0.9-3.3).[31]

Schroeder et al. (2002) found no excess risk of non-Hodgkin lymphoma among ever snuff and ever chewing tobacco users in a population-based, case-control study. The ORs for ever snuff users and ever chewing tobacco users, compared with never tobacco users, were 1.0 (95 percent CI: 0.7-1.4) and 1.3 (95 percent CI: 0.9-1.8), respectively.[32] For ever snuff users, there were 62 cases with 137 controls and there were 68 cases with 112 controls who ever used chewing tobacco.[33] These authors detected a significant excess risk (RR = 2.5) of the positive non-Hodgkin lymphoma subtype t(14;18) among chewing tobacco users who were 18 or younger (OR: 2.5, 95 percent CI: 1.0-6.0).[34] The authors noted that this result is "based on a small number of exposed cases."

Heineman et al. (1992) evaluated risk of myeloma among snuff or chewing tobacco users in the prospective cohort study of U.S. military veterans. No excess risk was detected among ST users compared with never users of tobacco (RR: 1.0, 95 percent CI: 0.4-2.3).[35] This result was based on six deaths among ST users.

Henley et al. (2005) assessed hematopoietic cancer risk in the CPS-II cohort. For hematopoietic cancers, the multivariate-adjusted HR for current ST users compared with that for never tobacco users was 0.95 (95 percent CI: 0.60-1.51).[36]

Lee and Hamling (2009b) conducted a systematic review and meta-analysis evaluating non-Hodgkin lymphoma risk among ST users. On the basis of risk estimates from three U.S.

[29] Adjusted for age, education and alcohol.
[30] Adjusted for age and location.
[31] Adjusted for age, location and alcohol.
[32] Adjusted for state, vital status, age.
[33] Numbers of cases and controls calculated from proportions provided in reference.
[34] Adjusted for state and age.
[35] Adjusted for age, calendar time, and year of questionnaire response.
[36] Adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use.

studies, these authors calculated the RR of non-Hodgkin lymphoma for ST users to be 1.45 (95 percent CI: 0.81-2.59). When the authors limited the analysis to studies that adjusted for smoking or included never-smoking ST users, they calculated a risk estimate of 2.07 (95 percent CI: 0.70-6.13). Considering data from U.S. and European studies, these authors concluded that there was "no significant relationship" between ST use and non-Hodgkin lymphoma; noting that the data "do not suggest any relationship" between ST use and other hematopoietic and lymphoid cancers.[37]

Table 7.5.6-1-16 summarizes published literature assessing the association between ST use and hematopoietic and lymphoid cancer.

[37] Note that this meta-analysis includes three older U.S. studies which we were unable to locate.

Table 7.5.6-1-16: Literature Evaluating the Relationship Between ST Use and Hematopoietic and Lymphoid Cancers

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Brown, 1992a)	Smoking and risk of non-Hodgkin's lymphoma and multiple myeloma.	Case-control study White men in Iowa and Minnesota NHL: N= 19 ST using cases, 23 controls Multiple myeloma: N= 5 ST using cases, 8 controls	"Risks [of multiple myeloma] were not significantly elevated for use of any tobacco product."	NHL: Ever ST use OR = 1.3 (0.7-2.5) Multiple myeloma: Ever ST use OR = 1.9 (0.5-6.6)	Age, location	Limitations Very few exposed cases/controls No adjustment for certain potential confounders
(Brown, 1992b)	Smoking and risk of leukemia.	Case-control study Iowa Health Registry (1981-1984) N= 24 ST using cases, 23 controls	No specific conclusions stated	All leukemia: Ever ST use OR = 1.8 (0.9-3.3)	Adjusted for age, state, and alcohol use	Limitations Very few exposed cases/controls
(Heineman, 1992)	A prospective study of tobacco use and multiple myeloma: evidence against an association.	Cohort study Cohort American veterans followed prospectively for 26 years Number of exposed participants not reported	"The risk of death from myeloma was not increased...among users of chewing tobacco or snuff"	Current ST users RR = 1.0 (0.4-2.3)	Age, calendar time, and year of questionnaire response	Limitations Results based on 6 deaths

Table 7.5.6–1-16: Literature Evaluating the Relationship Between ST Use and Hematopoietic and Lymphoid Cancers (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Schroeder, 2002)	A case-control study of tobacco use and other non-occupational risk factors for t(14;18) subtypes of non-Hodgkin's lymphoma (United States).	Case-control study National Cancer Institute's Factors Affecting Rural Men 11% of cases, 9% of controls reported chewing tobacco use 10% of cases, 11% of controls reported snuff use Total 622 cases, 1,245 controls	"Evidence of an association between chewing tobacco and t(14;18)-positive NHL, particularly among those who began use at an early age."	Snuff OR = 1.0 (0.7-1.4) Chewing tobacco OR = 1.3 (0.9-1.8) Chewing tobacco initiation >age 18 t(14:18)+ NHL, OR = 1.3 (0.6-2.9) t(14:18)- NHL, OR = 1.2 (0.6-2.2) Chewing tobacco initiation ≤age 18 t(14:18)+ NHL, OR = 2.5 (1.0-6.0) t(14:18)- NHL, OR = 1.0 (0.3-3.0)	State, vital status, age	<u>Limitations</u> Small number of exposed cases
(Bracci, 2005)	Tobacco use and non-Hodgkin lymphoma: results from a population-based case-control study in the San Francisco Bay Area, California.	Case-control study San Francisco Bay Area between (1988-1995) N= 7 exposed cases, 6 exposed controls	"ORs were increased for NHL among men who used…smokeless tobacco alone"	Current ST users OR = 4.0 (1.3-12.0)	Age, education and average weekly consumption of alcoholic beverages	<u>Limitations</u> Very small number of exposed cases/controls

Table 7.5.6–1-16: Literature Evaluating the Relationship Between ST Use and Hematopoietic and Lymphoid Cancers (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Henley, 2005)	Two large prospective studies of mortality among men who use snuff or chewing tobacco (United States).	Cohort study 1982 CPS-II Males <u>CPS-II</u> Exclusive snuff or chewing tobacco use: N = 3,327 No previous use of any tobacco product N = 111,482 18-year follow-up: N = 19,588 deaths “In both cohorts, ACS volunteers invited families of their friends, neighbors, and acquaintances to participate.”	"... inappropriate to recommend the use of spit tobacco as an alternative to tobacco smoking unless there is persuasive evidence that these products are less hazardous...”	CPS-II Current ST users (males) HR = 0.95 (0.60-1.51) Former ST users (males) HR = 1.16 (0.60-2.25)	CPS-II age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use	<u>Strengths</u> Large Samples size and prospective design <u>Limitations</u> Exposure assessment conducted only at baseline Participants more likely to be more educated, married, middle-class, and white than the general U.S. population

Table 7.5.6–1-16: Literature Evaluating the Relationship Between ST Use and Hematopoietic and Lymphoid Cancers (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Lee, 2009b)	Systematic review of the relation between smokeless tobacco and cancer in Europe and North America.	Meta-analysis Available published epidemiological cohort and case-control studies relating any form of cancer to ST use. Overall data: 3 estimates Smoking-adjusted data: 2 estimates Never-smoking data: 2 estimates	"The evidence for other endpoints – multiple myeloma, Hodgkin's disease, leukaemia, and overall haematopoietic cancer – is more limited, and does not suggest any relationship with ST use."	Overall data: RE RR/OR = 1.45 (0.81-2.59) Smoking-adjusted data: RE RR/OR = 2.07 (0.70-6.13) Never smokers: RE RR/OR = 2.07 (0.70-6.13)	N/A	Limitations [Limitations of studies included in meta-analysis] Small numbers of cases in many studies Unclear description of inclusion and exclusion criteria, Lack of clear description of ST type used Failure to adjust for confounders, especially smoking

While the overall data related to hematopoietic or lymphoid cancers and use of ST products in the U.S. are mixed, realistically, the data suggest no relevant elevated risk due to ST use (Table 7.5.6-1-17). Only one risk estimate (Bracci, 2005) from a small study with wide CIs indicated a difference between ST users and never tobacco users. All other studies found no significant association between ST use and hematopoietic and lymphoid cancer risk.

Table 7.5.6-1-17: Summary of Published Hematopoietic and Lymphoid Cancer Risk Estimates for ST Users

Cancer type	Study	Group	ST Exposure	Risk Estimate	95% Confidence Interval
Non-Hodgkin lymphoma	(Brown, 1992a)	Males	Ever ST	OR = 1.3	0.7-2.5
	(Schroeder, 2002)	Males	Ever snuff	OR = 1.0	0.7-1.4
			Ever chew	OR = 1.3	0.9-1.8
	(Bracci, 2005)	Males	Current ST	OR = 4.0	1.3-12.0
	(Lee, 2009b)	-	Overall data	RE RR/OR = 1.45	0.81-2.59
			Smoking adjusted	RE RR/OR = 2.07	0.70-6.13
			Never smokers	RE RR/OR = 2.07	0.70-6.13
Myeloma	(Brown, 1992a)	Males	Ever ST	OR = 1.9	0.5-6.6
	(Heineman, 1992)	Males	Current ST	RR = 1.0	0.4-2.3
Leukemia	(Brown, 1992b)	Males	Ever ST	OR = 1.8	0.9-3.3
Hematopoietic cancers	(Henley, 2005)	Males: CPS-II	Current ST	HR = 0.95	0.60-1.51

7.5.6-1.2.4.7. Relationship Between ST Use and Kidney Cancer

We identified seven publications studies that evaluated the relationship between ST use in the U.S. and risk of kidney cancer, including renal cell carcinoma (RCC). These include six case-control studies and one meta-analysis.

Based on the results of a case-control study on RCC, conducted in Oklahoma, Asal et al. (1988) concluded that "the association [of RCC] with snuff use is impressive and statistically significant." For RCC, the OR for ever snuff users compared with never tobacco users was 3.6 (95 percent CI: 1.2-13.3).[38] The number of snuff users among cases with controls was

[38] Adjusted for age, race, hospital, and time of admission.

not provided, and the exposures were assessed through hospital interviews and through home interviews for population controls.

Bennington and Laubscher (1968) concluded that "tobacco chewers…appear to have a greater risk of developing renal carcinoma than nonusers" based on five exposed cases and eight exposed controls. A risk estimate was not calculated, but the proportion of tobacco chewers between cases and controls was significant at the 0.05 level when using the chi-squared test.

Goodman et al. (1986) also reported a positive association between ever use of chewing tobacco and risk of renal cell cancer. This conclusion was based on a hospital-based case-control study (13 exposed male cases, with 4 exposed male controls) resulting in an OR of 4.00 (95 percent CI: 1.13-14.17).[39] Exposures were assessed based on personal interviews.

McLaughlin et al. (1984) conducted a population-based, case-control study (495 cases with 697 controls) that included ever snuff or chewing tobacco users. For RCC, the OR among ever snuff users (11 cases) compared with never tobacco users was 1.7 (95 percent CI: 0.5-6.0); the OR for ever chewing tobacco users (12 cases) was 0.4 (95 percent CI: 0.1-2.6).[40]

Muscat et al. (1995) found a statistically significant association between ever use of chewing tobacco and RCC in a hospital-based case-control study. Overall, the OR for ever chewing tobacco users compared with never tobacco users was 3.2 (95 percent CI: 1.1-8.7).[41] There was an apparent dose response, with the OR for those reporting using chewing tobacco less than 10 times per week being 2.5 (95 percent CI: 1.0-6.1) and for those using chewing tobacco 10 times per week or more being 6.0 (95 percent CI: 1.9-18.7). The authors noted that this result is based on only 14 exposed cases. Exposure was assessed using in-hospital interviews.

Yuan et al. (1998) reported that "no increased risk of RCC was observed for the use of…smokeless tobacco" in a case-control study that included 32 cases and 27 controls reporting ever use of snuff or chewing tobacco. The OR for RCC among ST users compared with that among never tobacco users was 1.02 (95 percent CI: 0.56-1.85).[42] The authors noted that few ST users were exclusive users.

Lee and Hamling (2009b) conducted a systematic review and meta-analysis evaluating kidney cancer risk among ST users. On the basis of risk estimates from eight U.S. studies, these authors calculated the RR of kidney cancer for ST users to be 1.52 (95 percent CI: 0.94-2.46). When the authors limited the analysis to the three studies that adjusted for smoking, they produced a risk estimate of 1.41 (95 percent CI: 0.64-3.10). One study included a kidney cancer risk estimate for never-smoking ST users of 4.80 (95 percent CI: 1.18-19.56). The authors noted that most risk estimates are elevated, although not statistically significant, and concluded that "there is a suggestion of a possible relationship [but] more data are needed before any firm conclusions can be reached."

[39] Adjusted for age, race, hospital, and time of admission.
[40] Adjusted for age and cigarette smoking.
[41] Adjusted for age and education.
[42] Adjusted for education, cigarettes per day, current smoking status

Table 7.5.6-1-18 summarizes published literature assessing the association between ST use and kidney cancer.

Table 7.5.6-1-18: Literature Evaluating the Relationship Between ST Use and Kidney Cancer

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Bennington, 1968)	Epidemiologic studies on carcinoma of the kidney. I. Association of renal adenocarcinoma with smoking.	Case-control study Patients with renal adenocarcinoma N= 5 exposed cases, 8 exposed controls	"While tobacco chewers also appear to have a greater risk of developing renal adenocarcinoma than nonusers of tobacco, it is not as great as for pipe or cigar smokers."	Not calculated	N/A	Limitations Very small number of exposed cases/controls
(McLaughlin, 1984)	A population--based case--control study of renal cell carcinoma.	Case-control study Minneapolis-St. Paul seven-county metropolitan area (1979-1980) 495 exposed cases/697 controls	The OR for snuff use, adjusted for age and cigarette smoking, was 1.7 (0.5-6.0), whereas the adjusted OR for use of chewing tobacco was 0.4 (0.1-2.6).	Snuff (ever use) OR = 1.7 (0.5-6.0) Chewing tobacco (ever use) OR = 0.4 (0.1-2.6)	Age, cigarette smoking	None
(Goodman, 1986)	A case-control study of factors affecting the development of renal cell cancer.	Case-control study 18 hospital centers in six U.S. cities (1977-1983) N= 13 exposed cases, 4 controls Controls matched for hospital, sex, race, age ±5 years, and time of admission)	"The use of chewing tobacco was positively associated with disease"	Males - ever chewing tobacco use OR = 4.0 (1.13-14.17)	Age, race, hospital, time of admission	Limitations Very small number of cases/controls

Table 7.5.6–1-18: Literature Evaluating the Relationship Between ST Use and Kidney Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Asal, 1988)	Risk factors in renal cell carcinoma: I. Methodology, demographics, tobacco, beverage use, and obesity.	Case-control study Patients from 29 hospitals in Oklahoma, including Tulsa and Oklahoma City. Number of exposed cases/controls not reported	"The association [of RCC] with snuff use is impressive and statistically significant."	Ever snuff use OR = 3.6 (1.2-13.3)	Age, race, hospital, time of admission	Limitations Difficult to interpret finding without sample size
(Muscat, 1995)	The epidemiology of renal cell carcinoma. A second look.	Case-control study Data from a hospital-based case-control study, 1977 to 1993 Among men, 2.6% of cases/1.0% of controls ever chewed tobacco regularly 543/529 male cases/controls No women reported having chewed tobacco or smoked pipes and cigars	"Among men, the OR associated with chewing tobacco was 3.2 (95% CI: 1.1-8.7)."	Overall OR = 3.2 (1.1-8.7) Uses per week <10 OR = 2.5 (1.0-6.1) ≥10 OR = 6.0 (1.9-18.7)	Age and education	Limitations Small number of exposed cases, controls

Table 7.5.6–1-18: Literature Evaluating the Relationship Between ST Use and Kidney Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Yuan, 1998)	Tobacco use in relation to renal cell carcinoma.	Case-control study Population-based study in Los Angeles, CA 32 exposed cases, 27 exposed controls (ever use)	"...no increased risk of RCC was observed for the use of ...ST."	Ever ST use OR = 1.02 (0.56-1.85)	Education, cigarettes per day, current smoking status	None
(Lee, 2009b)	Systematic review of the relation between smokeless tobacco and cancer in Europe and North America.	Meta-analysis Available published epidemiological cohort and case-control studies relating any form of cancer to ST use. Overall data: 8 estimates Smoking-adjusted data: 3 estimates Never-smoking data: 1 estimate	"While there is a suggestion of a possible relationship [between ST use and kidney cancer], more data are needed before any firm conclusions can be reached."	Overall data RE RR/OR = 1.52 (0.94-2.46) Smoking adjusted RE RR/OR = 1.41 (0.64-3.10) Never smokers RE RR/OR = 4.80 (1.18-19.56)	N/A	Limitations [Limitations of studies included in meta-analysis] Small numbers of cases in many studies Unclear description of inclusion and exclusion criteria, Lack of clear description of ST type used Failure to adjust for confounders, especially smoking

Overall, the data related to a potential association between ST use and kidney cancer are mixed (Table 7.5.6-1-19). We note that several small studies suggested the possibility that some association may exist; however, many of the studies contained small numbers of exposed cases, and some studies did not adjust for potential confounding variables.

Table 7.5.6-1-19: Summary of Published Kidney Cancer Risk Estimates for ST Users

Study	Group	ST Exposure	Risk Estimate	95% Confidence Interval
(McLaughlin, 1984)	Males	Ever snuff	OR = 1.7	0.5-6.0
		Ever chew	OR = 0.4	0.1-2.6
(Asal, 1988)	Males	Ever snuff	OR = 3.6	1.2-13.3
(Muscat, 1995)	Males	Ever chew	OR = 3.2	1.1-8.7
		<10 times per wk	OR = 2.5	1.0-6.1
		≥10 times per wk	OR = 6.0	1.9-18.7
(Goodman, 1986)	Males	Ever chew	OR = 4.0	1.13-14.17
(Yuan, 1998)	Males + females	Ever ST	OR = 1.02	0.56-1.85
(Lee, 2009b)	Meta-analysis	Overall data	RE RR/OR = 1.52	0.94-2.46
		Smoking adjusted	RE RR/OR = 1.41	0.64-3.10
		Never smokers	RE RR/OR = 4.80	1.18-19.56

7.5.6-1.2.4.8. Relationship Between ST Use and Prostate Cancer

Five studies, including a meta-analysis, have evaluated ST use as a risk factor for prostate cancer.

Accortt et al. (2005) evaluated the risk of prostate cancer incidence among ST users in the NHEFS. These authors concluded that there was "no substantial increase among ST users compared to non-tobacco users" for risk of prostate cancer. The adjusted HR was 1.2 (95 percent CI: 0.5-3.4) based on 19 cases.[43] This study relied on self-reporting for identification of risk factors, including ST use.

Hayes et al. (1994) reported the results of a population-based case-control study among 981 confirmed prostate cancer cases with 1,315 controls. The study sample included current and former chewing tobacco and snuff users. The OR for current chewing tobacco users (14 cases with 33 controls) compared with never tobacco users was 0.5 (95% CI: 0.2-1.0); for former chewing tobacco users (56 cases with 69 controls), the OR was 1.0 (95% CI: 0.6-1.5). For current snuff users (10 cases with 2 controls) and former snuff users (10 cases with 17

[43] Adjusted for age, race and poverty index ratio

controls) the ORs were 5.5 (95% CI: 1.2-26.2) and 0.6 (95% CI: 0.3-1.4), respectively.[44] The authors noted that the finding of excess risk among current snuff users could be due to "chance."

Hsing et al. (1990) assessed risk of prostate cancer among exclusive ST (snuff and chewing tobacco) users in the Lutheran Brotherhood Cohort. There were 10 prostate cancer deaths among exclusive ST users, and the age-adjusted RR, compared with that for never tobacco users, was 4.5 (95 percent CI: 2.1-9.7). When the analysis was expanded to include those who had ever used ST (42 prostate cancer deaths), the age-adjusted RR for prostate cancer was 2.1 (95 percent CI: 1.1-4.1). The risks for former and occasional users were not statistically different from those for never users (RR = 1.8 and 1.4, respectively). However, the RR for regular current ST users (24 prostate cancer deaths) was 2.4 (95 percent CI: 1.3-4.9). Tobacco use information was collected only at baseline, and the intensity and duration of ST use were not evaluated.

Hsing et al. (1991) also evaluated prostate cancer risk among ST users in the U.S. Veterans Cohort comprising 293,916 individuals who served in the military between 1917 and 1940. There were 4,607 prostate cancer deaths in this cohort, including 48 deaths among exclusive ST users. The age-adjusted RR for prostate cancer mortality among exclusive ST users was 1.17 (95 percent CI: 0.88-1.56).

Lee and Hamling (2009b) conducted a systematic review and meta-analysis evaluating prostate cancer risk among ST users. On the basis of risk estimates from five U.S. studies, these authors calculated the RR of prostate cancer mortality for ST users to be 1.20 (95 percent CI: 1.03-1.40).

Table 7.5.6-1-20 summarizes published literature assess the association between ST use and prostate cancer.

[44] Odds ratios adjusted for age, race, and study site.

Table 7.5.6-1-20: Literature Evaluating the Relationship Between ST Use and Prostate Cancer

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Hsing, 1990)	Diet, tobacco use, and fatal prostate cancer: results from the Lutheran Brotherhood Cohort Study.	Cohort study Epidemiological questionnaire (1966 and was followed until 1986) N = 17,633 white males age 35 or older 42 deaths among ever ST users (snuff or chewing tobacco)	"Risks were significantly elevated among persons who ever used any form of tobacco (RR = 1.8, 95% Cl, 1.1-2.9). both among cigarette smokers and users of smokeless tobacco."	Ever ST use RR = 2.1 (1.1-4.1 Former use RR = 1.8 (0.8-3.9) Occasional use RR = 1.4 (0.5-3.9) Regular use RR = 2.4 (1.3-4.9) Exclusive use RR = 4.5 (2.1-9.7)	Age, cigarette smoking	Strengths Large cohort size Prospective design 20 year follow-up Limitations Lack of adjustment for potential confounders Exposure assessed at baseline only
(Hsing, 1991)	Tobacco use and prostate cancer: 26-year follow-up of US veterans.	Cohort study 1954 or 1957 U.S. survey of Armed Forces veterans, 26-year follow-up N = 293,916 veterans aged 31-84 y (as of 1953) 48 deaths among ST only users	"There were small non-significant increases in the risk of prostate cancer…among users of smokeless tobacco"	Ever ST use RR = 1.17 (0.88-1.56)	Age	Strengths Prospective design 26 year follow up Limitations Lack of adjustment for potential confounders Exposure assessed at baseline only

Table 7.5.6–1-20: Literature Evaluating the Relationship Between ST Use and Prostate Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Hayes, 1994)	Tobacco use and prostate cancer in blacks and whites in the United States.	Case-control study Population-based study conducted in Atlanta, Detroit and 10 New Jersey counties 14 current chewing tobacco using cases, 33 controls 10 current snuff using cases, 2 controls	The risk associated with [current] snuff use was OR = 5.5 (1.2-26.2). This subgroup finding may have been due to chance	Current chew use OR = 0.5 (0.2-1.0) Current snuff use OR = 5.5 (1.2-26.2)	Age, race and study site	Limitations Small number of cases/controls
(Accortt, 2005)	Cancer incidence among a cohort of smokeless tobacco users (United States).	Cohort study First National Health and Nutrition Examination Survey Epidemiologic Followup Study (1971-1975) N = 414 ST users N = 2,979 non-ST users	No substantial increase among ST users compared with non-users	Ever ST use HR =1.2 (0.5-3.4)	Race and poverty index ratio	Strengths Based on a national probability sample Limitations Reliance on self-reporting for exposure assessment, potential confounders Ever use of ST as exposure category

Table 7.5.6–1-20: Literature Evaluating the Relationship Between ST Use and Prostate Cancer (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Lee, 2009b)	Systematic review of the relation between smokeless tobacco and cancer in Europe and North America.	Meta-analysis Available published epidemiological cohort and case-control studies relating any form of cancer to ST use. Overall data: 5 estimates Smoking-adjusted data: 4 estimates Never-smoking data: 3 estimates	Based on the five studies that provide usable data, the overall estimate is 1.20 (95% CI: 1.03-1.40).	Overall data: RE RR/OR = 1.20 (1.03-1.40) Smoking adjusted: RE RR/OR = 1.29 (1.07-1.55) Never smokers: RE RE/OR = 1.81 (0.76-4.30)	N/A	Limitations [limitations of studies included in meta-analysis] Small numbers of cases in may studies Unclear description of inclusion and exclusion criteria, Lack of clear description of ST type used Failure to adjust for confounders, especially smoking

Overall, the U.S. epidemiology data assessing the association between ST use and prostate cancer risk are mixed (Table 7.5.6-1-21). Four of the eight risk estimates presented in the individual studies are statistically significantly different from those for never tobacco users, although all have some uncertainty with associated wide CIs.

Table 7.5.6-1-21: Summary of Published Prostate Cancer Risk Estimates for ST Users

Study	Group	ST Exposure	Risk Estimate	95% Confidence Interval
(Hsing, 1990)	Males	Exclusive ST	RR = 4.5	2.1-9.7
		Ever ST	RR = 2.1	1.1-4.1
		Occasional ST	RR = 1.4	0.5-3.9
		Regular ST	RR = 2.4	1.3-4.9
(Hsing, 1991)	Males	Ever ST	RR = 1.17	0.88-1.56
(Hayes, 1994)	Males	Current chew	OR = 0.5	0.2-1.0
		Current snuff	OR = 5.5	1.2-26.2
(Accortt, 2005)	Males	Ever	HR = 1.2	0.5-3.4
(Lee, 2009b)	Meta-analysis	Overall data	RE RE/OR = 1.20	1.03-1.40
		Smoking adjusted	RE RE/OR = 1.29	1.07-1.55
		Never smokers	RE RE/OR = 1.81	0.76-4.30

7.5.6-1.2.4.9. Relationship between ST Use and Other Cancers

This section briefly summarizes investigations of association between ST use and specific cancer types not addressed in prior sections and for which the data are limited to one or two publications.

Nasal and paranasal sinuses

Brinton et al. (1984) evaluated risk of nasal and paranasal sinus cancers among male and female ever snuff users (23 cases with 28 controls) and chewing tobacco users (15 cases with 37 controls) in a case-control study. The RRs for nasal and paranasal sinus cancer for snuff users were 1.47 (95 percent CI: 0.8-2.8) and for chewing tobacco users 0.74 (95 percent CI: 0.4-1.5). The authors reported an elevated risk of nasal and paranasal adenocarcinoma among snuff users (RR: 3.06, CI was not provided). However, this result is based on six cases.

Soft tissue sarcoma

Zahm et al. (1989) published the results of a population-based, case-control study that investigated the relationship between ever use of ST products and soft tissue sarcoma (STS) (28 cases with 127 controls). Exposure to risk factors was assessed through in-person

interviews with patients or proxies. The authors detected a "significant excess risk associated with the use of chewing tobacco or snuff" (OR: .8, 95 percent CI: 1.1-2.9).

Zahm et al. (1992) reported that ever ST users in the U.S. Veterans Cohort had "a nonsignificant 40 percent excess of STS" mortality when compared with that for never tobacco users.[45] However, there were no STS deaths among the 2,308 participants who only ever used ST (snuff or chewing tobacco). Analyses of ever ST users stratified by age indicated that those using ST for less than 5 years had a significant excess risk of STS mortality (RR: 2.9, 95 percent CI: 1.3-6.3) based on nine STS deaths. An excess risk was also detected among participants who quit using ST between the ages of 25 to 29 years (OR: 3.9, 95 percent CI: 1.6-9.8).[1] The authors note that "findings may have been affected by limitations in the histories of tobacco use, the quality of death certificate data on STS, and the small number of STS deaths particularly among users of smokeless tobacco."

Breast cancer

A possible association between breast cancer and ST use was evaluated in a sample of 1,070 Cherokee women in North Carolina; of these women, 6 percent were current ST users, and 21 percent were former ST users (Spangler, 2001). Based on three exposed cases and two unexposed cases, the OR for breast cancer diagnosis before the age 55 years was 7.79 (95 percent CI: 1.05-66.0).

Brain glioma

Analysis by Zheng and colleagues (2001) of a population-based, case-control study with 375 brain glioma patients and 2,434 controls concluded that the study did "not support a major effect on the risk of brain cancer associated with… the use of other tobacco products [including smokeless tobacco products]."

Other cancers

Henley et al. (2005) included a category of "other cancers" in their analyses of ST users in the CPS-I and CPS-II cohorts based on an array of miscellaneous neoplasms. Based on 85 deaths among current ST users, the HR for mortality from these causes was 0.90 (95 percent CI: 0.71-1.14).[46] In the CPS-II, a significant excess mortality risk was detected among current ST users for "other cancers" (HR: 1.49, 95 percent CI: 1.04-2.14) based on 32 deaths.[47] It was noted that there was some difference between CPS-I and CPS-II in the ICD codes used to capture "other cancers" data.

7.5.6-1.2.4.10. Relationship between ST Use and Cardiovascular Disease

Three publications evaluated the association between CVD and use of ST products in the U.S. Data sets from four epidemiology studies were used in these publications.

[45] Adjusted for age and calendar time.
[46] Adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use.
[47] Adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat. consumption, fruit/vegetable intake, and aspirin use.

Henley et al. (2005) assessed CVD risk in the CPS-I and CPS-II cohorts. For current ST users in the CPS-I, the risk of CVD mortality was 1.18 (95 percent CI: 1.11-1.26) and in the CPS-II 1.23 (95 percent CI: 1.09-1.39).[48] There were 1,399 CVD deaths among current ST users in the CPS-I and 278 CVD deaths among current ST users in the CPS-II. The authors concluded that "men in both cohorts who reported current use of spit tobacco at the time of enrollment had significantly higher death rates from…all cardiovascular diseases than men who reported never using any tobacco product."

Accortt et al. (2002) evaluated mortality risk for diseases of the circulatory system among current ST users participating in the NHEFS. Overall, no excess CVD mortality risk was detected among current ST users (HR: 1.1, 95 percent CI: 0.8-1.5).[49] No excess CVD risk was seen in analyses limited to males with adjusted HRs of 1.0 (95 percent CI: 0.7-1.5) or in females with adjusted HRs of 1.2 (95 percent CI: 0.7-1.9).[2]

Yatsuya et al. (2010) assessed the risk of incident CVD[50] among 456 current ST users in the Atherosclerosis Risk in Communities (ARIC) Study. There were 131 CVD incidents among current ST users, and the CVD incidence HR for current ST users compared with never tobacco users was 1.27 (95 percent CI: 1.06-1.52).[51] The authors concluded that "current use of ST at baseline was associated with 1.27-fold greater CVD incidence."

Table 7.5.6-1-22 summarizes literature the association between ST use and CVD risk.

[48] CPS-I adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use. CPS-II adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use.
[49] Adjusted for age, race, and poverty index ratio.
[50] Cardiovascular disease was defined as hospitalized myocardial infarction, fatal coronary heart disease, electrocardiogram-confirmed myocardial infarction, cardiac procedure, or stroke.
[51] Adjusted for age, sex, race-center, educational level, total annual household income, usual alcohol consumption, physical activity, cigarette smoking status (never, past, or current smoker), pack years of smoking, and use of smokeless tobacco, past and current use of pipes and cigars, and secondhand smoke exposure (hours/week).

Table 7.5.6-1-22: Literature Summary Evaluating the Relationship Between ST Use and CVD

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Accortt, 2002)	Chronic disease mortality in a cohort of smokeless tobacco users.	Cohort study First National Health and Nutrition Examination Survey Epidemiologic Followup Study (1971-1975) N = 1,068 ST users N = 5,737 non-ST users	After adjustment for confounders, no association between ST use and…all cardiovascular mortality was found.	Overall: Ever ST use HR = 1.1 (0.8-1.5) Males: Ever ST use HR = 1.0 (0.7-1.5) Females: Ever ST use HR = 1.2 (0.7-1.9)	Age, race, and poverty index ratio	Strengths Based on a national probability sample Limitations Potential residual confounding Use of proxies for exposure assessment Exposure category based on ever use of ST rather than current use

Table 7.5.6–1-22: Literature Summary Evaluating the Relationship Between ST Use and CVD (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Henley, 2005)	Two large prospective studies of mortality among men who use snuff or chewing tobacco (United States).	Cohort study: males 1959 CPS-I or 1982 CPS-II <u>CPS-I</u> Exclusive snuff or chewing tobacco use: N = 7,745 No previous use of any tobacco product N = 69,662 12-year follow-up: N = 11,871 deaths <u>CPS-II</u> Exclusive snuff or chewing tobacco use: N = 3,327 No previous use of any tobacco product N = 111,482 18-year follow-up: N = 19,588 deaths "In both cohorts, ACS volunteers invited families of their friends, neighbors, and acquaintances to participate."	"A critical question is whether the association between the use of spit tobacco and increased risk of cardiovascular disease is causal, or merely reflects confounding by extraneous factors such as the lower socioeconomic status of men who use chewing tobacco or snuff."	CPS-I Current ST use (males) HR = 1.18 (1.11-1.26) CPS-II Current ST use (males) HR = 1.23 (1.09-1.39)	CPS-I age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use CPS-II age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use	<u>Strengths</u> Studies size and prospective design <u>Limitations</u> Exposure assessment conducted only at baseline Participants more likely to be more educated, married, middle-class, and white than the general U.S. population

Table 7.5.6–1-22: Literature Summary Evaluating the Relationship Between ST Use and CVD (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Yatsuya, 2010)	Risk of incident cardiovascular disease among users of smokeless tobacco in the Atherosclerosis Risk in Communities (ARIC) study.	Cohort study Atherosclerosis Risk in Communities (ARIC) Study (1987-1989) N = 14,498 men and women aged 45-64 Median follow-up of 16.7 years 456 current ST users 735 past ST users	"Current use of smokeless tobacco at baseline was associated with 1.27-fold greater CVD incidence" "Past use of smokeless tobacco was not associated with CVD incidence"	Current use HR = 1.27 (1.06-1.52)	Age, sex, race-center, educational level, total annual household income, usual alcohol consumption, physical activity, cigarette smoking status (never, past, or current smoker), pack years of smoking, and use of ST, past and current use of pipes and cigars and secondhand smoke exposure (h/wk)	<u>Strengths</u> Prospective design Long follow-up period <u>Limitations</u> No assessment of the quantity or duration of ST use Potential misclassification of tobacco use Relatively small number of ST users

Overall, data from U.S. epidemiology studies with ST show mixed results (Table 7.5.6-1-23). The three largest published studies provide some evidence of an elevated risk of CVD among ST users, but the risk estimates were relatively low.

Table 7.5.6-1-23: Summary of Published Cardiovascular Disease Risk Estimates for ST Users

Study	Group	ST Exposure	Risk Estimate	95% Confidence Interval
(Accortt, 2002)	Males + females	Ever ST	HR = 1.1	0.8-1.5
	Males		HR = 1.0	0.7-1.5
	Females		HR = 1.2	0.7-1.9
(Henley, 2005)	Males: CPS-I	Current ST	HR = 1.18	1.11-1.26
	Males: CPS-II		HR = 1.23	1.09-1.39
(Yatsuya, 2010)	Males + females	Current ST	HR = 1.27	1.06-1.52

7.5.6-1.2.4.11. Relationship Between ST Use and Ischemic Heart Disease

Four publications evaluated the association between ischemic heart disease (IHD) (including coronary heart disease [CHD]) and use of ST products in the U.S. These include analyses of three cohort sources and two meta-analyses.

Henley et al. (2005) assessed CHD mortality risk in the CPS-I and the CPS-II cohorts. For current ST users, the risks of CHD mortality were 1.12 (95 percent CI: 1.03-1.21) in the CPS-I and 1.26 (95 percent CI: .08-1.47) in the CPS-II.[52] There were 799 CHD deaths among current ST users in the CPS-I and 172 CHD deaths among current ST users in the CPS-II. The authors concluded that "the associations seen between current use of chewing tobacco or snuff and CHD…were considerably weaker than the association of these endpoints with current cigarette smoking in CPS-I and CPS-II."

Accortt et al. (2002) evaluated mortality risk for IHD (ICD-9 Codes 410-414) among current ST users participating in the NHEFS. The authors concluded that "ST use was not associated with significant increases in mortality for ischemic heart disease." The HR for male never-smoking ST users compared with male never tobacco users was 0.6 (95% CI: 0.3-1.2);[53] the HR for male ever-smoking ST users was 1.0 (95% CI: 0.6-1.7). The IHD mortality HRs were 1.4 (95% CI: 0.8-2.2) for female never-smoking ST users and 1.1 (95% CI: 0.4-3.2) for female ever-smoking ST users.

[52] CPS-I adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use. CPS-II adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use.
[53] Adjusted for age, race, poverty index ratio, alcohol, recreational physical exercise, fruit/vegetable intake, systolic blood pressure, serum cholesterol, and body mass index

Boffetta and Straif (2009) conducted a meta-analysis of IHD risk among ST users in the U.S. using data from the publications described above. The resulting risk estimate was 1.11 (95 percent CI: 1.04-1.19). Lee (2007) also conducted a meta-analysis of the same data, arriving at a risk estimate of 1.14 (95 percent CI: 1.06-1.22) using a fixed-effects model and of 1.14 (95 percent CI: 0.96-1.34) using a random-effects model.

Table 7.5.6-1-24 summarizes published literature assessing the association between ST use and IHD risk.

Table 7.5.6-1-24: Literature Evaluating the Relationship Between ST Use and IHD

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Accortt, 2002)	Chronic disease mortality in a cohort of smokeless tobacco users	Cohort study First National Health and Nutrition Examination Survey Epidemiologic Followup Study (1971-1975) N = 1,068 ST users N = 5,737 non-ST users	ST use was not associated with significant increases in mortality for IHD or stroke in either gender	Males Never smokers: HR = 0.6 (0.3-1.2) Ever smokers: HR 1.0 (0.6-1.7) Females Never smokers: HR = 1.4 (0.8-2.2) Ever smokers: HR = 1.1 (0.4-3.2)	Age, race, poverty index ratio, alcohol, recreational physical exercise, fruit/vegetable intake, systolic blood pressure, serum cholesterol, and body mass index	Strengths Based on a national probability sample Limitations Potential residual confounding Use of proxies for exposure assessment Exposure category based on ever use of ST rather than current use

Table 7.5.6–1-24: Literature Evaluating the Relationship Between ST Use and IHD (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Henley, 2005)	Two large prospective studies of mortality among men who use snuff or chewing tobacco (United States).	Cohort study: males 1959 CPS-I or 1982 CPS-II <u>CPS-I</u> Exclusive snuff or chewing tobacco use: N = 7,745 No previous use of any tobacco product N = 69,662 12-year follow-up: N = 11,871 deaths <u>CPS-II</u> Exclusive snuff or chewing tobacco use: N = 3,327 No previous use of any tobacco product N = 111,482 18-year follow-up: N = 19,588 deaths "In both cohorts, ACS volunteers invited families of their friends, neighbors, and acquaintances to participate."	The associations seen between current use of chewing tobacco or snuff and CHD or stroke were considerably weaker than the association of these endpoints with current cigarette smoking in CPS-I and CPS-II	<u>CPS-I</u> Current ST use (males) HR = 1.12 (1.03-1.21) <u>CPS-II</u> Current ST use (males) HR = 1.26 (1.08-1.47)	CPS-I age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use CPS-II age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use	<u>Strengths</u> Studies size and prospective design <u>Limitations</u> Exposure assessment conducted only at baseline Participants more likely to be more educated, married, middle-class, and white than the general U.S. population

Table 7.5.6–1-24: Literature Evaluating the Relationship Between ST Use and IHD (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Lee, 2007)	Circulatory disease and smokeless tobacco in Western populations: a review of the evidence.	Meta-analysis U.S. studies 3 estimates	"...the combined evidence…gave some indication of a weak association of IHD or AMI with current ST use in never smokers..."	Fixed effect RR/OR = 1.14 (1.06-1.22) Random effect RR/OR = 1.14 (0.96-1.34)	N/A	Strengths Substantial total number of cases in the meta-analyses Availability of estimates adjusted for a wide range of relevant potential confounding variables in the majority of the studies Long-term follow-up in all the cohort studies. Limitations Variable definitions of exposure and of disease endpoints Small numbers of individuals and lack of confounder control in a few studies Lack of good recent data from the United States. Dependence on the two CPS studies (76% of the total studied cases of IHD or AMI)

Table 7.5.6–1-24: Literature Evaluating the Relationship Between ST Use and IHD (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Boffetta, 2009)	Use of smokeless tobacco and risk of myocardial infarction and stroke: systematic review with meta-analysis.	Meta-analysis Selected studies that provided a quantitative estimate of the association between ever use of ST products and occurrence (incidence or mortality) of myocardial infarction or stroke among never smokers.	"An increased risk of fatal myocardial infarction was present in studies from…the United States..."	U.S. studies Any MI RR = 1.11 (1.04-1.19) Fatal MI RR 1.11 (1.04-1.19)	Not applicable	Limitations [Of studies included in meta-analysis] Potential confounding by active smoking Exposure generally assessed at baseline

Overall, published epidemiology data with U.S. ST products relating to IHD risk and ST use are mixed (Table 7.5.6-1-25). As seen with CVD risk, both of the larger CPS-I and CPS-II identified a slightly elevated risk for IHD with ST use.

Table 7.5.6-1-25: Summary of Published Ischemic Heart Disease Risk Estimates for ST Users

Study	Group	ST Exposure	Risk Estimate	95% Confidence Interval
(Accortt, 2002)	Males	Ever ST, never smoker	HR = 0.6	0.3-1.2
		Ever ST, ever smoker	HR = 1.0	0.6-1.7
	Females	Ever ST, never smoker	HR = 1.4	0.8-2.2
		Ever ST, ever smoker	HR = 1.1	0.4-3.2
(Henley, 2005)	Males: CPS-I	Current ST, never smoker	HR = 1.12	1.03-1.21
	Males: CPS-II		HR = 1.26	1.08-1.47
(Lee, 2007)	Fixed-effects model	-	RR/OR = 1.14	1.06-1.22
	Random-effects model	-	RR/OR =1.14	0.96-1.34
(Boffetta, 2009)	Random-effects model	-	RR = 1.11	1.04-1.19

7.5.6-1.2.4.12. Relationship Between ST Use and Stroke

Four publications evaluated the association between risk of stroke and use of ST products in the U.S. These publications include analyses of data from three cohort studies and two meta-analyses.

Henley et al. (2005) assessed cerebrovascular mortality risk in the CPS-I and CPS-II cohorts. The risk of cerebrovascular disease mortality was 1.46 (95 percent CI: 1.31-1.64) for current ST users in the CPS-I and was 1.40 (95 percent CI: 1.10-1.79) for current ST users in the CPS-II.[54] There were 460 cerebrovascular disease deaths among current ST users in the CPS-I and 71 cerebrovascular disease deaths among current ST users in the CPS-II. The authors concluded that current ST users in the CPS-I and CPS-II had "significantly higher death rates than never users from…cerebrovascular disease."

Accortt et al. (2002) evaluated mortality risk for stroke among current ST users participating in the NHANES I and NHEFS. The authors concluded that "ST use was not associated with significant increases in mortality for...stroke in either gender." When compared with the HH for never tobacco users, the HR was 0.7 (95% CI: 0.2-2.0)[55] for male never-smoking ST

[54] CPS-I adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use. CPS-II adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use.
[55] Adjusted for age, race, poverty index ratio, alcohol, recreational physical exercise, fruit/vegetable intake, systolic blood pressure, serum cholesterol, and body mass index.

users and 0.7 (95% CI: 0.3-1.5) for male ever-smoking ST users. The stroke mortality HRs were 1.0 (95% CI: 0.3-2.9) for never-smoking female ST users and 1.7 (95% CI: 0.4-7.0) for ever-smoking female ST users.

Boffetta and Straif (2009) conducted a meta-analysis of fatal stroke risk among ST users in the U.S. using data from Henley et al and Accortt et al. The resulting RR estimate was 1.39 (95 percent CI: 1.22-1.60). Lee (Lee, 2007) also conducted a meta-analysis of the same data, arriving at a RR/OR risk estimate of 1.44 (95 percent CI: 1.30-1.60) when using a fixed-effects model and a risk estimate of 1.41 (95 percent CI: 1.17-1.71) when using a random-effects model.

Table 7.5.6-1-26 summarizes published literature assessing the association between ST use and risk of stroke.

Table 7.5.6-1-26: Literature Evaluating the Relationship Between ST Use and Stroke

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Accortt, 2002)	Chronic disease mortality in a cohort of smokeless tobacco users	Cohort study First National Health and Nutrition Examination Survey Epidemiologic Followup Study (1971-1975) N = 1,068 ST users N = 5,737 non-ST users	ST "use was not associated with significant increases in mortality for...stroke in either gender."	Males Never smokers HR = 0.7 (0.2-2.0) Ever smokers HR = 0.7 (0.3-1.5) Females Never smokers HR = 1.0 (0.3-2.9) Ever smokers HR= 1.7 (0.4-7.0)	Age, race, poverty index ratio, alcohol, recreational physical exercise, fruit/vegetable intake, and systolic blood pressure	Strengths Based on a national probability sample Limitations Potential residual confounding Use of proxies for exposure assessment Exposure category based on ever use of ST rather than current use

Table 7.5.6–1-26: Literature Evaluating the Relationship Between ST Use and Stroke (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Henley, 2005)	Two large prospective studies of mortality among men who use snuff or chewing tobacco (United States).	Cohort study: males 1959 CPS-I or 1982 CPS-II <u>CPS-I</u> Exclusive snuff or chewing tobacco use: N = 7,745 No previous use of any tobacco product N = 69,662 12-year follow-up: N = 11,871 deaths <u>CPS-II</u> Exclusive snuff or chewing tobacco use: N = 3,327 No previous use of any tobacco product N = 111,482 18-year follow-up: N = 19,588 deaths "In both cohorts, ACS volunteers invited families of their friends, neighbors, and acquaintances to participate."	[In CPS-I] Men who reported current use of spit tobacco had statistically significantly higher death rates than never users from …cerebrovascular disease [In CPS-II] Current users of any type of spit tobacco had statistically significantly higher death rates than never users from ...cerebrovascular disease	CPS-I Current ST use (males) HR = 1.46 (1.31-1.64) CPS-II Current ST use (males) HR = 1.40 (1.10-1.79)	CPS-I age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use CPS-II age, race, educational level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use	<u>Strengths</u> Studies size and prospective design <u>Limitations</u> Exposure assessment conducted only at baseline Participants more likely to be more educated, married, middle-class, and white than the general U.S. population

Table 7.5.6–1-26: Literature Evaluating the Relationship Between ST Use and Stroke (continued)

Author	Title	Study Type and Sample	Findings	Risk Estimate (95% CI)	Adjustments	Comments
(Lee, 2007)	Circulatory disease and smokeless tobacco in Western populations: a review of the evidence.	Meta-analysis U.S. studies 3 estimates	"The associations with stroke...among never smokers were somewhat more clearly seen."	U.S. studies Fixed effect RR/OR = 1.44 (1.30-1.60) Random effect RR/OR 1.41 (1.17-1.71)	N/A	Limitations Dependence on the two CPS studies (90% of total studied stroke cases)
(Boffetta, 2009)	Use of smokeless tobacco and risk of myocardial infarction and stroke: systematic review with meta-analysis.	Meta-analysis Selected studies that provided a quantitative estimate of the association between ever use of ST products and occurrence (incidence or mortality) of myocardial infarction or stroke among never smokers.	"The studies from...the United States...showed an increased risk of death from...stroke."	U.S. studies Any stroke RR = 1.39 (1.22-1.60) Fatal stroke RR = 1.39 (1.22-1.60)	N/A	Limitations [Of studies included in meta-analysis] Potential confounding by active smoking Exposure generally assessed at baseline

Overall, the published epidemiology data with U.S. ST products relating to stroke risk and ST use are mixed (Table 7.5.6-1-27). However, the meta-analysis, driven by the CPS-I and the CPS-II, suggests an increased risk of stroke associated with ST use.

Table 7.5.6-1-27: Summary of Published Stroke Risk Estimates for ST Users

Study	Group	ST Exposure	Risk Estimate	Confidence Interval
(Accortt, 2002)	Males	Never smoker	HR: 0.7	0.2-2.0
	Males	Ever smoker	HR: 0.7	0.3-1.5
	Females	Never smoker	HR: 1.0	0.3-2.9
	Females	Ever smoker	HR: 1.7	0.4-7.0
(Henley, 2005)	Males: CPS-I	Current ST	HR: 1.46	1.31-1.64
	Males: CPS-II	Current ST	HR: 1.40	1.10-1.79
(Lee, 2007)	Fixed-effects model	-	RR/OR:1.44	1.30-1.60
	Random-effects model	-	RR/OR:1.41	1.17-1.71
(Boffetta, 2009)	Random-effects model	-	RR: 1.39	1.22-1.60

CPS-I = Cancer Prevention Study I; CPS-II = Cancer Prevention Study II; HR = hazard ratio; RR/OR = Random effects relative risk/odds ratio, RR = Relative risk; ST = smokeless tobacco.

7.5.6-1.2.4.13. Relationship between ST Use and Respiratory System Diseases[56]

Two studies have evaluated respiratory system disease risk, such as COPD, among ST users.

Accortt et al. (2002) evaluated the mortality risk from respiratory system disease among ST users compared with non-users in the NHEFS. No excess risk was detected, and the HR for male ST users was 0.9 (95 percent CI: 0.3-2.5), and the HR for female ST users was 0.6 (95 percent CI: 0.1-2.3).[57]

Henley et al. (2005) also assessed the mortality risk for respiratory system diseases (including COPD) in the CPS-I and CPS-II cohorts. For current ST users in the CPS-I, the risk of respiratory disease mortality was 1.28 (95% CI: .03-1.59) and in the CPS-II it was 1.11 (95% CI: 0.84-1.45).[58] There were 123 respiratory system disease deaths among current ST users in the CPS-I and 56 respiratory system disease deaths among current ST users in the CPS-II. COPD mortality was considered separately from other respiratory diseases in this

[56] Such as: Acute respiratory infections, pneumonia and influenza, COPD and allied conditions, pneumoconioses and other lung diseases due to external agents, other diseases of respiratory system
[57] Adjusted for age, race, and poverty index ratio.
[58] CPS-I adjusted for age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake, and aspirin use. CPS-II adjusted for age, race, educational level, body mass. index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use.

study. An excess risk of COPD was detected among exclusive current ST users in the CPS-I cohort (HR: 1.86, 95% CI: 1.12-3.06). However, the COPD mortality risk HR among current ST users in the CPS-II was 1.28 (95% CI: 0.71-2.32). The possibility of misclassification in the CPS-I is strongly suggested in the results. We are not aware that a biological plausible mechanism has been proposed for such a finding, nor that any similar associations between ST use and COPD have been reported elsewhere.

7.5.6-1.2.4.14. Relationship between ST Use and Non-neoplastic Oral Diseases

The U.S. Surgeon General has established a causal association between ST use and leukoplakia in 1986 (U.S. Dept. Health Human Services, 1986). ST products marketed in the U.S. are required by law to carry labels warning of the oral health consequences associated with ST use.

In 2008, Philip Morris International (Kallischnigg, 2008) published an exhaustive review of the published literature investigating the relationship between ST use and non-neoplastic oral diseases. Experimental and epidemiological studies published in 1963–2007 were identified that related risk of oral lesions to smokeless tobacco use. Data were assessed separately for oral mucosal lesions, periodontal and gingival diseases, dental caries and tooth loss, and oral pain. Kallischnigg et al. (2008) concluded that the current scientific evidence supports an association between ST use and oral mucosal lesions and suggests an association between snuff use and gingival recession and attachment loss.

We do not include in this MRTPA an exhaustive review of the current literature on ST use and oral disease, instead deferring to the extensive review by Kallischnigg et al. (2008).

Brief summaries of the analysis by Kallischnigg et al. for key non-neoplastic oral diseases are as follows:

Oral mucosal lesions: A total of 24 U.S. studies investigated the potential association between ST use and oral mucosal lesions. The prevalence of oral mucosal lesions across these studies ranged from 30 percent to 70 percent. Kallischnigg et al. (2008) noted that prevalence of lesions tends to be lower among chewing tobacco users than among snuff users.

Periodontal and gingival diseases: A total of 13 U.S. studies were identified that evaluated a variety of periodontal and gingival health indices. These included plaque, gingivitis, gingival bleeding, gingival recession, pocket depth, attachment loss, and periodontal disease. Three studies found a significant association between gingival recession and ST user, two studies found increased risk of attachment loss associated with ST use, and one study reported increased risk of periodontal disease. The remainder of the comparisons across studies were not different between exposed and unexposed participants. However, several of the reported ORs were elevated, but with wide CIs due to very small numbers of exposed subjects exhibiting the endpoints of interest.

Tooth loss: Four U.S. studies were identified that assessed the presence of teeth among ST or chewing tobacco users. Two studies reported no significant difference in tooth loose for snuff users compared with unexposed participants. Two studies found a significantly increased risk for tooth loss hazard among snuff or chewing tobacco users.

Dental caries/decayed or filled teeth: Seven studies in the U.S. evaluated the incidence of dental caries or decayed or filled teeth among ST users. Positive associations were noted for chewing tobacco users, but not for snuff (ST) users.

7.5.6-1.2.4.15. Relationship Between ST Use and Other Conditions

This section reviews published literature examining the relationship between ST use in the U.S. and disease conditions not summarized elsewhere.

Peripheral arterial disease

Agarwal et al. (2009) analyzed pooled data from the 1999-2000, 2001 to 2002, and 2003 to 2004 NHANES to assess the potential relationship between ST use and peripheral arterial disease (defined as low ankle brachial index). No risk estimate was calculated. However, the authors concluded that "no significant differences were observed in the distribution of...snuff users, chewing tobacco users...between participants with and without [peripheral arterial disease] PAD." The authors noted that there were few ST users in this study, a fact that limited the power to detect an association.

Endocrine, nutritional, metabolic diseases and immunity diseases

In their analysis of the NHEFS data set, Accortt et al. (2002) determined the association between ST use and endocrine, nutritional, and metabolic diseases as well as immunity disorders. The authors concluded that "male ST users experienced statistically nonsignificant increases in mortality from endocrine, nutritional, and metabolic diseases and immunity disorders." When compared with the HR for non- users, the HR for male ST users was 2.4 (95 percent CI: 0.7-8.8), and the HR for female ST users was 1.4 (95 percent CI: 0.1-13.5).[59]

Nervous system diseases

Accortt et al. (2002) also evaluated the association between nervous system disorders and use of ST in the NHEFS. There was no evidence of an association. The HR for males was 1.1 (95 percent CI: 0.2-5.2) and for females 0.6 (95 percent CI: 0.1-2.6).

Benedetti et al. (2000) conducted a case-control study that included an evaluation of the association between snuff or chewing tobacco use and Parkinson disease. ST use "was significantly more common in control subjects than cases" (OR: 0.18, 95 percent CI: 0.04-0.82). However, this result is based on 3 cases and 13 controls.[60]

Digestive system diseases

Accortt et al. (2002) also evaluated digestive system disease mortality among ST users in the NHEFS. Among males, the HR for the comparison with never tobacco users was 1.9 (95 percent CI: 0.4-9.8).[5] There were no digestive system disease deaths among female current ST users.

[59] Adjusted for age, race, and poverty index ratio.
[60] Numbers calculated from proportions provided in reference.

Henley et al. (2007) evaluated the mortality risk from digestive system diseases among current ST users in the CPS-I and CPS-II cohorts. An excess risk was detected in the CPS-I cohort (HR = 1.49, 95 percent CI: 1.14-1.93) but not in the CPS-II cohort (HR = 1.38, 95 percent CI: 0.92-2.07).[4] There were 85 and 25 digestive system disease-related deaths among current ST users in CPS-I and CPS-II, respectively.

7.5.6-1.2.4.16. Summary of Epidemiology

The health risk epidemiology literature we identified and reviewed informs the assessment of the candidate MRTP. Our review indicated mixed or equivocal evidence about the association between ST use and all-cause mortality, risk of all cancers, oropharyngeal cancer, lung cancer, esophageal cancer, digestive cancers, kidney cancer, prostate cancer, and various CVD endpoints. On the basis of the existing evidence with U.S. products, there appears to be little, if any association between ST use and bladder cancers, pancreatic cancer, or hematopoietic or lymphoid cancers. For many of these endpoints, the available U.S. epidemiology data demonstrate relatively low RR estimates, wide CIs, inconsistency between studies, and lack of adequate adjustment for known confounding factors.

There is clear evidence of an association between ST use and oral lesions, gingival recession and tooth loss based on the temporal associations, consistency between studies, and strength of the association.

7.5.6-1.2.5. Nonclinical Models

Nonclinical data can be useful in disease assessment since they can provide evidence of plausibility and address potential mechanistic aspects related to disease development. There are numerous nonclinical studies using ST products marketed and used worldwide. To focus on what is considered to be the most relevant data related to the candidate MRTPs, we limited our literature review to studies using only U.S. reference or commercial MST products or to extracts derived from those MST products (Section 6.1.2).

The majority of nonclinical studies we identified investigated the potential adverse effect of exposing oral mucosa (*in vivo*) or oral-derived tissue or cells (*in vitro*) to MST or MST extract. In fact, greater than 75 percent of nonclinical studies investigate aspects related to oral injury or disease. We provide an evidence table (Table 7.5.6-1-28) detailing methods and findings for each of the 107 publications in the literature review on nonclinical research which included laboratory animal studies (*in vivo*) and tissues or cell-based studies (*in vitro*).

Topic narratives include summaries of findings from publications related to known or potential clinical toxicities of MST related to:

- general toxicology studies (long-term exposure of laboratory animals and genotoxicity studies);
- carcinogenicity studies (complete or modulatory activity);
- oral injury and dental-related studies;
- immune system studies;

- cardiovascular studies; or
- reproductive/developmental studies.

Not all publications included in the evidence table have been included in the topic discussions, since not every nonclinical study appeared to be related to a known or proposed diseases typically identified with ST use (e.g., hepatotoxicity, gene expression, or receptor studies).

As previously described in Section 7.5.6-1.1.3, there are several important and challenging issues that warrant consideration when interpreting the nonclinical study findings and its relevance to human data, including exposure duration, exposure dose, exposure extract preparation, and product storage and freshness.

7.5.6-1.2.5.1. General Toxicology Studies

7.5.6-1.2.5.1.1. Long-term Exposure of Laboratory Animals

Two studies have been published evaluating the effect of feeding MST or MST extract to hamsters for 2 years (Homburger, 1976) or rats and mice for 90 days (Theophilus, 2012). Overall, these studies found that chronic exposure to MST resulted in plasma nicotine levels relevant to human exposure did not result in a significant adverse effects in any target organ examined (as compared with nicotine-treated animals or control animals).

Homberger et al. (1976) investigated the potential carcinogenic effect of a diet containing 20 percent MST (commercial product, brand not disclosed) in male Syrian hamsters over a 2-year period. This chronic-feeding study showed a similar tumor spectrum and incidence for animals fed diets containing 20 percent MST or 20 percent methylcellulose (negative control). The authors noted that “Clearly, snuff was not carcinogenic.” Based on tissue histopathology results, this study also concluded that chronic MST exposure does not result in “non-neoplastic lesions,” as compared with the results in control animals. Since accurate plasma nicotine or cotinine levels were not clearly reported, the relevance of MST exposure in this hamster study to human exposure is not known.

Theophilus et al. (2012) described a comprehensive study examining the potential toxic effect of three separate doses of MST or MST extract (the MST source was not disclosed) in the diet of rats or mice for 90 days. Measured plasma levels of nicotine and cotinine confirmed the relevance of the dosing regimens to human exposure. In fact, the plasma nicotine level observed with the highest dose of MST in rats or mice was approximately threefold to fivefold higher than that observed for MST users. The key effect observed with MST exposure was lower body weight, which paralleled that of nicotine treatment alone. Overall, considering tissue histopathology, clinical chemistry, hematology, coagulation, and urinalysis evaluations, the authors concluded that “the [MST] doses evaluated were confirmed to span the no observable adverse effect level, the lowest observable adverse effect level and the maximum tolerated dose.”

Two rat studies from the same laboratory investigated the potential carcinogenic effect of lifetime (2 years) MST exposure (lip canal model) (Johansson, 1989; Johansson, 1991c). Using an identical study design, these studies reported similar findings; the MST treatment

group demonstrated no difference in mean survival time as compared with that in the control group. The MST treatment group did show significantly lower body weights (20 percent) after 2 years of exposure, suggesting that the rats received an MST (nicotine) dose relevant to human MST users.

7.5.6-1.2.5.1.2. Genotoxicity

Seven peer-reviewed publications (Guttenplan, 1987; Rickert, 2009; Rickert, 2007; Shirname-More, 1991a, 1991b; Stamm, 1994; Whong, 1984) from four different laboratories were identified that investigated the effect of MST extracts on mutagenesis using bacterial assays (Ames Salmonella/microsomes). In these studies, a wide variety of U.S. commercial MST products were examined using a variety of extraction methods/solvents, including aqueous-based solvents: artificial saliva, water, 10 mM phosphate buffer, and organic solvents (dimethyl sulfoxide [DMSO], dichloromethane [DCM], acetone/methanol, methanol). The overall findings, using aqueous-based MST extracts (without pH modifications, addition of nitrite or addition of DMSO) in the standard Ames assay, suggest that MST contains weak or no mutagenic activity.

Two studies have been published describing the mutagenic effects of different U.S. commercial products (brands not disclosed) ST extracted with DCM or methanol/acetone (Stamm, 1994; Whong, 1984). Weak mutagenic activity was observed for MST extracts using the Ames Salmonella/microsome assay (with four different bacterial strains). An exception was with bacterial strain YG 1024 (developed to improved sensitivity in detecting aromatic amines and nitroarenes) in which MST extracts were found to be mutagenic.

A short communication publication reported that aqueous extracts of five different U.S. commercial brands (brands not disclosed) were mutagenic in the Ames assay (strain TA 100 with microsomes) (Guttenplan, 1987). However, the author noted that the Ames assay conditions were modified to maximize mutagenesis by nitrosamines (acidic pH, addition of nicotinamide adenine dinucleotide phosphate, and preincubation). When these modifications were omitted, the observed mutagenic activity of MST extracts was weak or absent.

A study published by Shirname-More (1991a) investigated the mutagenic potential (using Salmonella strain TM 677) of water extracts of several U.S. commercial MST products (brands not disclosed). The author reported that the aqueous MST extracts “did not induce significant mutagenicity either in the presence or absence of metabolic activation.”

Finally, MST products have been components of studies comparing the mutagenic activity of multiple tobacco products (Rickert, 2009; Rickert, 2007). A 2007 study by Rickert et al. (2007) demonstrated weak mutagenic activity (less than twofold increase as compared with the increase in controls) for DMSO extracts of MST using Salmonella strains TA 100 + microsomes and TA 98 + microsomes. In a 2009 study by Rickert et al. (2007), a wide variety of U.S. and Canadian commercial MST were tested. MST extracts were prepared with DMSO, DCM, and artificial saliva. The Ames mutagenicity assay was conducted with five *Salmonella* strains, including TA 100 + microsomes and TA 98 + microsomes. The authors noted that, for some unknown reason, the results were often highly variable for replicates of the same product. The overall conclusion from this study was that distinguishing

differences in mutagenic activity between MST products was difficult due to the weak activity observed with MST products in general.

Seven publications investigated the genotoxic effect of MST extracts in mammalian cells using a variety of *in vitro* techniques such as sister chromatid exchange assay, micronucleus assessment, comet assays, and *in vivo* DNA adduct measurement (Barley, 2004; Coppe, 2008; Gao, 2014; Rickert, 2009; Shirname-More, 1991b; Smith, 1997; Tucker, 1985). In these seven studies, a wide variety of U.S. commercial MST products were examined using various extraction methods/solvents, including aqueous-based solvents: artificial saliva, water, cell culture medium, and organic solvents (DMSO, DMSO/mineral oil, DCM, DCM/acetone/methanol). The overall findings, using aqueous-based MST extracts, indicate that MST exposure can result in a positive genotoxic response in mammalian cells.

In a 2009 study by Rickert et al. (Rickert, 2009), a wide variety of U.S. and Canadian commercial MST were tested. MST extracts were prepared with DMSO, DCM, and artificial saliva. Using an *in vitro* micronucleus assay (using Health Canada official method T-503), the authors noted a dose-dependent biological effect (increase in percent micronuclei formation) after MST exposure. Similar findings were observed with all U.S. commercial MST products tested, irrespective of the extraction solvent used.

Three different laboratories (Barley, 2004; Coppe, 2008; Gao, 2014) used the *in vitro* comet assay to investigate the ability of MST extracts to induce DNA strand breaks in oral cavity-derived cell cultures. The study from Gao et al. (Gao, 2014) showed that artificial saliva extracts of reference MST (high dose: 3 percent) resulted in a weak or no effect in gingival-derived cells but a significant increase in DNA breaks in an oral carcinoma cell line. Similarly, the study by Coppe et al. (2008) demonstrated that normal human oral mucosa fibroblasts exposed to cell culture medium extracts of Copenhagen® MST clearly resulted in DNA strand breaks in a dose-response manner (threefold and sevenfold increase with 2 percent and 4 percent extract, respectively). Finally, the comet assay study by Barley et al. (2004) demonstrated a dose-dependent (3 percent to 25 percent Copenhagen® MST extract) increase in DNA strand breaks in treated immortalized hamster cheek pouch cells. The biological plausibility of MST exposure resulting in a positive genotoxic response was also supported by the *in vivo* study of Smith et al. (1997) This study found that a 10-week exposure to reference MST (rat lip canal model) resulted in DNA adduct formation in the oral cavity.

Overall, the various assays investigating potential MST genotoxicity provide mixed evidence that MST itself is genotoxic. While some studies indicated a dose-response relationship, the clinical relevance of the MST extract concentrations used to induce the observed genotoxic responses is not established. Furthermore, several studies described above used DMSO as the extraction solvent (Rickert, 2009; Rickert, 2007), or as a vehicle to reconstitute the dried MST extract then added to cells (Guttenplan, 1987; Stamm, 1994; Whong, 1984). There are published accounts of the presence of DMSO enhancing mutagenesis in bacterial mutation assays (Gatehouse, 1987).

7.5.6-1.2.5.1.3. Carcinogenicity Studies

Carcinogens are often classified as complete carcinogens or as a modulators of carcinogenesis. A complete carcinogen is an agent that induces tumors, itself, usually with properties of initiating, promoting, and progressor agents. A modulator of carcinogenesis does not initiate the carcinogenic process, but instead promotes or stimulate the carcinogenic process.

We review the evidence derived with hamster, rat, and *in vitro* models for MST, or MST extracts, to act as either a complete carcinogen or modulator of carcinogenesis (promotion or progression).

7.5.6-1.2.5.1.4. Complete Carcinogen

7.5.6-1.2.5.1.4.1. *In Vivo* Models

Researchers have found that mimicking human exposure to MST in laboratory animal models is very challenging. Typically, studies investigating the ability of agents to cause cancer (complete carcinogen) administer an agent to laboratory animals over a lifetime (18 to 24 months in rats and hamsters). About half of the *in vivo* studies identified administered MST to the oral cavity for less than 18 months, complicating the interpretation of the findings. Additionally, some studies used unorthodox MST exposure technique (immobilization of the animal), which was deemed not applicable to human studies and appeared to be unrelated to typical MST use (Homburger, 1971).

Homburger et al. (1976) examined tissues from Syrian hamsters for the presence of cancer after a diet containing 20 percent MST for 2 years (lifetime). This chronic-feeding study failed to show the ability of MST to cause cancer in any tissue, as compared with that in control animals (fed a 20 percent methylcellulose diet). The authors noted that “Clearly, snuff was not carcinogenic.”

Three separate rat studies (applying MST to test lip canals once or twice a day, 5 days per week for 2 years) reported no significant difference (as compared with that for controls) in tumor incidence in numerous tissues, including the digestive system and the lung (Hecht, 1986; 1991b; Johansson, 1991c). The MST treatment group did show significantly lower body weights (20 percent) after 2 years of exposure, suggesting that the rats received an MST (nicotine) dose relevant to human MST users (Johansson, 1991b; 1991c).

Eighteen published studies from 11 different laboratories investigated the potential ability of chronic MST to specifically cause oral cancer in laboratory animals. Many of these studies used a hamster cheek pouch model, where the test substance in placed in the animals mouth for a period of time.

The hamster cheek pouch model for investigating chemical carcinogenesis was established in the 1970s. During this time, it was shown that swabbing the cheek pouch (a pocket the hamster uses to store food) with the chemical 7,12-dimethylbenz(a)anthracene (DMBA), resulted in mucosa epithelial tumor formation. Using this technique, Barley et al. (2004) swabbed the cheek pouches of hamsters with DMSO/mineral oil extracts of Copenhagen® MST for 10 months (three times a week). Histopathological results from this study found no

tumor formation but found mild epithelial dysplastic (precancerous) changes in the cheek pouch mucosa.

In other studies using the hamster cheek pouch model, investigators placed MST into the cheek pouch for various lengths of time (once or twice daily for up to 24 months) and determined the presence of cancerous or precancerous lesions in the area. It was observed that MST placed in the cheek pouch is removed and ingested by the hamster within a couple of hours (Shklar, 1985). An exposure regimen that better simulates human use would be to place a fresh sample of MST into the cheek pouch every 50 minutes for 8 hours daily (no MST overnight) over a 24-month period (Mitchell, 2010). Unfortunately, such a study design does not appear technically feasible in this animal model.

Using the hamster cheek pouch model, Peacock et al. (Peacock, Jr., 1959; Peacock, Jr., 1960) found no evidence of neoplasia after 12 to 18 months of MST exposure (1 application of MST was sewed into the pouch and left for 12-18 months). Greater than 50 percent of the animals died from infection or nicotine poisoning in this very early study. In 1985, Shklar et al. (1985) published the results of their study, showing no gross or microscopic changes in the oral mucosa of hamsters given a single, daily application of Copenhagen® MST (100 mg) to the cheek pouch for 20 weeks. Park et al. (1986) found that commercial MST (brand not disclosed) placed in the cheek pouch (150 mg) twice a day, 5 days per week for 6 months, did not induce precancerous or neoplastic changes in the oral mucosa of these hamsters. In 1992, Summerlin et al. (1992) reported that MST lacked carcinogenic potential. They found “no significant difference in the rate of dysplastic change or formation of carcinoma in pouch mucosa” in hamster cheek pouches that received Skoal MST (200 mg) once daily, 5 days per week for 6 months. Finally, the Ashrafi laboratory (Alonge, 2003; Ashrafi, 1992; Colvard, 2006) published three separate studies with an identical study design; Skoal MST (2 g) was applied to cheek pouches once a day, 5 days per week for 24 months. The gross and histopathological findings in the oral mucosa were similar for all three studies. No tumors were observed, oral mitotic activity was not observed, and ultrastructural changes in the cheek pouch epithelium were similar to human leukoplakia.

Researchers investigated the potential carcinogenic effect of MST in eight separate rat studies from four laboratories using several exposure techniques, including swabbing the oral cavity with MST extract (Brunnemann, 1987; Hecht, 1986), applying MST to the buccal folds (Chen, 1989), or applying MST to a lip canal (Hecht, 1986; Johansson, 1989, 1991b; Johansson, 1991c; Schwartz, 2010).

In rat studies in which the oral cavity was swabbed with aqueous extracts of commercial MST (two times per day), the authors reported that no tumors were found in the oral cavity or the lung after 2 years of treatment. They concluded that MST extract is not tumorigenic when applied by swabbing (Brunnemann, 1987; Hecht, 1986). Another chronic rat study by Chen et al. (1989) also found that MST treatment did not induce oral cancer. The authors reported that U.S. commercial MST applied weekly for a year to a rat’s mandibular mucobuccal folds resulted in a whitish buccal mucosa without tumor formation. The authors noted that the applied MST was gradually cleared from the oral cavity by the rat and disappeared in several hours.

In 1986, Hecht et al. (1986) published a study in which a test canal was surgically created in the low lip of rats, and U.S. commercial MST was placed daily 5 days per week for 116 weeks. At sacrifice, the authors observed that 3 out of 32 animals showed tumors in the oral cavity; however, the tumor incidence rate was not statistically significant (as compared with that for controls). The authors concluded that "these findings strongly indicate that these tumors were induced by snuff and were not fortuitous occurrences."

The Johansson laboratory published two separate studies in 1989 and 1991 with near identical study design (Johansson, 1989; Johansson, 1991c). In both, U.S. commercial MST was applied to rat test lip canals, two times per day and 5 days per week for 104 weeks. According to the authors, results from the 1989 study suggested that MST exerted tumorigenic effects in the oral cavity. However, statistical confirmation of this conclusion was not obvious. Histopathological analyses demonstrated precancerous pathology in that MST-induced lip squamous cell hyperplasia (24 out of 29 rats) and squamous cell dysplasia (10 out of 29 animals) were reported. In the 1991 study, the data showed lip tumors in 4 out of 29 animals. The authors reported that rats treated with MST had a significantly higher number of squamous-cell tumors and hyperplastic squamous lesions of the lip than controls.

In 1991, Johansson et al. (1991c) also published a study with the same exposure design as described above, except University of Kentucky reference MST[61] was used (instead of commercial MST). A total of 26 percent of the rats exposed to reference MST for 2 years presented with lip sarcoma at sacrifice. On the basis of these findings, the authors concluded that "snuff is a carcinogen for the lip and oral cavity."

More recently, Schwartz et al. (2010) published a study in which rats were exposed to various commercial MSTs (including Copenhagen® and Skoal) in the test lip canal model. In this study, MST was applied two times per day, 5 days a week for 12 months. At sacrifice, gross observations indicated no visible tumors. Oral mucosa histopathological findings with MST (Copenhagen® and Skoal) exposure, resulting in significant differences from controls including moderate to severe dysplasia (premalignancy), irreversible dysplasia remaining 3 months after exposure) and regions of abnormal epithelium/dysplasia demonstrated characteristics of malignancy (significant increase in proliferation markers and mitotic figures, and significant decrease in p16 positive cells).

7.5.6-1.2.5.1.4.2. *In Vitro* Models

Murrah et al. (1993) investigated the effect of aqueous extract of reference MST on the morphology and growth of primary human oral epithelial cells over 10 weeks in culture. The authors found that "cells exposed to...moist and dry extract continued to divide, maintained a differentiated phenotype...and displayed focal growth and morphologic changes suggestive of early stages in cell transformation." However, this study lacked appropriate statistical analysis to adequately determine differences between the control and treated cells. Hence, these data appear to demonstrate a "trend" toward extended cell longevity with MST

[61] Reference MST produced by commercial manufacturing has been available for researcher through the University of Kentucky. For additional detail see: http://www.tobacco.ncsu.edu/strp.html

exposure. MST treatment did not result in the development of irreversibly transformed oral epithelial cultures in this study.

7.5.6-1.2.5.1.5. Modulating Carcinogenesis

7.5.6-1.2.5.1.5.1. *In Vivo* Models

One long-term study investigated the possibility of MST to promote systemic toxicity after tumor induction with 20-methylcholanthrene (MC) (Homburger, 1976). In this study, Syrian hamsters were given oral gavages of MC, and were fed a diet containing 20 percent MST for 2 years (lifetime). This chronic-feeding study failed to show the ability of MST to modulate (stimulate) MC-induced tumors, as compared with results for control animals (fed a 20 percent methylcellulose diet). The authors noted that "20% snuff in the diet is neither carcinogenic nor co-carcinogenic in these animals."

Thirteen published *in vivo* studies from four laboratories investigated the potential ability of MST exposure to promote (stimulate) oral carcinogenesis initiated by a chemical or virus. The initiators included TSNAs (Brunnemann, 1987; Hecht, 1986; Prokopczyk, 1987); ethanol (Summerlin, 1992); DMBA (Johansson, 1991c); 4-nitroquinoline-N-oxide (4-NQO) (Johansson, 1989, 1991b; Johansson, 1991c); and herpes simplex virus (HSV-1) (Park, 1988; Park, 1985; Park, 1987; Park, 1986; Stich, 1987).

The Hoffmann laboratory reported, in three separate studies, that MST exposure did not promote or stimulate NNN- or NNK-induced tumor formation. Instead, MST appears to protect rats from TSNA-induced carcinogenesis. Two rat studies (Brunnemann, 1987; Hecht, 1986) examined swabbing (two times per day for 2 years) the oral cavity with aqueous extracts of U.S. commercial MST enriched with NNN and NNK at the same level of an aqueous solution of NNN plus NNK. The authors reported that the MST-containing solution produced fewer tumors in the oral cavity and the lung than were produced with TSNAs alone. Definitive conclusions are difficult since the studies did not provide statistical analyses. A third study (Prokopczyk, 1987) found that "components within the snuff extract altered the genotoxic potential of NNK." The investigators administered, by oral gavage, aqueous extracts of commercial MST to rats (two times per day for 2 weeks), followed by a single oral dose of NNK. The levels of DNA methylation (measure of potential NNK-induced carcinogenesis) in the oral cavity and the liver were found to be much lower in rats pretreated with MST extract. Similar to the previous publication, interpretation is difficult since the study did not provide statistical analyses but does support the conclusion of the earlier studies (Brunnemann, 1987; Hecht, 1986). Collectively, these studies suggest that MST does not promote (stimulate) TSNA-mediated carcinogenesis.

Summerlin et al. (1992) exposed the cheek pouch of hamsters to MST (daily, 5 days per week for 6 months) alone, to ethanol (2 mL per day, 5 days per week for 6 months) or to MST in combination with ethanol (same dosing regimen as above). The authors reported that MST in combination with ethanol treatment did not produce neoplastic or preneoplastic alterations in hamster pouch mucosa or the stomach. No modulation in organ histopathology was observed for the cheek pouch, liver, intestines, or stomach.

Johansson et al. (1991c) using the test lip canal model in rats, investigated the ability of MST exposure (two times per day, 5 days per week) to promote oral tumors initiated by DMBA (3 times per week, 4 weeks in the test canal prior to MST treatment). Cancer-promoting ability was not observed since no oral tumors were present after 2 years of MST exposure.

The Johansson laboratory published two separate rat studies with near identical designs (Johansson, 1989, 1991b). In both studies, 4-NQO was swabbed on the oral palate mucosa (0.13 mg per application, three times per week for 4 weeks), and then U.S. commercial MST was applied to rat test lip canals (two times per day and 5 days per week for 104 weeks). The authors concluded that MST exposure did not display any significant tumor-promoting activity. However, statistical analysis was not provided.

In 1991, Johansson et al. (1991c) published a study with similar exposure design as those described in their 1989 study (Johansson, 1989, 1991b) with some modifications. Reference MST was used (instead of commercial MST), and 4-NQO was applied to cotton (70 mg per application or 50-fold higher compared with what was used in the previous studies) and placed in the test lip canal of rats. On the basis of the findings from this study, the authors stated that "The results show that snuff has a strong promoting capability with regard to development of lip sarcomas after 4-NQO initiation." A limitation of this study was the very high dose of 4-NQO administered. The overall conclusion from studies using 4-NQO as an initiator is that there exists inadequate evidence to suggest that MST may act as a promoter of carcinogenesis, due to conflicting studies.

The Park laboratory published a series of five studies investigating the effect of HSV-1 inoculation and commercial MST or aqueous MST extract application alone and in combination on histopathological changes in the oral mucosa of mice and hamsters (Park, 1988; Park, 1985; Park, 1987; Park, 1986). Using a mouse model, investigators inoculated lips with HSV-1 and/or then topically applied MST extract to the inoculation site (three times per day, 5 days per week for 2 to 3 months) (Park, 1985; Park, 1987). The histopathological findings indicated that MST or HSV-1 exposure alone resulted in no epithelial dysplasia observed. In contrast, the combination of these agents produced epithelial dysplasia and other pathological changes such as hyperplasia. Using the hamster cheek pouch model, the authors demonstrated that neither MST or HSV-1 treatments alone induced neoplastic changes in the cheek pouch (Park, 1986). However, in combination, these treatments produced epithelial dysplasia and invasive squamous cell carcinoma in the pouches of greater than 50 percent of the animals. The exposure regimen for this hamster study included placing commercial MST in the cheek pouch (two times per day, 5 days per week for 6 months) and/or inoculating the cheek pouch with HSV-1 once per month for 6 months. Other studies published by the Park laboratory (Park, 1987; Park, 1986) investigated the effect of Copenhagen® MST on viral growth and viral-induced lesions using the hamster cheek pouch model. The findings from these studies indicated that MST inhibited both the development of viral lesions as well as viral growth in the pouch.

The overall finding from the Park studies is that there is sufficient evidence to suggest that MST may promote viral-mediated carcinogenesis.

7.5.6-1.2.5.1.5.2. *In Vitro* Studies

Ten published *in vitro* studies from six laboratories investigated the effect of MST extracts on viral activity and on the proliferation of cells derived from the oral cavity. Collectively, the results from these studies provide inadequate evidence (due to conflicting studies) to suggest that MST might promote carcinogenesis.

Five publications studied the mode of action of the *in vitro* effect of MST extracts on HSV activity (Oh, 1990; Park, 1988; Stich, 1987) or Epstein-Barr virus (Jenson, 1999a; 1999b). The studies from the Park laboratory reported that reference or commercial MST extracts inhibits HSV DNA replication by altering viral protein synthesis and inhibits the cytolytic activity. Jenson et al. (Jenson, 1999a; 1999b) found that aqueous extracts of reference MST did not re-activate latent Epstein-Barr virus.

Four publications investigated the ability of MST extract to modulate cell proliferation (oral-derived cells). Using hamster oral epidermoid carcinoma cell cultures, Muns et al. (1994) reported that aqueous extracts of reference MST (one dose, 1 percent) had no significant effect on cell number over a 72-hour incubation. Similarly, Coppe et al. (2008) observed that human oral fibroblasts, exposed to an aqueous extract of Copenhagen® MST (6 doses) over a 10-day incubation period, did not proliferate at a rate faster than vehicle control-treated cells. In fact, five out of six MST doses inhibited cell proliferation in this study. Wang et al. (2001), using organotypic cultures exposed to aqueous extracts of reference MST (4 doses tested), showed stimulation of fibroblast cell growth at all MST concentrations and stimulation of normal human epidermal keratinocyte proliferation at the low dose but suppression of growth at higher concentrations. Finally, Rubinstein (2000) investigated the mechanism of reference MST (aqueous extract)-induced promotion of cell growth in cultured chemically transformed hamster oral keratinocytes noting that aqueous extract of ST potentiated DNA synthesis elicited by vasoactive intestinal peptide.

7.5.6-1.2.5.2. Summary

Overall, *in vivo* (with laboratory animal) and *in vitro* studies provide conflicting results on the potential carcinogenic effect of MST exposure in the oral cavity.

The two *in vivo* model systems used to investigate the potential for MST to act as a complete carcinogen provide contradictory results. Studies with the hamster cheek pouch model provide little reason to conclude that MST may act as a complete carcinogen. However, on the basis of rat studies using the lip canal model, there appears to be evidence that supports a role for MST as a complete carcinogen in oral tissue.

The examination of MST to act as a modulator of carcinogenicity also provides mixed outcomes, which appear to be dependent on the initiating agent used in the study. For DMBA or ethanol, the evidence does not suggest that MST modulates carcinogenesis. For the initiating agent, 4-NQO, there is inadequate evidence for such an effect due to conflicting studies. There is sufficient evidence to suggest that MST may promote viral-mediated carcinogenesis; in contrast, however, there is sufficient evidence to suggest that MST does not promote TSNA-induced tumor formation. In fact, the evidence suggests that MST may even inhibit TSNA-induced carcinogenesis.

The unknown factor with these studies is the relevance of the MST-exposure regimen and material preparation compared with the MST-exposure situation encountered by humans. Oral cancer studies in animal models are exceedingly difficult to conduct without creating some unique exposure system or dosing regimen that does not fit the human situation. Similarly, chemical extraction of MST before *in vitro* work may or may not actually represent human exposure situations. The clinical relevance of the nonclinical work with MST to human exposure is not known.

7.5.6-1.2.6. Oral Injury and Dental-Related Studies

The nonclinical studies described in the following section, discuss scientific evidence demonstrating biological plausibility for MST-induced adverse effects on periodontal tissue. However, the clinical relevance of the MST extract concentrations often used to induce these periodontal-associated responses is not known.

7.5.6-1.2.6.1. Periodontal Disease

Periodontal disease is associated with the loss of periodontal attachment and bone loss.

We reviewed six *in vitro* studies from three different laboratories related to this topic. Four of these studies were conducted in the Ramp laboratory and investigated the effect of reference MST extracts on collagen synthesis in osteoblast-like cells isolated from 17-day-old chick embryo bones (Galvin, 1988, 1991; Galvin, 1992; Lenz, 1992). The 1988 published study used cell culture medium to extract MST, whereas the other three studies used methanol as the extraction solvent. A significant inhibition of bone collagen synthesis after a 48-hour exposure to MST extracts was reported in all studies, regardless of the extraction solvent. A 1995 study by Henderson et al. (1995) showed that cultured osteoblasts isolated from chick embryos exposed (for 1 to 7 days) to an aqueous extract of reference MST results in cell proliferation. An increase in functional osteoblast cell number would be expected to promote bone formation. This increase, however, would contradict the Ramp laboratory's findings. The final study and the most recent publication (Andersson, 2006) describe the use of primary human periodontal ligament fibroblast cultures. These cells, exposed to aqueous extracts of reference MST for 24 hours, demonstrated a reduction in cell growth and a reduction in cellular alkaline phosphatase production.

Together, the findings from these studies suggest that MST can inhibit processes important in bone formation.

7.5.6-1.2.6.2. Dental Caries

No preclinical studies were found that directly investigated the effect of MST on the formation of dental caries. One short communication provided limited methodology details investigating the effect of MST on a process associated with human dental caries (Falkler, 1987). This study demonstrated that aqueous extracts of U.S. commercial MST can serve as a substrate for the growth of oral bacteria commonly associated with dental caries in humans.

7.5.6-1.2.6.3. Vascular System Effects

Ten publications (eight *in vivo* and three *in vitro* studies) from four different laboratories investigated the vascular integrity of the oral mucosa during exposure to MST or MST extracts.

In 1993, Huckabee et al. (1993) published a study demonstrating that dose-independent vasodilation was observed at the site of reference MST application in anesthetized dogs. This observation led to a series of *in vivo* studies conducted by the Rubinstein laboratory showing that reference MST exposure in hamsters (cheek pouch model) results in the acute increase in macromolecular efflux from the oral mucosa (Gao, 1999; Gao, 1997a, 1997b; 1997c; Rubinstein, 2000; Rubinstein, 1998; Rubinstein, 2002; Suzuki, 1996). Studies from this laboratory confirmed a "leaky site formation" at the site of MST application and investigated the mode of action for this observation (Gao, 1997a, 1997b; 1997c; Suzuki, 1996). One study from this laboratory showed that the microorganism species, *Bacillus megaterium*, present in commercial MST, causes plasma exudation from blood vessels in the intact hamster cheek pouch (Rubinstein, 2002). Other laboratories have published several *in vitro* studies related to vascular integrity. For example, Furie et al. (2000) demonstrated that, in cultured human vascular endothelial cells, exposure to reference MST aqueous extracts resulted in "leaky" endothelial monolayers, which enabled inflammatory cells to migrate through the monolayer. Ljungberg et al. (2013) reported that acute *in vitro* exposure to commercial MST aqueous extracts inhibited platelet activation.

The relevance of these findings to human exposure is unknown.

7.5.6-1.2.6.4. Oral Cytotoxicity

Ten publications from five different laboratories investigated the *in vitro* toxic effect of aqueous extracts of reference MST on cells derived from the oral cavity. Many of these *in vitro* studies also investigated the mode of action for MST-induced toxicity.

Overall, the findings from these studies (individual studies are shown in Table 7.5.6-1-28) provide scientific evidence demonstrating that MST exposure can result in both apoptotic and nonapoptotic cell death. Evidence also supports the role of oxidative stress, osmotic stress, and physical disruption of the plasma membrane in this toxic process. The clinical relevance of the MST extract concentrations used *in vitro* to induce the observed toxic responses is not known.

7.5.6-1.2.6.5. Cell Death

Seven *in vitro* studies have been published investigating the ability of MST extracts to cause cell death. Cells derived from the oral cavity were used in these studies, including primary human oral keratinocytes (Bagchi, 1999; Bagchi, 1997; Bagchi, 2001; Mitchell, 2010), immortalized human oral keratinocytes (Mitchell, 2010), human oral squamous cell carcinoma cells (Gao, 2013), and immortalized hamster cheek pouch cells (Mangipudy, 1999). These studies clearly demonstrated a dose-dependent increase in apoptotic cell death (Bagchi, 1999; Bagchi, 2001; Mangipudy, 1999) or nonapoptotic cell death (Bagchi, 1996; Bagchi, 1997; Mitchell, 2010) following exposure to MST extracts for 3 to 96 hours.

7.5.6-1.2.6.6. Mode of Action for MST Extract's Toxic Effect on Oral Cavity-Derived Cells

Nine separate studies investigated the mode of action for MST extract's toxic effect on oral cavity-derived cells. The vast majority of these studies reported evidence for the role of oxidative stress (reactive oxygen species, nitric oxide formation, lipid peroxidation) in MST-induced cell death (Bagchi, 1996; Bagchi, 1999; Bagchi, 1997; Bagchi, 2001; Mangipudy, 1999; Mitchell, 2010). Two studies provided evidence to support the role of osmotic stress (hyperosmolarity) in MST mediated cytotoxicity (Lombard, 2010; Tobey, 1988). The physical disruption of cellular plasma membranes by MST itself was also shown to play an important role in the loss of cell viability (Joyce, 2010).

7.5.6-1.2.7. Immune System Studies

The possible effect of MST or MST extract exposure on the immune system response has been investigated. Overall, findings from these studies provide scientific evidence to support a role for MST exposure in stimulating a proinflammatory response and in inhibiting the cytotoxic activity of immune system cells. The clinical relevance of the MST extract concentrations used *in vitro* to induce the observed immune system responses is not known.

7.5.6-1.2.7.1. Immunotoxicity

Three studies (two *in vivo* and one *in vitro*) investigated the ability of MST or MST extracts to alter the cytolytic function of immune cells. Johansson et al. (1991a) reported that the cytotoxic activity of peripheral blood-derived natural killer cells (lymphocyte sub-population) were significantly reduced following a 15-week exposure to oral U.S. commercial MST (rat lip canal model). Similarly, the Shklar laboratory showed that macrophages isolated from hamsters exposed for 20 weeks to oral MST (cheek pouch model) had a significant reduction in cytotoxic activity (Antoniades, 1984). In both *in vivo* studies, the immune cell cytotoxic activity was determined *in vitro*, against tumor target cells. The *in vitro* study of Lindemann and Park (1988) found that human lymphokine-activated killer cell activity was inhibited after exposure to reference MST extracts (extract with cell culture medium), in a dose-dependent manner.

7.5.6-1.2.8. Inflammation Mediators

Fourteen studies (one *in vivo* study) from seven different laboratories have been published investigating the ability of aqueous MST extracts to stimulate a proinflammatory response in a variety of model systems. The experimental systems include the activation of complement (Chan, 1999; Chang, 1998), primary human oral keratinocyte cultures (Johnson, 1996), transformed hamster oral epidermoid cells (Vishwanatha, 2003), human peripheral blood monocytes (Arimilli, 2012; Arimilli, 2013; Bernzweig, 1998; Payne, 1994), primary rat macrophages (Hassoun, 1995), and primary mouse splenic mononuclear cells (Goud, 1993; Petro, 2003; Petro, 2002; Petro, 1999; Petro, 1997). Collectively, the findings from these studies clearly demonstrate that exposure of human immune cells or oral-derived cells to aqueous MST extracts result in a significant increase in the production and secretion of a variety of cytokines/chemokines. These proinflammatory mediators include nitric oxide

production, interleukin-8, 1, cyclooxygenase-2, prostaglandin-2, and complement activation to name a few.

7.5.6-1.2.9. Studies on Cardiovascular Effects

There are no known published nonclinical studies solely designed to investigate the potential adverse effects of chronic MST exposure on the cardiovascular (CV) system. However, the findings from two MST feeding studies in three different species indicate the absence (as compared with that in controls) of significant microscopic changes in the heart (cardiomyopathy) following 90 days (Theophilus, 2012) or 2 years (Homburger, 1976) exposure to MST or MST extract in the diet. In 1976, Homburger et al. (1976) reported that the heart rate, blood pressure, and electrocardiogram tracings were unaffected in hamsters after 24 months of snuff feeding.

Squires et al. (1984) reported that dogs exposed for 20 minutes to MST in the buccal, gingival fold showed significant changes in several CV endpoints. Significant increases were seen in heart rate, blood pressure, left ventricular pressure, and left ventricular end diastolic pressure. Overall, acute MST exposure in dogs resulted in physiological CV effects similar to that expected from nicotine exposure, but no pathogenic effects were noted.

7.5.6-1.2.10. Reproductive and Developmental Studies

There were six publications identified in this category; all from the same laboratory (Paulson, 1994a; Paulson, 1989; Paulson 1994b; Paulson, 1992; Paulson, 1991; Paulson, 1993). Every study investigated potential fetal or pup developmental effects of MST extracts. Female rats or mice were administered water extracts of reference MST by oral gavage during prebreeding, breeding, or during gestation. MST treatment groups included low and high MST extract dosing regimens. In mouse studies, MST treatment resulted in a steady-state plasma nicotine level of 99 to 300 ng/mL and 481 to 623 ng/mL, respectively. In the rat study, low and high MST treatment resulted in a steady-state plasma nicotine level of 283 ng/mL and 846 ng/mL, respectively. Since a steady-state plasma nicotine concentration of 10 to 50 ng/mL has been reported for MST users, it appears that findings from the low MST treatment groups may provide greater relevance to human studies (Theophilus, 2012).

Developmental studies in mouse and rat models measured maternal and fetal endpoints including maternal weight gain, death and placental weight, litter size, total implantations, fetal weight, placental weight, resorbed, dead and malformed fetuses, skeletal abnormalities, and level of skeleton ossification (Paulson, 1994a; Paulson, 1989; Paulson, 1992; Paulson, 1991). The results from these studies demonstrated a negligible effect of low-dose MST exposure on mouse and rat fetuses and dams. In contrast, high-dose MST demonstrated significant embryotoxicity, fetal growth retardation, decrease in ossification, and maternal toxicity.

For developmental studies in rat pups (Paulson, 1994b; Paulson, 1993), the endpoints measured include pup weight, physical landmark development and behavioral performance. The results of these studies demonstrated that, with low-dose MST exposure, (equal to 1.33 mg/kg nicotine) pups were more active, but there were no significant differences in physical development, behavioral or cognitive performance. Higher doses (equal to 4 or 6 mg/kg

nicotine) resulted in maternal toxicity (lower weight gain), as well as lower weight gain and increased mortality in the pups. Overall findings from laboratory animal studies with MST extracts indicate the potential for an adverse developmental effect of MST exposure is minimal.

Table 7.5.6-1-28: Summary of Relevant Published Literature - Nonclinical Endpoints

Author	Title	Topic	Methods	Findings	Comments
(Peacock, Jr., 1959)	An evaluation of snuff and tobacco in the production of mouth cancer	Complete carcinogenesis	*In vivo* (hamster cheek pouch, n = 50/group) Snuff, chewing tobacco, chemical carcinogens, controls implanted once in left cheek pouch and remained for up to 2 years.	The authors found that " ... there has been no evidence of neoplasia in 12 animals which lived over 1 year following implantation of snuff and tobacco."	High mortality among the animal groups.
(Peacock, Jr., 1960)	The effect of snuff and tobacco on the production of oral carcinoma: an experimental and epidemiological study	Complete carcinogenesis	*In vivo* (hamster cheek pouch, n = 124) Snuff or chewing tobacco implanted one time in one cheek pouch *chewing gum and sand in the other) and remained for a lifetime (up to 2 years.)	The authors reported "Twenty-one of the hamsters which were implanted with snuff and 21 of the hamsters which were implanted with tobacco survived for over one year. No neoplasms were found in any of the pouches. Apparently snuff and tobacco were not carcinogenic when applied to the oral mucous membranes of golden hamsters during the major portion of their life span."	High mortality among the animals groups due to infection or nicotine poisoning.
(Homburger, 1971)	Mechanical irritation, polycyclic hydrocarbons, and snuff. Effects on facial skin, cheek pouch, and oral mucosa in Syrian hamsters	Complete carcinogenesis General toxicity	*In vivo* (hamster cheek pouch, n = 24-60/group) Insertion of cotton, cotton impregnated with DMBA, benzo[a]pyrene, or snuff 30 min/d, 5 d/wk for up to 1 year.	The authors reported "… exposure to tobacco caused no changes more intense that those seen in animals biting on cotton-containing bits."	The authors noted that the "…experiment suffers from the limitation of the length of exposure which was tolerable to the hamsters. Immobilization necessary for adequate exposure was tolerated for no more than 45 min. each day."

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Homburger, 1976)	Absence of carcinogenic effects of chronic feeding of snuff in inbred Syrian hamsters	Complete carcinogenesis Modulating carcinogenesis	*In vivo* (hamster feeding, n = 50/group) Feeding study with (a) 20% moist snuff, (b) 20-methylcholanthrene (MC), (c) MC with a cellulose-containing diet, (d) MC with a diet containing 20% moist snuff.	The authors reported "These chronic feeding studies failed to reveal any carcinogenic or co-carcinogenic effects of ...20% snuff in the diet."	Cotinine blood levels were higher than observed in daily tobacco users. Histopathology findings are not presented other than statement of "no pathological changes."
(Antoniades, 1984)	Effects of smokeless tobacco on the immune system of Syrian hamsters	Immunotoxicity	*In vivo* (hamster cheek pouch, n = 60, 20/group) Approximately 0.07 g snuff, chewing tobacco, and control, placed in right buccal pouch daily for 20 weeks.	"The buccal pouch mucosa revealed no evidence of either dysplasia or neoplasia." "No significant pathologic changes were seen" in major tissues.	Snuff placed in cheek pouch only remained for several hours before finally being chewed and swallowed. 20-week treatment
(Squires, 1984)	Hemodynamic effects of oral smokeless tobacco in dogs and young adults	Cardiovascular	*In vivo* (dogs, n = 10) 2.5 g of ST was placed in the left buccal, gingival fold of anesthetized animals CV-related measurements were taken each minute for 20 minutes.	"Significant increases were seen in heart rate, blood pressure, left ventricular pressure, left ventricular end diastolic pressure, and left ventricular dP/dt. Significant decreases in flow were noted in the coronary circumflex, renal, and femoral arteries. The flow reduction was thought to have been mediated by an alpha adrenergic mechanism."	Acute exposure demonstrating physiological CV effects.
(Whong, 1984)	Mutagenicity of tobacco snuff: possible health implications for coal miners	Genotoxicity	*In vitro* (Salmonella typhimurium: TA100, TA98, TA1535; with and without S9 activation) Commercial MST extracts were prepared using dichloromethane, then acetone plus methanol (1:1); or water.	"No mutagenic activity was found for tobacco snuff extracts without pH adjustment ." "…mutagenic substances were formed from tobacco snuff extracts in an acidic environment."	A potential mechanism for the observed effect at pH 3 is from tobacco-specific N-nitrosamine (TSNA) formation via nitrosylation (in the presence of nitrite), which is enhanced

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
					under acidic conditions.
(Park, 1985)	Combined effect of herpes simplex virus and tobacco on the histopathologic changes in lips of mice	Modulating carcinogenesis	*In vivo* (mouse, n = 20/group) Snuff water extract, smoking tar condensate in acetone, acetone, or distilled water was brushed on the mucosa and skin of the upper lips three times a day, 5 d/wk for 2 months. The topical applications began 1 day after mock, or HSV-1 inoculation.	"Two months' exposure to tobacco or HSV-1 inoculation alone did not induce dysplasia…" "…HSV-1 inoculation combined with snuff water extract or smoking tar condensate produced epithelial dysplasia and other histomorphologic changes (ie. hyperkeratosis, increased granular cell layer thickness, acanthosis, and increased inflammatory cell infiltration in a significant number of animals)."	Short treatment period. The commercial snuff can was opened to the air for 2 weeks prior to extract preparation (in the hope of increasing TSNA content).
(Shklar, 1985)	Effects of smokeless tobacco and snuff on oral mucosa of experimental animals	Complete carcinogenesis	*In vivo* (hamster cheek pouch, n = 20/group) Approximately 0.07 g of Copenhagen®, or Hawken Rough Cut, was placed in buccal pouch daily for up to 20 weeks.	The authors concluded the following: "Histologic study of the buccal pouch mucosa revealed no significant pathologic changes at either 10 or 20 weeks after the start of the experiment." "Our findings suggest that chewing tobacco and snuff may not be direct carcinogens when kept in apposition to oral mucosa for short periods, or that they are at most extremely mild carcinogens."	Plasma nicotine levels were not measured and thus the relevance of MST exposure regimen in this study to MST users is unknown. Twenty weeks of MST exposure is likely not sufficient time to assess complete carcinogenesis.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Tucker, 1985)	Induction of sister chromatid exchanges by coal dust and tobacco snuff extracts in human peripheral lymphocytes	Genotoxicity	*In vitro* (sister chromatid exchange: SCE) MST extracted with organic solvent.	"Snuff was found to induce a 59% increase above the background SCE frequency at the highest dose (average of 3 donors). Coal dust alone nearly doubled the SCE frequency while coal dust plus snuff together nearly tripled the SCE frequency."	Description of methods in regard to exposure time to extracts etc. was limited.
(Hecht, 1986)	Induction of oral cavity tumors in F344 rats by tobacco-specific nitrosamines and snuff	Complete carcinogenesis Modulating carcinogenesis	*In vivo* (rat: surgical lip canal, n = 10-32/group) Study 1: Male F344 rats received either water, water extract of snuff, water extract of snuff enriched with 10 times indigenous concentration of NNN and NNK, or with NNN and NNK in water applied by swab to the oral cavity in divided doses over 131 weeks. Study 2: A test canal was surgically created in the lower lip of groups of rats and either snuff, water extracted snuff or snuff enriched with its own water extract was inserted in the test canal 5 times weekly for 116 weeks.	The authors concluded that this study has demonstrated that snuff and tobacco-specific nitrosamines can induce tumors in the rat oral cavity. They also suggested that that components of snuff extract may inhibit tumor induction by tobacco-specific nitrosamines. In the first experiment, the incidence of oral cavity tumors in the rats treated with NNN and NNK was 8/30, as compared with 3/30 in rats treated with snuff extract enriched with NNN and NNK. The authors reported that this "suggests that NNN and NNK were less tumorigenic when administered together with snuff extract than when administered alone," but not in the rats treated with snuff extract alone. Oral tumor response in the second experiment was: 3/32 snuff 2/21 water extract 1/32 water extract enriched snuff	None

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Park, 1986)	Oral cancer induced in hamsters with herpes simplex infection and simulated snuff dipping	Modulating carcinogenesis	*In vivo* (hamster cheek pouch, n = 15-20/group) Commercial snuff (150 mg) was placed in both cheek pouches twice a day for 6 months. HSV-1, HSV-2 or culture medium inoculations were completed once a month for 6 months.	The authors found that "Neither simulated snuff dipping nor HSV infection alone induced neoplastic changes in hamster buccal pouches. However, HSV infection in combination with simulated snuff dipping resulted in epithelial dysplasia and invasive squamous cell carcinoma in more than 50% of the animals." Animals treated with infection and snuff combined showed mild reactive changes, while those with HSV infection alone developed severe inflammatory lesions. Moreover, none of the animals that received snuff died, in spite of the repeated HSV infection.	The exposure period of this study was limited to 6 months.
(Brunnemann, 1987)	A study of snuff carcinogenesis	Complete carcinogenesis Modulating carcinogenesis	*In vivo* (rat – surgical lip canal, n = not reported) Oral surfaces were swabbed twice daily with an aqueous solution of NNN (135 ppm) and NNK (27.5 ppm), snuff extract enriched with NNN and NNK, snuff extract alone or control for 120 weeks. Five U.S. commercial brands were included.	"The concentrations of TSNA are similar in dry snuff and in the more popular moist snuff. After oral swabbing with a mixture of NNN and NNK, rats developed tumours of the oral cavity and lung, showing that these TSNAs are not only organ-specific carcinogens but can also induce local tumours. After swabbing an extract of snuff containing the same concentrations of NNN and NNK, significantly fewer tumours were induced in the oral cavity and lung, indicating inhibition of the tumourigenic activity of the TSNAs by other snuff constituents."	Exposure duration of 120 weeks was likely sufficient time to assess complete carcinogenic effect in animals.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Falkler, 1987)	The effect of smokeless-tobacco extracts on the growth of oral bacteria of the genus Streptococcus	Dental carries	*In vitro* (streptococci: mutans, salivarius and sanguis) Growth inhibition studies were conducted with extracts of Skoal, Copenhagen®, Kodiak and Hawken.	The authors concluded that "extracts of Kodiak, Skoal and Copenhagen® can serve as substrates for the growth of Strep. mutans and Strep. salivarius." These Streptococci species are associated with dental caries.	Statistical methods are lacking.
(Guttenplan, 1987)	Mutagenic activity in smokeless tobacco products sold in the USA	Genotoxicity	*In vitro* (Salmonella typhimurium: TA 100 with and without S9 activation) Aqueous extracts of U.S. commercial ST (brands not disclosed) were used.	The author concluded that "...aqueous extracts of five smokeless tobacco products are mutagenic without further treatment…" Further noting that "…the mutagenesis behavior is typical of nitrosamines, and suggests that N-nitrosamines are responsible for at least some of the mutagenic activity in the extracts."	Extraction procedure (24-hour extraction in phosphate buffer at 37 degrees) could stimulate microbial growth. The assay conditions were altered by the author to maximize mutagenicity by TSNAs. When these conditions were returned to normal (standard Ames assay), mutagenicity observed was eliminated or weak.
(Park, 1987)	Effect of tar condensate from smoking tobacco and water-extract of snuff on the oral mucosa of mice with latent herpes simplex virus	Modulating carcinogenesis	*In vivo* (mouse, n = 20/group) One month following HSV-1 or mock inoculation, snuff (commercial) extract (20 g/100 mL water), condensate in acetone (50% v/v acetone in water), acetone, or distilled water were applied on the labial mucosa of the upper lips with a brush 3 times per day, 5 d/wk for 2 or 3 months.	"Three months' exposure to tobacco produced epithelial dysplasia and other changes in a significant number of latent HSV-infected mice, whereas tobacco alone did not induce dysplasia in the labial epithelium of uninfected mice."	Limited exposure period. Application of the solvent (acetone) induced significant histologic changes in the lip.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Prokopczyk, 1987)	Effect of snuff and nicotine on DNA methylation by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone	Modulating carcinogenesis	*In vivo* (rat: surgical lip canal, n = 6/group) Animals received either snuff extract (2 times per day, Monday through Friday, and once daily on weekends) via gavage), or nicotine (drinking water) for 2 weeks. Animals were subsequently gavaged once with NNK. DNA isolation and DNA methylation analyses were performed on tissues removed from rats killed 4 hours after NNK gavage.	"Formation of 7-methylguanine in the liver, nasal mucosae and oral cavity and of O^6-methylguanine in the liver and oral cavity was much lower in the rats pretreated with snuff extract than in those not pretreated. On the other hand, pretreatment of the rats with nicotine had no significant effect…" "These data suggest that snuff pretreatment does not accelerate repair of O^6-MeGua to a similar extent in all organs and that factors other than an increase in repair are responsible for the decreased levels of O^6-MeGua in the nasal cavity."	Small study.
(Stich, 1987)	Effect of smokeless tobacco on the replication of herpes simplex virus *in vitro* and on production of viral lesions in hamster cheek pouch	Modulating carcinogenesis	*In vivo* (hamster cheek pouch, n = 12/group) Animals were inoculated with either HSV-1 or HSV-2 and received snuff via cheek pouch twice daily for 7 days. *In vitro* (DNA synthesis): Confluent Vero cell monolayers inoculated with HSV for 1 hour and then incubated with snuff extract for 24 hours.	"Snuff-extract inhibited the replication of HSV-1 and HSV-2 in Vero cell monolayers…" "Simulated snuff-dipping not only inhibited the development of viral lesions in the hamster pouch, but also significantly suppressed the growth of HSV there."	The relevance of extract concentrations is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Galvin, 1988)	Smokeless tobacco contains a nonnicotine inhibitor of bone metabolism	Periodontal disease	*In vitro* (tibiae of chick embryos) MST extracts (including Skoal Bandits Wintergreen and University of Kentucky Reference MST*) were added to bone cultures for 5 hours.	The authors concluded that findings suggest the following conclusions: (1) both nicotine and MST extract at concentrations found in the saliva of smokeless tobacco users, stimulate glycolysis and markedly inhibit bone collagen synthesis and mitochondrial activity; (2) effects of MST extract on bone are not due to nicotine; (3) under conditions studied, bone partially recovers from the effects of MST extract.	The relevance of this method is unknown.
(Lindemann, 1988)	Inhibition of human lymphokine-activated killer activity by smokeless tobacco (snuff) extract	Immunotoxicity	*In vitro* (LAK, cytotoxicity assays) Human peripheral blood lymphocytes incubated with MST extract (University of Kentucky reference 1S3) for 3 days.	Snuff extract inhibited both LAK cytotoxicity and DNA synthesis in a dose-dependent fashion leading the authors to conclude "…snuff extract can inhibit lymphocyte activation *in vitro*, apparently by immune suppression, which may be related to an increase in the risk of neoplasia."	The relevance of this method is unknown.
(Park, 1988)	Smokeless tobacco carcinogenesis: the role of viral and other factors	Modulating carcinogenesis	*In vivo* (hamster cheek pouch n = not reported) Hamsters were infected with either HSV-1 or HSV-2 and received snuff twice daily for 7 days or received snuff for 40 weeks (twice a day for 5 d/wk) *In vitro* (Green monkey kidney cell monolayers): The virus was mixed with 2% snuff extract, incubated with the kidney cells and cytolytic activity of the virus determined	The authors found the following: "Snuff extracts inhibited the growth of both HSV-1 and HSV-2 in green monkey kidney cell monolayers cultures in a dose-dependent manner." "Snuff extracts selectively inhibit viral growth in concentrations that do not interfere with cellular growth and replication." "…water-soluble component of snuff is capable not only of inhibiting the growth of HSV in cells but also of inactivating HSV in cell-free conditions."	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
				They also noted that "…snuff extract, at a concentration that inhibits the cell lysis caused by herpes virus, does not totally abolish gene expression of the virus and may therefore increase the oncogenic capacity of HSV."	
(Tobey, 1988)	The acute effects of smokeless tobacco on transport and barrier function of buccal mucosa	Oral cytotoxicity	*In vitro* (cell injury/hyperosmolarity) Buccal mucosa obtained from mongrel dogs was exposed to ST extracts (Skoal Bandit Wintergreen) for 1 hour.	The authors concluded that ST can acutely alter buccal transport and barrier function by creating through electrolyte release, electrochemical and osmolar gradients across the tissue.	The effects are possibly related to hyperosmolarity, although the publication does not clearly stated as such.
(Chen, 1989)	Effects of smokeless tobacco on the buccal mucosa of HMT rats	Complete carcinogenesis	*In vivo* (Harwell mouth tumor rats: HMT, n = 30) Both sides of the mandibular mucobuccal fold were treated weekly with ST for 1 year. Rats were followed for an additional observation period of 6 months.	The authors concluded that "…smokeless tobacco to the buccal mucosa of HMT rats did not result in dysplasia or carcinoma during the interval examined." "Hyperorthokeratosis, acanthosis, numerous binucleate spinous cells, and subepithelial connective tissue hyalinization were observed, whereas verrucous carcinoma and squamous cell carcinoma were not seen."	Dosage and frequency of application may not have been sufficient to induce carcinoma, although other changes were induced.
(Johansson, 1989)	Snuff-induced carcinogenesis: effect of snuff in rats initiated with 4-nitroquinoline N-oxide	Complete carcinogenesis Modulating carcinogenesis General toxicity	*In vivo* (rat, n = 30/group) ST (commercially available) was applied 2 times per day, 5 d/wk for up to 104 weeks, or propylene glycol applied 3 times per week. 4-NQO dissolved in propylene glycol was applied 4 weeks prior to ST exposure (5 d/wk for 104 weeks)	The authors noted that snuff and 4-NQO by themselves have the potential to induce malignant tumors. Initiation with 4-NQO followed by snuff did not significantly enhance tumor formation.	Plasma nicotine levels were not measured.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Paulson, 1989)	Effect of smokeless tobacco on the development of the CD-1 mouse fetus	Reproductive/ developmental	*In vivo* (mouse, n = 18-34/group) ST extract (University of Kentucky reference* containing nicotine at the equivalent of 4 mg/kg body weight (1 x), 12 mg/kg (3 x), and 20 mg/kg (5 x) was administered orally three times per day by oral gavage during gestational Days 1-16.	The authors concluded that the lowest ST dose (1 x) produced a "negligible effect" on CD-1 mouse fetus and the dam, whereas the 5 x concentration (highest dose) demonstrated embryotoxicity, growth retardation, few malformations, hemorrhages, and maternal toxicity. A dose of 3 x) produced "a range of effects between the highest and lowest doses to both the fetus and dam."	The lowest MST dosage, which produced mean nicotine plasma level of 99 ng/mL, approximated human exposure levels.
(Oh, 1990)	Effect of snuff extract on the replication and synthesis of viral DNA and proteins in cells infected with herpes simplex virus	Modulating carcinogenesis	*In vitro* (Vero cell monolayer) Cells were inoculated with HSV-1, and the effects of snuff extract on the replication of HSV-1, on viral DNA synthesis, and on the synthesis of different classes of viral proteins were determined following a 24-h incubation.	"Snuff extract inhibited the replication of HSV-1 in Vero cell monolayers…" "In view of the fact that HSV must be inactivated and lose its cytolytic activity to be oncogenic, we assume that snuff extract can increase the carcinogenic capacity of HSV by inactivating HSV."	The relevance of this method is unknown.
(Galvin, 1991)	Comparison of the effects of smokeless tobacco extract with the effects of prolyl hydroxylase inhibitors on collagenous and noncollagenous protein synthesis by osteoblasts	Periodontal disease	*In vitro* (chick embryo periodontal/bone effects) Confluent cultures were prepared from osteoblast-like cells isolated from chick embryos. ST extracts (UK reference*, 2,2'-dipyridyl, or pyridine 2,5-dicarboxylate were added to the cultures for 48 hours.	"These findings clearly suggest that smokeless tobacco contains a component (or components) which inhibits collagen production by bone-forming cells. Consequently, the bone loss associated with the periodontal damage seen in smokeless tobacco users may be related to this component(s)."	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Johansson, 1991a)	Effect of repeated oral administration of tobacco snuff on natural killer-cell activity in the rat	Complete carcinogenesis Immunotoxicity	*In vivo* (rat: surgical lip canal, n = 38) American brand snuff (150 mg snuff was applied 2 times per day, 5 d/wk for 15 weeks)	"NK cell activity was significantly reduced in the snuff-treated group." This reduction occurred rapidly, within 4 days of exposure and maintained for 15 weeks. "No significant changes were identified histologically. None of the animals developed detectable neoplasms during the 15 weeks."	Exposure time may be too short for cancer formation.
(Johansson, 1991b)	Lack of promoting ability of snuff in rats initiated with 4-nitroquinoline-N-oxide	Complete carcinogenesis Modulating carcinogenesis	*In vivo* (rat: surgical lip canal, 29-30/group) Snuff was applied 2 times per day, 5 d/wk for up to 104 weeks, or propylene glycol applied 3 times per week. 4-NQO dissolved in propylene glycol was applied prior (3 times per week for 4 weeks). There were 5 treatment groups and they received different combination treatments over the course of 104 weeks.	"Rats treated with snuff only, 4-NQO followed by snuff and 4-NQO only had a significantly higher number of squamous-cell tumours and hyperplastic squamous lesions of the lip, oral and nasal cavity and forestomach than solvent or untreated controls." "Snuff appears to have a general tumorigenic effect but lacks promoting ability after initiation with 4-NQO."	None

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Johansson, 1991c)	Promoting effect of snuff in rats initiated by 4-nitroquinoline-N-oxide or 7,12-dimethylbenz(a)anthracene.	Complete carcinogenesis Modulating carcinogenesis	*In vivo* (rat: surgical lip canal, n = 30-40/group) Snuff (UK reference 1S3) was applied in the lip canal, 2 times daily, 5 d/wk week for up to 104 weeks. Rats were initiated with DMBA 3 times per week for 4 weeks and then received either cotton pellet dipped in saline (daily, 5 d/wk for 104 weeks), MST (2 times a day, 5 d/wk for up to 104 weeks). Rats were initiated with 4-NQO 3 times per week for 4 weeks and then received a cotton pellet dipped in saline (daily 5 d/wk for 100 weeks or MST (2 times a day, 5 d/wk for up to 100 weeks. Control rats treated with the cotton pellet dipped in saline 5 d/wk for up to 100 weeks.	"It was found that rats exposed to snuff with or without initiation had significantly lower weight gains." "These results show that snuff has strong promoting capability with regard to the development of lip sarcomas after 4-NQO initiation but not after DMBA initiation." Snuff by itself was shown to be carcinogenic for the lip and oral cavity.	In a previous publication, the authors found that snuff did not promote 4-NQO–induced cancer. Increasing the dose of 4-NQO (by eightfold) in this study produced a modulating effect of snuff. The authors suggest that ST storage conditions during this 2-year study may have resulted in increased TSNA levels.
(Paulson, 1991)	Pre- and post-conceptional tobacco effects on the CD-1 mouse fetus.	Reproductive/ developmental	*In vivo* (mouse, n = 15-26/group) Aqueous extracts of UK reference ST 1S3, or water were administered three times daily by oral gavage for 2 weeks before breeding, during breeding and during Gestational Days 1-17.	"The low dose produced a negligible effect on the CD-1 mouse fetus and the dam. The high dose demonstrated growth retardation, increased embryotoxicity and a significant decrease in ossification."	Plasma nicotine and cotinine levels were measured during the study, but the lowest extract dose produced plasma nicotine levels that were not relevant to human exposure.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Shirname-More, 1991a)	Forward mutation of S. typhimurium by smokeless tobacco extracts.	Genotoxicity	*In vitro* (Salmonella typhimurium: TM677 with and without metabolic activation, and with and without nitrite treatment)	The authors concluded that the results indicate that MST contain polar and nonpolar chemicals that become mutagenic to S. typhimurium under nitrosation conditions	None
(Shirname-More, 1991b)	Smokeless tobacco extracts mutate human cells.	Genotoxicity	*In vitro* (cell viability and mutation fraction in human lymphoblast cell lines, TK-6 and AHH-1) Cultures were treated with aqueous tobacco extracts (two commercial products) for 28 hours, and then incubated for 2 weeks.	"…extracts tested were found to be detectably mutagenic in the range 1-3 mg/mL extractable solids. The mutagenicity of both extracts for both cell lines was markedly decreased by treatment at neutral pH with nitrite and acidic treatments. Treatment of extracts with nitrite at pH 3 did not have any effect on the mutagenicity of the untreated extracts for TK-6."	Extract concentration given in mg/mL extractable solids.
(Ashrafi, 1992)	A light, transmission and scanning electron microscope study of snuff-treated hamster cheek pouch epithelium.	Complete carcinogenesis	*In vivo* (hamster cheek pouch, n = 8-24/group) Approximately 2 g of snuff (including Skoal MST) were placed into the blind end of the right buccal pouch of the experimental animals once a day, 5 d/wk for 24 months.	No tumors were observed. The authors reported that MST-treated animals had whitish patches that correlated with hyperorthokeratosis, prominent granular cell layers with increased keratohyalin granules and hyperplasia. Ultrastructural changes included wider intercellular spaces filled with microvilli, numerous shorter desmosomes, many thin tonofilament bundles, increased number of mitochondria, membrane-coating granules, and keratohyalin granules.	Plasma nicotine levels were not obtained and therefore the relevance of exposure (one application per day) to MST users is not known.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Galvin, 1992)	Smokeless tobacco contains an inhibitor of prolyl hydroxylase activity.	Periodontal disease	*In vitro* (chick embryo periodontal/bone) Prolyl hydroxylase (extracted from chick embryos) activity was determined during a 30-minute incubation in the presence and absence of MST extract (UK reference*), salt solutions with or without zinc, nicotine, anabasine, varying substrates, and cofactor concentrations.	The authors concluded that the "…mechanism of inhibition of avian collagen formation involves specific inhibition of prolyl hydroxylase activity by STE extract. Therefore, some of the toxic effects of smokeless tobacco may be due to a component which directly alters collagen formation by inhibition of hydroxylation of proline."	The relevance of this method is unknown.
(Lenz, 1992)	Inhibition of cell metabolism by a smokeless tobacco extract: tissue and species specificity	Periodontal disease	*In vitro* (cultures of osteoblast-like cells harvested from chick embryos) and mouse fibroblasts Extracts of ?? Cells culture were exposed to ST extract (UK reference) for 2 days at concentrations 10, 15, 25, or 30 µL/mL.	"In the present study, ST extract inhibited collagen synthesis in frontal bone and sternal cartilage from chick embryos. The extract also inhibited AIPase activity and collagen synthesis in embryonic chick osteoblast-like cells and inhibited collagen synthesis in collagen-secreting embryonic mouse fibroblasts. The latter data indicate that inhibition of collagen synthesis by the inhibitor is not specific for one species or cell type."	ST effect on human collagen-producing cells is unknown
(Paulson, 1992)	Alcohol and smokeless tobacco effects on the CD-1 mouse fetus	Reproductive/ developmental	*In vivo* (mouse, n = 14-19/group) Mice were gavaged three times per day on Gestational Days 6-15 with either ST extract (UK reference 1S3) equivalent to 8 mg/kg nicotine, ethanol, a combination of ST extract and ethanol, or D-glucose (control, to supply calories similar to ethanol). On Gestational Day 17, all dams were sacrificed.	The authors concluded that "…in terms of fetal growth and ossification, ST had the greatest effect, followed by ETOH and ST+ETOH."	The ST dose used resulted in a plasma nicotine level of 321 ng/mL. This level is not relevant to human exposure.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Summerlin, 1992)	Histologic effects of smokeless tobacco and alcohol on the pouch mucosa and organs of the Syrian hamster	Complete carcinogenesis Modulating carcinogenesis	*In vivo* (hamster cheek pouch, n = 20/group) Animals received ST (Skoal 200 mg 5 times per week), alcohol (2 mL of 15% ethanol five times per week), tobacco and alcohol, or mechanical stimulation of the pouch (negative control) for 26 weeks.	"The only statistically significant histologic feature produced by this study was the presence of increased epithelial thickness in..." the groups exposed to tobacco alone as well as tobacco and alcohol. "No statistical difference was observed in the rate of dysplastic change or was the formation of carcinoma noted in the pouch mucosa." "Alterations were observed in the abdominal organs, but not of statistical significance."	The 26-week exposure period may not be sufficient time for the induction of cancer.
(Goud, 1993)	Immunostimulatory potential of smokeless tobacco extract in *in vitro* cultures of murine lymphoid tissues	Inflammation	*In vitro* (mouse splenic or mesenteric lymph node lymphocytes) Purified B cells and T cells were cultured with ST extract (UK reference*) at various dose levels to measure lymphocyte proliferation, polyclonal antibody responses, induction of IL-1 or 2, and mobilization of intracellular calcium.	"The results indicate that ST extract has a strong polyclonal mitogenic potential on B lymphocytes, inducing both proliferation and differentiation. It also induced proliferation of T cells which appeared not to be mediated by IL-2."	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Huckabee, 1993)	Effects of snuff on regional blood flow to the cheek and tongue of anesthetized dogs	Vascular effects	*In vivo* (dogs, n = 31) snuff or smokeless tobacco (UK reference*) was placed in the right buccal space that had been moistened with warm saline solution. Radioactive microspheres were injected into the left ventricle to measure regional blood flow at the peak blood pressure response to snuff, which usually occurred 2 to 3 minutes after snuff was placed in the buccal space.	The authors noted that changes in blood flow and vasodilation that appeared to correlate with blood nicotine levels. They concluded that the data "…appear to rule out ischemia as a direct cause of oral lesions at the site of snuff application."	Plasma nicotine levels were determined and indicate that dosing levels of 3.12, 6.25 and 12.5 mg snuff/kg resulted in plasma nicotine levels that are relevant to snuff users. Dosing levels of 25 mg/kg and above resulted in plasma levels above 100 ng/mL (not relevant).
(Murrah, 1993)	Morphologic and growth effects of tobacco-associated chemical carcinogens and smokeless tobacco extracts on human oral epithelial cells in culture	Complete carcinogenesis	*In vitro* (oral keratinocytes grown from explants of human labial and gingival mucosa) Cell monolayers were treated for 1 hour at weekly intervals with aqueous extracts of loose leaf tobacco, moist snuff, or dry snuff (UK reference*) , or with NNK, NNN or benzo(a)pyrene. After treatment, cultures were washed with media. Cultures were maintained for several weeks.	"Even though the controls and most treatment groups terminally differentiated, cells exposed to NNK, NNN and ST and dry ST extract continued to divide, maintained a differentiated phenotype for 8.5 to 10 weeks in culture, and displayed focal growth and morphologic changes suggestive of early states in cell transformation."	Statistical results seem to contradict overall conclusions of a ST effect on longevity and enhanced growth.
(Paulson, 1993)	Behavioral effects of prenatally administered smokeless tobacco on rat offspring	Reproductive/ developmental	*In vivo* (rat, n = 12-39) ST extract (UK reference 1S3) was given three times per day by oral gavage on Gestational Days 6-20. Dams were allowed to deliver, and pups were monitored for weights, physical landmark development, and behavioral performance preweaning	The authors concluded that "…maternal exposure to ST has dose-related effects on the physical development of the neonate and young rats." Some effects on body weight and growth were noted. "No treatment-related differences were seen in the rats' performance on two cognitive tests, the active avoidance shuttle	Although plasma nicotine levels were not measured, based on the estimated levels from previous experiments, blood levels likely attained this experiment exceeded typical human MST

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
			and postweaning periods.	box and the Cincinnati Water Maze."	users (fourfold to fivefold at the low-dose level).
(Bagchi, 1994)	Smokeless tobacco induced increases in hepatic lipid peroxidation, DNA damage and excretion of urinary lipid metabolites	General toxicity	*In vivo* (rat, n = 4-6/group) Rats were treated orally (oral gavage) with a single dose standardized smokeless tobacco (moist snuff) and STE (UK reference*) in phosphate buffer, control animals received vehicle. Animals were sacrificed 24 hours after treatment and liver microsomes, mitochondria and nuclei were isolated and examined for lipid peroxidation and DNA single-strand breaks.	Dose dependent increases in hepatic mitochondrial and microsomal lipid peroxidation and increases in hepatic DNA single-strand breaks occurred after MST extract treatment relative to control values. MST administration also resulted in significant dose-dependent increases in the excretion of the urinary lipid metabolites. The results suggest the involvement of an oxidative stress in the toxicity of MST.	Plasma levels for nicotine or cotinine were not measured, therefore relevance to MST consumers is unknown.
(Muns, 1994)	Effects of smokeless tobacco on chemically transformed hamster oral keratinocytes: role of angiotensin I-converting enzyme	Modulating carcinogenesis	*In vitro* (7, 12-Dimethylbenz[a]anthracene-transformed golden Syrian hamster oral keratinocytes: HCPC-1) Cells were incubated with 5 different concentrations of ST extract (UK reference*) for up to 72 hours. The effect of ST extract or bradykinin on cell proliferation was determined. The effect of ST extract on ACE activity in cell lysates was determined.	The authors found that "… ST extract induced a significant concentration- and time-dependent decrease in ACE activity in cultured HCPC-1 cells." "ST extract alone had no significant effect on cell number. Bradykinin alone induced a slight, but significant, increase in cell number. These effects were significantly potentiated by ST extract."	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Paulson, 1994a)	Prenatal smokeless tobacco effects on the rat fetus	Reproductive/ developmental	*In vivo* (rat, n = 15-23/group) ST extract (UK reference*) was given by oral gavage on Gestational Days 6-18 (MST low dose = 1.33 mg/kg nicotine, high dose = 6.0 mg/kg nicotine). Dams were sacrificed on Gestational Day 19; fetuses and placentas were weighed; and resorptions, deaths, or malformations were noted.	The authors concluded that under these experimental conditions the effects of MST at the low dose are minimal, whereas the high MST dose resulted in significant growth retardation and decreased ossification levels.	Plasma nicotine levels in this experiment exceeded typical human MST users (fourfold to fivefold at the low-dose level).
(Paulson, 1994b)	Behavioral effects of smokeless tobacco on the neonate and young Sprague Dawley rat	Reproductive/ developmental	*In vivo* (rat, n = 10-17/group) ST extract (UK reference*) was given by oral gavage on Gestational Days 6-18 (ST low dose = 1.33 mg/kg nicotine, mid dose = 4.0 mg/kg, high dose = 6.0 mg/kg nicotine). Dams were dosed three times per day by oral gavage on Gestational Days 6-20 with extracts or distilled water (control). Dams were allowed to deliver, and weights, physical landmark development, and behavioral performance of pups were monitored during preweaning and postweaning periods.	"High dose ST reduces pre- and post-weaning offspring weight gain and increases fetal mortality. ST at the low dose level appears to have the opposite effect, in that these offspring weights actually exceed control weights. ST also alters success in surface righting, activity levels and swimming development while no differences are noted in the rats' performance in active avoidance tests on learning."	Plasma nicotine levels in this experiment exceeded typical human MST users (fourfold to fivefold at the low-dose level).
(Payne, 1994)	Smokeless tobacco effects on monocyte secretion of PGE_2 and IL-1 β	Inflammation	*In vitro* (peripheral blood monocytes) Cells were treated for 24 hours with ST extract (UK reference*) with or without bacterial LPS. Monocyte secretion of inflammatory mediators, PGE_2 and IL-1β, were measured.	In summary, 1% aqueous snuff extract was a potent stimulator of PGE_2 release by peripheral blood monocytes obtained from nonsnuff users. Furthermore, 1% snuff extract significantly potentiated LPS-stimulated PGE_2 release. No effect was observed for lower concentrations of snuff extract for IL-1β.	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Stamm, 1994)	Mutagenicity of coal-dust and smokeless-tobacco extracts in *Salmonella typhimurium* strains with differing levels of *O*-acetyltransferase activities	Genotoxicity	*In vitro* (Salmonella typhimurium: TA98 and YG1024) ST extracts were tested as is (nonnitrosated) and after treatment with nitrite (nitrosated).	The authors concluded that this study with nitrosated and non-nitrosated ST extract indicated that aromatic amines and nitroarenes are probable sources of the mutagenic activity of low pH ST extracts.	An organic solvent extract was used and the relevance to water extract is unknown.
(Bagchi, 1995a)	Protective effects of free radical scavengers and antioxidants against smokeless tobacco extract (STE)-induced oxidative stress in macrophage J774A.1 cell cultures	Oral cytotoxicity	*In vitro* (Macrophage J774A.1 cell cultures) Cells were incubated for 24, 48, and 72 hours with various concentrations of ST extracts (UK reference*). Various concentrations of free radical scavengers and antioxidants were incubated with macrophages alone or in combination with ST extracts. Cell viability was determined by measuring LDH leakage and trypan blue exclusion.	The authors concluded that the results indicate that ST extract activates macrophage cells, resulting in the production of reactive oxygen species." Further suggesting that oxygen free radicals may be responsible for tissue damaging effects including membrane damage and selected oxygen free radical scavengers and antioxidants can attenuate these tissue damaging effects.	The study provided no direct measurement of oxidative stress or damage induced by MST extract exposure.
(Bagchi, 1995b)	Chronic effects of smokeless tobacco extract on rat liver histopathology and production of HSP-90	General toxicity	*In vivo* (rat, n = not reported) Rats received ST (UK reference*: 125 mg/kg in phosphate buffer) via oral gavage every other day for 90 days. Rats were sacrificed on Days 30, 45, 60, and 90 and the livers were processed for light and transmission electron microscopy (TEM) and Western blot analysis for heat shock/stress protein 90 (HSP90).	"Under light microscopy, no histopathological changes were observed. However, TEM analysis revealed time-dependent changes in the liver following chronic exposure to ST. Changes in cellular shape, cell to cell contact and the distribution of mitochondria and endoplasmic reticulum as well as disappearance of microvilli, suggest progressive and significant hepatocellular damage." "The expression of (liver) HSP90 increases up to 3-fold following chronic ST administration."	Morphological changes noted suggest slight injury, which is most likely reversible. None mentioned.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Fox, 1995)	Effect of smokeless tobacco extract on HT 1080 cell adhesion, pp125FAK phosphorylation and apoptosis	General toxicity	*In vitro* (HT 1080 fibrosarcoma cells) Cells were cultured with ST extract (UK reference*) at various concentrations. Cell adhesion, adhesion maintenance, phosphotyrosine phosphorylated proteins and cell death were measured over a 24-h exposure time.	The authors found that "…smokeless tobacco extract can cause a time and concentration dependent loss of cellular adhesiveness of HT 1080 cells to a variety of matrices." They conclude that "Cell death resulting from long term incubation with extract does not appear to be via an apoptotic pathway."	The relevance of this method is unknown.
(Hassoun, 1995)	Effect of vitamin E succinate on smokeless tobacco-induced production of nitric oxide by rat peritoneal macrophages and J774A.1 macrophage cells in culture	Inflammation	*In vivo* (rat, 4/group) Rats were given a single dose of 250 mg/kg ST extract (UK reference*) in phosphate buffer. Some treatment groups were given vitamin E succinate for 4 days prior to ST extract administration. After 24 hours, rats were sacrificed; peritoneal exudate cells (primarily macrophages) were isolated and cultured; and the nitric oxide production was determined. *In vitro* (macrophages) Various levels of ST extract were incubated with cells for 24, 48, and 72 hours, and nitric acid levels (as nitrite) were measured in the medium.	A significant increase in NO production was observed in macrophages from both the *in vivo* and the *in vitro* experiments. When the antioxidant vitamin E succinate was preadministered to rats, a marked decrease in NO production was observed.	Plasma nicotine levels were not measured.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Henderson, 1995)	The effects of smokeless tobacco extract on bone nodule formation and mineralization by chick osteoblasts *in vitro*	Periodontal disease	*In vitro* (chick embryo periodontal/ bone) Osteogenic cells were incubated with ST extracts (UK reference 1S3), IGF-1, nicotine, and acid- or heat-treated ST extract for up to 21 days. Cell proliferation, cell protein content, cell alkaline phosphatase activity, and bone nodule formation were measured.	The authors reported a number of changes in cell proliferation, alkaline phosphatase activity, and bone nodule formation, concluding that "…ST extract may contain a peptide capable of significantly stimulating osteoblast proliferation, differentiation and metabolism similar to the effects of insulin growth factor 1 (IGF-1)."	The relevance of this method is unknown.
(Bagchi, 1996)	*In vitro* effects of a smokeless tobacco extract on the production of reactive oxygen species by human oral epidermal cells and rat hepatic mitochondria and microsomes, and peritoneal macrophages	Oral cytotoxicity	*In vitro* (oral epidermal carcinoma KB) cells, rat peritoneal macrophages, hepatic mitochondria and microsomes) Cells were cultured with ST extract (UK reference*) for determination of cytotoxicity (oral cells only) and oxidative stress (chemoluminescence, macrophages only). The effect of various antioxidants on STE-induced lipid peroxidation was determined in hepatic mitochondria and microsomes.	The authors concluded that "…oral cells, peritoneal macrophages and hepatic mitochondria and microsomes produce reactive oxygen species following *in vitro* incubation with an aqueous extract of ST. Tissue damage in response to STE may occur as the result of reactive oxygen species production."	The relevance of this method is unknown.
(Gao, 1997c)	Mechanisms of smokeless tobacco-induced oral mucosa inflammation: role of bradykinin	Inflammation	*In vivo* (hamster in situ, n = 76) The left cheek pouch membrane between two chambers was removed, which allowed for placement of a plastic chamber filled with suffusion fluid containing smokeless tobacco extract (UK reference 1S3) and drugs. After a 20-minute exposure, bradykinin-like immunoreactivity was measured in the suffusate.	"Taken together, these data suggest that smokeless tobacco elicits plasma exudation in the oral mucosa in vivo in a specific fashion, and that this response is mediated by bradykinin."	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Johnson, 1996)	Effect of smokeless tobacco extract on human gingival keratinocyte levels of prostaglandin E_2 and interleukin-1	Inflammation	*In vitro* (primary human gingival keratinocyte) Keratinocyte cultures were exposed to UK reference* ST extract (0%, 2.5%, 5%, 10%) for 30, 90, 150, and 240 minutes. Cell viability (MTS assay), cytokines: PGE_2 and IL-l levels and mitochondrial dehydrogenase activity were measured.	The authors found that "… aqueous extract of ST is capable of stimulating PGE_2 release by human gingival keratinocytes in culture. Interleukin-1 levels also were increased at selected concentrations of tobacco stimulation."	The relevance of this method is unknown.
(Suzuki, 1996)	Aqueous smokeless tobacco extract impairs endothelium-dependent vasodilation in the oral mucosa	Vascular effects	*In vivo* (hamster in situ, n = 64) The left cheek pouch was exposed between two chambers, which allowed continuous suffusion of ST extract (UK reference*) and drugs into the suffusate (5-minute exposure).	"We found that the ST extract had no significant effects on diameter of resistance arterioles in the hamster cheek pouch. However, it significantly attenuated vasodilation elicited by two endothelium-dependent agonists, acetylcholine and bradykinin."	The relevance of this method is unknown.
(Bagchi, 1997)	*In vitro* free radical production in human oral keratinocytes induced by smokeless tobacco extract	Oral cytotoxicity	*In vitro* (human oral keratinocyte cells) Cell cultures were treated with ST extracts (UK reference*) for 24 hours. The generation of reactive oxygen species, protein kinase C activity, DNA damage, and collagen production was measured.	The authors found STE "…enhanced production of ROS, enhanced intracellular oxidized states of the cell, increased DNA damage and enhanced synthesis of mRNA that encodes for type IV collagen."	The relevance of this method is unknown.
(Gao, 1997a)	Angiotensin-converting enzyme and neutral endopeptidase modulate smokeless tobacco-induced increase in macromolecular efflux from the oral mucosa in vivo	Vascular effects	*In vivo* (hamster in situ, n = 42) The left cheek pouch membrane between two chambers was removed, which allowed for placement of a plastic chamber filled with suffusion fluid containing smokeless tobacco extract (UK reference 1S3) and drugs. After a 20-minute exposure, bradykinin-like immunoreactivity was measured in the	The authors reported that the "… data suggest that ACE and NEP each play a role in modulating a ST induced increase in macromolecular efflux from the in situ oral mucosa, in part by regulating local bradykinin catabolism."	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
			suffusate.		
(Gao, 1997b)	Purified ACE attenuates smokeless tobacco-induced increase in macromolecular efflux from the oral mucosa	Vascular effects	*In vivo* (hamster in situ, n = 39) The left cheek pouch membrane between two chambers was removed, which allowed for placement of a plastic chamber filled with suffusion fluid containing smokeless tobacco extract (UK reference 1S3) and drugs. After a 20-minute exposure, bradykinin-like immunoreactivity was measured in the suffusate.	The authors reported that the "…data suggest that exogenous ACE attenuates ST-induced increase in macromolecular efflux from the in situ oral mucosa in part by promoting local bradykinin catabolism."	The relevance of this method is unknown.
(Petro, 1997)	The effect of smokeless tobacco extract on murine T cell cytokine production	Inflammation	*In vitro* (mouse spleen cells) Murine T cells in whole splenic mononuclear cell populations and enriched T cells, costimulated with anti-CD28 were exposed to ST extract (UK reference*) and stimulated with anti-CD3. IL-2, IL-4, IFN-gamma, and IL-10 were measured.	The authors reported that "…expression of key cytokines, IFN-gamma and IL-10 are consistently decreased upon exposure to ST extract while IL-2 is increased."	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Smith, 1997)	Detection of DNA adducts by ^{32}P postlabeling following chronic exposure of rats to snuff	Complete carcinogenesis Genotoxicity	*In vivo* (rat surgical lip canal, n = 10) Animals were given snuff (UK reference*) twice a day, 5 d/wk for 10 weeks. A cotton pellet dipped in water was used as a control (in place of snuff). The animals were sacrificed after 10 weeks and mucosal tissues, the liver and kidneys were processed for DNA adduct analysis.	The authors found polar DNA adducts in all tissues examined, whereas DNA adducts derived from aromatic carcinogens were not detected. This suggested "non-aromatic agents initiated carcinogenesis following exposure to snuff." The authors conclude that "…adduction to DNA in organs of the gastrointestinal tract and the kidneys indicates that snuff usage results in systemic exposure to carcinogens and may contribute to the incidence of neoplasms in organs outside the oral cavity."	The study involved a very small number of animals and a short exposure period. The data may be insufficient to make any conclusions about DNA adduct formation that may lead to cancer, a chronic disease.
(Bagchi, 1998)	Subchronic effects of smokeless tobacco extract (STE) on hepatic lipid peroxidation, DNA damage and excretion of urinary metabolites in rats	General toxicity	*In vivo* (rat, n = 4/group) Rats were treated orally (oral gavage) with 25 mg STE/kg (UK reference 1S3) in phosphate buffer every other day for 105 days, control animals received vehicle; Rats (number/treatment or time not disclosed) were sacrificed on Days 0, 15, 30, 45, 60, 75, 90; and 105; liver microsomes, mitochondria, and nuclei were isolated and examined for lipid peroxidation and DNA single-strand breaks.	"Time dependent increases in hepatic mitochondrial and microsomal lipid peroxidation" and increases in hepatic DNA single-strand breaks occurred after MST extract treatment relative to control values. "STE administration also resulted in significant time-dependent increases in the excretion of the urinary lipid metabolites."	None

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Bernzweig, 1998)	Nicotine and smokeless tobacco effects on gingival and peripheral blood mononuclear cells	Inflammation	*In vitro* (human peripheral blood and gingival tissue) Cell cultures from nonsmoking adult periodontitis patients were exposed for 24 h with medium alone, 1% ST extract (UK reference 1S3), 100 µg/mL nicotine, 1 µg/mL LPS, or LPS and either nicotine or ST extract. Enzyme immunoassays were used to quantify PGE_2 and IL-1β secretion.	The authors noted that "…data indicate that while nicotine and ST can stimulate PBMC to secrete PGE_2, they cannot activate further mononuclear cells extracted from gingiva, possibly due to maximal previous stimulation in the periodontitis lesion."	The authors mentioned that it would be best to examine PGE2 secretion by naive cell populations, rather than cells derived from the periodontitis lesion.
(Chang, 1998)	Smokeless tobacco extracts activate complement *in vitro*: a potential pathogenic mechanism for initiating inflammation of the oral mucosa	Inflammation	*In vitro* (sensitized sheep erythrocytes) MST extracts (UK reference*) were added to normal human serum and total hemolytic complement in the serum was assayed using sensitized sheep erythrocytes.	The authors conclude that "…smokeless tobacco extracts activate the alternative pathway and also suggest some measure of classical pathway activation. Activation of complement by STE may be a mechanism for initiating inflammation of the oral mucosa."	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Rubinstein, 1998)	Smokeless tobacco-exposed oral keratinocytes increase macromolecular efflux from the in situ oral mucosa	Vascular effects	*In vivo* (hamster in situ, n = 48) Animals' body temperature was kept constant at 37°C throughout the experiment using heating pad and heated microscope stage. The cheek pouch membrane was spread over a small plastic base plate After suffusion of buffer for 30 minutes, animals were injected with FITC labeled dextran. The number of leaky sites and clearance of labelled FITC dextran were determined for 30 minutes. Supernatants of HOK exposed to ST extracts (UK reference 1S3), cultured media (but not HOK) exposed to ST extracts, or media for 72 hours were suffused for 40 minutes The number of leaky sites were determined every minute for seven minutes at five-minute intervals over a 140-minute period. *In vitro* (subconfluent monolayers of HOK) Cells were incubated with STE or media and supernatants were collected for 24, 48, and 72 hours. Proteolytic activity was determined in the supernatants.	"These data suggest that oral keratinocytes modulate smokeless tobacco-induced increase in macromolecular efflux from the in situ oral mucosa in part by elaborating proteases that may account for local bradykinin production."	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Bagchi, 1999)	Smokeless tobacco, oxidative stress, apoptosis, and antioxidants in human oral keratinocytes	Oral cytotoxicity	*In vitro* (normal human oral keratinocyte cells) Cell cultures were treated with ST extracts (UK reference 1S3) for 24 hours, and superoxide anion production, oxidative tissue damage (by lipid peroxidation and DNA fragmentation) and apoptosis were measured.	The authors concluded that ST extract produces oxidative tissue damage and apoptosis which can be attenuated by antioxidants including vitamin C, vitamin E, a combination of vitamins C plus E and grape seed proanthocyanidin extract.	The relevance of this method is unknown.
(Chan, 1999)	Initial characterization of the complement activating compounds in extracts of smokeless tobacco	Inflammation	*In vitro* The molecular size of the complement activating compounds in 3 different UK reference compounds: aqueous extracts of loose leaf chewing tobacco (1S1), dry snuff (1S2), and moist snuff (1S3). were determined by fractionation by gel filtration chromatography. Complement activation was determined by a hemolytic assay.	The authors concluded that "…the complement activating substances in smokeless tobacco extracts may be large (>400 kDa) polyphenol-containing compounds (i.e., tannins)."	The relevance of this method is unknown.
(Gao, 1999)	Dexamethasone attenuates acute macromolecular efflux increase evoked by smokeless tobacco extract	Vascular effects	*In vivo* (hamster in situ, n = 36) The left cheek pouch membrane between two chambers was removed, which allowed for placement of a plastic chamber filled with suffusion fluid. After equilibration period, FITC-dextran was injected and the number of leaky sites and clearance of FITC-dextran were determined for 30 minutes. Two concentrations of ST extracts were suffused for 20 minutes each. The number of leaky sites was determined every minute for seven minutes and at five-minute	"We found that 20 min. suffusion of ST elicited significant, concentration-related leaky formation and an increase in clearance of FITC-dextran from the in situ hamster cheek pouch. This response was significantly attenuated by dexamethasone."	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
			intervals for 60 minutes thereafter.		
(Jenson, 1999a)	Effects of smokeless tobacco and tumor promoters on cell population growth and apoptosis of B lymphocytes infected with Epstein-Barr virus types 1 and 2	Modulating carcinogenesis	*In vitro* (B lymphocytes) Two strains Epstein-Barr virus (EBV type 1 and type 2) were placed in cell lines and the effect of ST extracts (3 types of STE were used: UK reference dry snuff, moist snuff, or loose leaf tobacco), NNN and NNK, TPA and n-butyrate were investigated on cell population growth, cell death, and apoptosis.	At concentrations used in these experiments, there appears to be an EBV type-specific response to chemical induction, with greater susceptibility of lytic EBV type 1 to ST extracts and lytic EBV type 2 to TPA and n-butyrate. "The absence of significant effects with NNK and NNN suggest that these properties reside with other compounds present in tobacco extracts."	The relevance of this method is unknown.
(Jenson, 1999b)	Evaluation of the effect of smokeless tobacco purified products and extracts on latent Epstein-Barr virus	Modulating carcinogenesis	*In vitro* (Raji lymphoid cell) A human EBV positive cell line, was suspended and treated alone or in combination with NNN, NNK, BaP, n-butyric acid, or 2% ST extract (ST extracts: dry snuff UK reference 1S2, moist snuff 1S3, and loose leaf tobacco 1S1) for 6-7 days to determine cell viability and EBV early antigen (measure of latent EBV infection).	The authors found "no discernable effect for the 6-7 day duration of treatment on viability of Raji cells or on induction of latent EBV in Raji cells." They concluded that "There does not appear to be an *in vitro* effect of ST constituents on EBV infected lymphocytes that may contribute to the development of oral cancers."	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Mangipudy, 1999)	Role of nitric oxide in the induction of apoptosis by smokeless tobacco extract	Oral cytotoxicity	*In vitro* (hamster cheek pouch cell: HCPC-1) Cell cultures were exposed to various ST extracts UK reference*1S3 (moist snuff) extracts at different concentrations) for 24-96 hours. After each exposure, various parameters of cell proliferation and cell death were measured.	No significant alterations were observed in cell cycle progression and cell proliferation as a result of exposure to ST extract. LDH leakage measured indicated no significant effect (necrotic cell death) with lower doses (0.5, 1.0, 2.5%). ST extract did cause significant rates of apoptotic cell death, with maximal apoptosis noted between 48 and 96 h incubation. NO levels (measured as nitrate) were significantly elevated at the doses that caused an induction of apoptosis.	There appears to be an association between an increase in NO (nitrite) levels and an increase in apoptotic cell death. However, the authors have not provided sufficient data to demonstrate a causal link.
(Petro, 1999)	Smokeless tobacco and nicotine bring about excessive cytokine responses of murine memory T-cells	Inflammation	*In vitro* (murine splenic T-cells) Cell populations were exposed to ST extract (UK reference*1S3), nicotine or medium during 4 days of stimulation with anti–CD-3. After washing, cells were restimulated with anti–CD3 and anti-CD28 in the absence of treatment.	ST extract, unlike nicotine administration, did not exhibit residual expression of cytokine MRNA after 4 days of primary stimulation. Like nicotine, however, restimulated ST extract treated cells exhibited maximum cytokine mRNA levels at 48 h.	The authors suggest the altered T-cell cytokine expression pattern (excessive and prolonged) may potentially influence oral cancer and periodontal disease.
(Demirci, 2000)	Smokeless tobacco extracts modulate exogenous gene expression in early passage cultured human oral epithelial cells: an *in vitro* system to study chemical and viral enhancer/promoter interaction	General toxicity	*In vitro* (human epithelial gingival tissue cells) Transfected (plasmids containing viral enhancer/promoter) cells and untransfected cell were cultured and treated with ST extracts (UK reference*) every 3 days for 12 days.	"…ST extracts modified exogenous gene expression under control of the cytomegalovirus immediate early enhancer/promoter." "…suggest that the increased longevity of virus-infected human epithelial cells treated with ST extracts is the morphological reflection at least in part, of the influence of ST extracts on viral enhancers/promoters."	This appeared to be mainly a methodology study

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Furie, 2000)	Extracts of smokeless tobacco induce pro-inflammatory changes in cultured human vascular endothelial cells	Vascular effects	*In vitro* (HUVEC) Cell cultures were exposed to 3 types of ST extracts (aqueous extracts of chewing tobacco, dry snuff, and moist snuff UK reference*) for up to 24 hours. Cell viability, transendothelial electrical resistance, migration of neutrophils across HUVEC cultures, expression of adhesion molecule E-selectin, and the production of IL-8 and MCP-1 were measured.	The authors state: "…aqueous extracts of STE induce human umbilical vein endothelial cells (HUVEC) to express the adhesion molecule E-selectin and to secrete the chemokines IL-8 and MCP-1. "We also provide evidence that bacterial LPS is a major, but not the sole, factor accounting for the pro-inflammatory activities of ST."	The relevance of this method is unknown.
(Rubinstein, 2000)	Smokeless tobacco potentiates VIP-induced DNA synthesis and inactivates NEP 24.11 in oral keratinocytes	Modulating carcinogenesis	*In vitro* (hamster oral keratinocytes: HCPC-1) 7, 12-Dimethylbenz[a]anthracene-transformed cells were incubated with 3 different concentrations of ST extract (UK reference*) or human VIP for 24-72 hours. DNA synthesis and NEP 24.11 activity were measured.	"…ST extract potentiates VIP-induced DNA synthesis in cultured oral keratinocytes and this response is temporally related to ST extract induced inactivation of NEP 24.11 in these cells."	The relevance of this method is unknown.
(Arredondo, 2001)	A receptor-mediated mechanism of nicotine toxicity in oral keratinocytes	General toxicity	*In vitro* (human oral keratinocytes) Cells were exposed to nicotine, or aqueous ST extracts (UK reference*) to measure changes in nACh receptors, cell cycle progression and cell differentiation.	The authors reported that "…chronic stimulation of oral keratinocyte cells with nicotine alters the genetically determined program of the cell differentiation-dependent expression of nAChR subunits."	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Bagchi, 2001)	Protective effects of antioxidants against smokeless tobacco-induced oxidative stress and modulation of Bcl-2 and p53 genes in human oral keratinocytes	Oral cytotoxicity	*In vitro* (human oral keratinocytes) Cultures were treated with ST extract (UK reference*) for 24 h for measurement of changes in cell viability and the expression of Bcl-2, p53 and c-myc genes following treatment with various antioxidants.	The authors concluded that "…antioxidant protection of ST extract-induced cellular injury is associated with alterations in Bcl-2 and p53 expression."	The relevance of this method is unknown.
(Wang, 2001)	Smokeless tobacco extracts modulate keratinocyte and fibroblast growth in organotypic culture	Modulating carcinogenesis	*In vitro* (human epidermal keratinocytes) Cultures with fibroblasts were exposed to various doses of 3 ST extracts (loose-leaf chewing tobacco (STEl), dry snuff (STE2), and moist snuff (STE3). (UK reference*: 0.25%, 0.5%, 1.0%, 2.0%) for 72 hours. Changes in morphology and proliferation of human keratinocytes and fibroblasts were determined.	The authors found the "… elevated doses of ST extract limited keratinocyte growth, low doses strongly stimulated keratinocyte proliferation." "In contrast, ST extracts promoted fibroblast proliferation at all concentrations."	The relevance of this method is unknown.
(Petro, 2002)	Smokeless tobacco extract decreases IL-12 production from LPS-stimulated but increases IL-12 from IFN-gamma-stimulated macrophages	Inflammation	*In vitro* (murine splenic T-cells and macrophages) T-cells were stimulated with anti-CD3 while splenic macrophages were stimulated with LPS in the presence and absence of ST extracts (UK reference 1S3). The production of IL-12 p40 and p70 were measured.	The authors stated that "ST extract has a unique suppressive influence upon the macrophage's innate immune response to LPS that is opposite to its influence on macrophage response to a component of adaptive immunity, IFN-gamma. Both effects could significantly influence the outcome of periodontal disease."	The relevance of this method is unknown.
(Rubinstein, 2002)	Bacillus species are present in chewing tobacco sold in the United States and evoke plasma exudation from the oral mucosa	Complete carcinogenesis	*In vivo* (hamster in situ) Bacterial colonies were isolated and identified from agar plates inoculated with ST extracts (Skoal Cherry and	The authors noted five distinct *Bacillus* species, and noted that suffusion of the *Bacillus* significantly increases macromolecular efflux from the cheek pouch.	The authors noted a concern of the publication reviewer in terms of the findings being relevant to ST

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
			Skoal Spearmint). The most frequently isolated Bacillus species was grown in broth and the effect of this Bacillus supernatant on plasma exudation (leaky sites) from the intact oral mucosa microcirculation of the hamster cheek pouch was determined (20-minute exposure).		users. Specifically, the concern was whether the bacterial count/load examined in this study was relevant to that found in a ST user's oral cavity.
(Alonge, 2003)	Mitochondrial volume densities in the smokeless tobacco-treated hamster cheek pouch epithelium	Complete carcinogenesis	*In vivo* (hamster cheek pouch: n = 24 treated, 8 controls) Two grams of ST (Skoal) was placed into the right buccal pouch of experimental animals for 5 d/wk for 24 months, No ST was given to control animals. After 24 months, animals were sacrificed, and the oral mucosa specimens from the buccal pouches of control and experimental animals were obtained and processed for transmission electron microscopy analysis. Volume densities of mitochondria were assessed by morphology.	The authors noted that ST-treated animals "…displayed more mitochondria than control, and the granular epithelial cell layer in experimental group showed a significantly higher mean mitochondrial volume density than the control group." "It was concluded that ST treatment of hamster cheek pouch epithelium for 24 months produced hyperplastic and hyperkeratotoic condition and the number of mitochondria was increased in the granular cells."	This study did not include sham treatment of the control pouch.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Petro, 2003)	Modulation of IL-12 p35 and p40 promoter activity by smokeless tobacco extract is associated with an effect upon activation of NF-kappaB but not IRF transcription factors	Inflammation	*In vitro* (RAW264.7 cells) Cells were stimulated with ST extract (UK reference 1S3) alone or in the presence of IFN-gamma. LPS and the activities of p35 and p40 promoter reporter plasmids were determined. In addition, nuclear localization of NF-kB p50, p65, and IRF-1, -2, -8 were evaluated in this experimental system.	The authors found that "…ST extract stimulation of bioactive IL-12 production is correlated with its impact upon both p35 and p40 and can be attributed in part through an effect upon NF-kappaB p50 nuclear localization."	This is a mechanistic/signaling study.
(Vishwanatha, 2003)	Modulation of annexin I and cyclooxygenase-2 in smokeless tobacco-induced inflammation and oral cancer	Inflammation	*In vivo* (hamster cheek pouch model, n = 10) Animals were exposed to ST extract (UK reference 1S3) or saline (vehicle) for 20 minutes. After which, the animals were sacrificed for immunohistochemistry analysis, RNA isolation, and Western blot analysis. *In vitro* (DMBA-transformed golden Syrian hamster oral epidermoid carcinoma cells: HCPC-1) Cells were incubated in the presence and absence of MST extracts for 24, 48, and 72 hours. At the designated time point, measurements of annexin I levels, PGE2 production were performed.	The authors found that "…exposure to smokeless tobacco results in loss of the anti-inflammatory activity of annexin I and up-regulation of the pro-inflammatory COX-2 in oral cells."	Duration of exposure of cheek pouch to MST extract was very short.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Barley, 2004)	Tobacco-related-compound-induced nitrosative stress injury in the hamster cheek pouch	Complete carcinogenesis Genotoxicity	*In vivo* (hamster cheek pouch) ST extract (Copenhagen®, 1:2 DMSO/mineral oil), vehicle control, nicotine, NNN, or NNK were brushed on the cheek pouch 3 times per week for 10 months. At the end of the exposure time, cheek pouches were excised and prepared for histopathological and immunohistochemical analysis [3-nitrotyrosine (3-NT) reactivity]. *In vitro* (immortalized hamster cheek pouch cells) Cells were exposed to ST extract (Copenhagen®, 1:2 DMSO/mineral oil) at concentrations up to 25%. Cell viability (MTT assay) and presence of DNA single strand breaks (Comet assay) were measured.	The authors reported "…a dose-dependent decrease in cheek pouch cell viability with increasing tobacco-related compound concentrations as well as a dose-dependent increase in DNA strand breaks." "Histopathologic findings in tobacco-related compound treated hamster cheek pouch mucosa were consistent with mild epithelial dysplasia."	The study used a DMSO/mineral extraction, which may or may not reflect human use conditions.
(Andersson, 2006)	The effect of Swedish and American smokeless tobacco extract on periodontal ligament fibroblasts *in vitro*		*In vitro* (human periodontal ligament cells) Cells from three young adults were exposed to snuff extract (UK reference*) for 30 minutes, 120, minutes, and 24 hours at 0.3%, 1%, and 3% ST extracts. Cell growth, cell morphology, and alkaline phosphatase production were measured.	The authors concluded that "…that smokeless tobacco has biological effects in terms of reduced periodontal ligament cell growth and production of alkaline phosphatase."	There was high variability due to cells being obtained from 3 different young adults.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Colvard, 2006)	Smokeless tobacco-induced lamellar body abnormalities	Periodontal disease	*In vivo* (hamster cheek pouch, n = 16/group) Animals received 2 g. ST (Skoal) in their right pouch, 5 d/wk, for 24 months while no ST (but mechanical stimulation of the pouch) was given to the control group. After 24 months, the cheek pouch epithelial tissues were obtained and prepared for histopathological analysis and processing for electron microscopy. Morphological analysis of lamellar bodies (Lb) were conducted using stereological point counting methodology.	The authors reported the following: "Commercial alkaline ST may have contributed to the abnormal accumulation of Lb in the granular cell layer and affected the extrusion process of Lb to form an incomplete permeability barrier in the oral epithelium." "No evidence of oral mitotic activity related to carcinogenesis was observed in this study."	The sample size of the study was small, but the exposure period was likely sufficient to induce carcinogenesis.
(Rickert, 2007)	A comparative study of the mutagenicity of various types of tobacco products	Genotoxicity	*In vitro* (Salmonella typhimurium: TA98 with S9 and TA100 with S9) DMSO extracts from two North American commercial products (brands unknown, numerous combustible tobacco products, numerous noncombustible products not traditional ST were tested for mutagenic activity was assessed per OECD Guideline 471 using the Ames assays.	The authors compared mutagenic potency of mainstream smoke condensate from smoking articles with that of ST products based on a nicotine basis. They concluded that "…some of the smokeless products assayed would result in less mutagenicity transmitted to the user than would occur with smoking products. Furthermore, we were not able to detect significant mutagenicity when the extracts of the ST products were tested with TA98+S9."	The study used a DMSO/mineral extraction, which may or may not reflect human use conditions.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Coppe, 2008)	A role for fibroblasts in mediating the effects of tobacco-induced epithelial cell growth and invasion	Modulating carcinogenesis Genotoxicity	*In vitro* (human skin and oral fibroblasts) Cells were cultured alone and as cocultures with ST extract (Copenhagen®), 0.1 to 4% concentration incubated with cells for 2 hours to 6 days. The effect of MST extract concentrations on fibroblast proliferation, fibroblast ROS production and oxidative DNA damage and secretory phenotype of fibroblasts were determined.	ST extracts elevated the levels of intracellular reactive oxygen, oxidative DNA damage, and DNA double-strand breaks in a dose-dependent manner. The authors concluded that "tobacco-exposed fibroblasts disrupt epithelial cell-cell interactions and stimulate epithelial migration and proliferation."	The relevance of this method is unknown.
(Rickert, 2009)	Chemical and toxicological characterization of commercial smokeless tobacco products available on the Canadian market	Genotoxicity	*In vitro* (mutagenicity, cytotoxicity, genotoxicity) DMSO extracts of commercial ST products including (Copenhagen® and Skoal) investigated for metal levels, BaP and TSNA levels, cytotoxicity (neutral red assay), mutagenesis (with activation) and genotoxicity (micronucleus assay) using Health Canada Official Methods.	The authors found that bioassays (cytotoxicity, clastogenicity, and mutagenicity) failed to distinguish among the different types of ST products. .	The study used a DMSO/mineral extraction, which may or may not reflect human use conditions.
(Joyce, 2010)	Role of plasma membrane disruption in reference moist smokeless tobacco-induced cell death	Oral cytotoxicity	*In vitro* (Het-1A immortalized human esophageal cells) Cells were exposed to MST and MST extract (UK reference 1S3, NC State) in an *in vitro* exposure system that directly exposes on a rocking platform to simulate the abrasion that might be experienced in the oral cavity when using MST.	Cell wounding was caused by the nonchemical properties of MST. Subsequent experiments revealed that cell wounding during simultaneous exposure to an aqueous MST extract resulted in greater than additive cell death when compared with treatment with washed MST or MST extract alone. The high levels of free calcium in MST extract were found to have an important role in this cytotoxicity.	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Lombard, 2010)	Reference moist smokeless tobacco-induced apoptosis in human monocytes/macrophages cell line MM6	Oral cytotoxicity	*In vitro* (Monocyte/macrophage cell line: MM6) Cells were exposed to STE extract (UK reference* 1S3), nicotine, and Osmotic Solution for 18 hours. Cell viability, apoptosis, and osmolarity were measured..	Exposure of MM6 cells to various concentrations of ST extract, led to a significant and dose-related decrease in cell viability. MST extract exposure induced apoptosis. The authors concluded that ST induced "… osmotic stress, but not exposure to nicotine, plays an important role in STE induced apoptosis of MM6 cells."	The relevance of this method is unknown.
(Mitchell, 2010)	Role of oxidative stress and MAPK signaling in reference moist smokeless tobacco-induced HOK-16B cell death	Oral cytotoxicity	*In vitro* (human oral keratinocyte cell line: HOK-16B) Cells were exposed to ST extract (UK reference*, NC State) for 3 hours. Cell death and reactive oxygen species (ROS) were measured from 30 minutes to 3 hours of ST extract exposure. Protective abilities of various antioxidant and iron chelators were determined.	The authors concluded that "…the acute exposure of HOK-16B cells to ST extract leads to cell death, at least in part, through oxidative stress via activation of ASK1 and the JNK 1/2 and p38 pathways."	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Schwartz, 2010)	Brand specific responses to smokeless tobacco in a rat lip canal model	Complete carcinogenesis	*In vivo* (rat: surgical lip canal, n = 15/group) ST (150-200 mg: Copenhagen®, Skoal, Ettan Swedish Snus and Stonewall) was placed in the lip canal, two times per day, 5 d/wk, for 12 months. Control rats received cotton.	The authors found that ST "…produced changes in the mucosa marked by increases in S phase and M phase cells for the Skoal and Copenhagen® exposed rats. This correlated with the high level of TSNAs and nicotine in these products." They concluded that "…the Skoal and Copenhagen brands, the two higher TSNA/unprotonated nicotine products, produced more histopathologic changes of the epithelium consistent with pre-malignancy."	The study utilized commercial products purchased at the beginning of the study (150 cans of each ST), mixed, and stored at 6 °C for use throughout the study. Long term storage might result in constituent (e.g., increase in TSNAs), microbial or pH changes in the ST used in this study. Composition analysis at the beginning and end of the study was noted in the methods, but data were not reported for the end of the study results.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Arimilli, 2012)	Evaluation of cytotoxicity of different tobacco product preparations	Inflammation	*In vitro* (HL60 cells, THP-1 cells, and human peripheral blood mononuclear cells (PBMC) Cytotoxicity was measured following 24-hour exposure to MST preparations (UK reference* 2S3 moist snuff), smoke total particulate matter , and cigarettes (3R4F reference University of Kentucky).	The authors reported that "…all three TPPs [tobacco product preparations] induced detectable levels of DNA damage and IL8 secretion, the combustible TPPs were significantly more potent than the ST preparation." They concluded "…relative cytotoxic and other cell biological effects of TPPs are dose-dependent and that ST extract is the least cytotoxic TPP tested in this study."	The authors discussed the minimal cytotoxicity observed in this study, as compared with greater cytotoxicity observed with ST extracts in the study of Mitchell et al. 2010. The difference may be related to the presence of 10% fetal bovine serum in this study and its absence in the Mitchell study.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Theophilus, 2012)	Toxicological evaluation of smokeless tobacco: 90-day rodent feeding studies	General toxicity	*In vivo* (rat and mouse, n = 40/group) This 90-day feeding study involved multiple treatment groups for assessment of standard toxicity endpoints. Rats: (1) negative control (NP-2000 diet, no additions) (2) positive control (nicotine hydrogen tartarate salt, 6 mg/kg/d) (3-5) ST blend in diet at 0.3, 3, 6 mg nicotine/kg/d) (6-8) ST extract in diet (0.3, 3, 6 mg nicotine/kg/d). Mice: (1) negative control (NP-2000 diet, no additions) (2) positive control (nicotine hydrogen tartarate salt, 120 mg/kg/d) (3-5) ST blend in diet at 6, 60, 120 mg nicotine/kg/d) (6-8) ST extract in diet (6, 60, 120 mg nicotine/kg/d).	The authors considered the plasmas nicotine levels attained by the animals to be relevant to those of typical consumers of ST products. The authors concluded that macroscopic and microscopic changes seen at termination were "…neither toxicologically nor biologically significant (not nicotine, tobacco blend, or tobacco extract related)". High-dose and positive-control effects included body weight reductions and organ weight changes. The organ weight changes were attributed mainly to the lower body weights of treated vs. control groups.	The study involved multiple species and multiple doses. However, the exposure period was limited to 90 days.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Arimilli, 2013)	Combustible and non-combustible tobacco product preparations differentially regulate human peripheral blood mononuclear cell functions	Inflammation	*In vitro* (T cells and NK cells) Cytolytic ability was assessed in the presence of tobacco product preparations (NCSU 3R4F reference cigarettes, 2S3 reference ST extract in medium, 67-hour exposure). Cytokines secretion (IL-1B, IL-6, IL-8, IL-10, IL-12, TNF alpha) were measured.	The authors noted that a "… marked reduction of the expression of intracellular IFN-gamma and TNF alpha was evident in NK cells and T cells treated" with whole smoke and TPM. "Although interference from the vehicle confounded the interpretation of effects of (ST extract), some effects were evident only at high concentrations." "Nicotine treatment minimally impacted expression of cytokines and cytolytic activity."	The artificial saliva used to produce ST extracts appears to have biological activity itself (e.g., induces IL-8 secretion), thus the findings for ST extract at high levels (and high vehicle levels) may be the result of a vehicle effect.
(Gao, 2013)	Differential cell-specific cytotoxic responses of oral cavity cells to tobacco preparations	Oral cytotoxicity	*In vitro* (two oral squamous cell carcinoma cells lines and normal human gingival epithelial cells) Cells were treated for up to 48 hours with TPM, ST extract (UK reference* 2S3), nicotine or whole smoke conditioned medium.	The authors found that TPM, but not ST extract, or whole smoke significantly activated caspase-3 in all three cell types. The authors also reported that ST extract elicited only very low cytotoxicity even at the highest dose with up to 450 µg/mL nicotine units delivered and at the longest exposure times (48 h) in all cell types.	The relevance of this method is unknown.
(Ljungberg, 2013)	Effects of nicotine, its metabolites and tobacco extracts on human platelet function in vitro	Vascular effects	*In vitro* (platelets) The effects of tobacco products (including ST extract: Copenhagen® fine cut) on platelet aggregation, static platelet adhesion, platelet P-selectin surface expression were measured.	The authors found that "Tobacco extracts inhibit platelet activation during short-term in vitro challenge. As only limited effects of nicotine and nicotine metabolites were seen, the tobacco-induced platelet inhibition are likely induced by other compounds present in tobacco and tobacco free snuff."	The relevance of this method is unknown.

Table 7.5.6–1-28: Summary of Relevant Published Literature - Nonclinical Endpoints (continued)

Author	Title	Topic	Methods	Findings	Comments
(Gao, 2014)	Combusted but not smokeless tobacco products cause DNA damage in oral cavity cells	Genotoxicity	*In vitro* (human gingival epithelial cells: HGEC, and human gingival fibroblasts: HGF, oral carcinoma cell line 101A) Cells were exposed for 24 hours to ST extract (UK reference* 2S3), TPM or nicotine. DNA strand breaks by Comet assays and gamma-H2AX assays were conducted.	The Comet assays indicated that TPM, but not ST extract or nicotine, caused substantial DNA breaks in cells (only the high ST extract concentration caused weak DNA damage). These results from H2AX assays confirmed the findings.	The relevance of this method is unknown.
(Malpass, 2014)	Regulation of gene expression by tobacco product preparations in cultured human dermal fibroblasts	General toxicity	*In vitro* (human dermal fibroblasts) Cultured cells were exposed to 1% ST extract (NC State reference 2S3), 4 µg/mL nicotine, TPM (at a nicotine level of 4 µg/mL or vehicle) for 1 to 5 hours. Gene expression arrays (human signal transduction pathway finder and human transcription factors), proinflammatory cytokine release assays, nitric oxide and ROS assays were conducted.	The authors concluded that "…ST extracts and TPM alter the expression of some critical immediate early genes involved in the inflammatory response in adult human dermal fibroblasts." "These findings suggest that changes in the expression of certain pro-inflammatory cytokines and related genes in human dermal fibroblasts can be used in the investigation of cellular responses to the exposure of different tobacco products."	This study followed gene expression over a very short time (1 and 5 h. incubation) and a single dose of TPM or ST extract. Additionally, since no cytotoxicity data were reported, the effect of cell death or diminished cell viability cannot be assessed.

* Note: Specific identification of references ST products used in the nonclinical studies is inconsistently reported (for instance: "UK reference" or UK reference 1S3). Given the time frame of the studies, it is likely that these are the same reference product produced in 1986 and made available through the University of Kentucky. Subsequently, new reference products, including 2S3, were produced and maintained by the North Carolina State University. Some authors may also refer to this as a UK reference. For additional detail see: http://www.tobacco ncsu.edu/strp.html

7.5.6-1.3. The Health Risks Associated with Use of the MST Product versus Using Other Tobacco Products on the Market, Including Those within the Same Class of Products

ST products currently sold in the U.S. include chewing tobacco (loose leaf, plug, or twist), snuff (moist or dry), snus, and dissolvables. Chewing tobacco typically uses almost-dark, air-cured tobacco, that which are not ground before use and are generally chewed to release its flavor. Snuff is finely ground or cut tobacco that can be dry, moist, and packaged in pouches or packets. Dry snuff is a finely ground tobacco powder that is usually applied orally or nasally. Tobaccos used in the production of moist snuff include primarily dark air-cured and dark fire-cured (Wahlberg, 1999). Snus tobaccos generally comprise tobaccos selected for low nitrosamine characteristics. Dissolvable tobaccos comprise finely ground tobacco pressed into shapes, such as tablets, sticks, or strips.

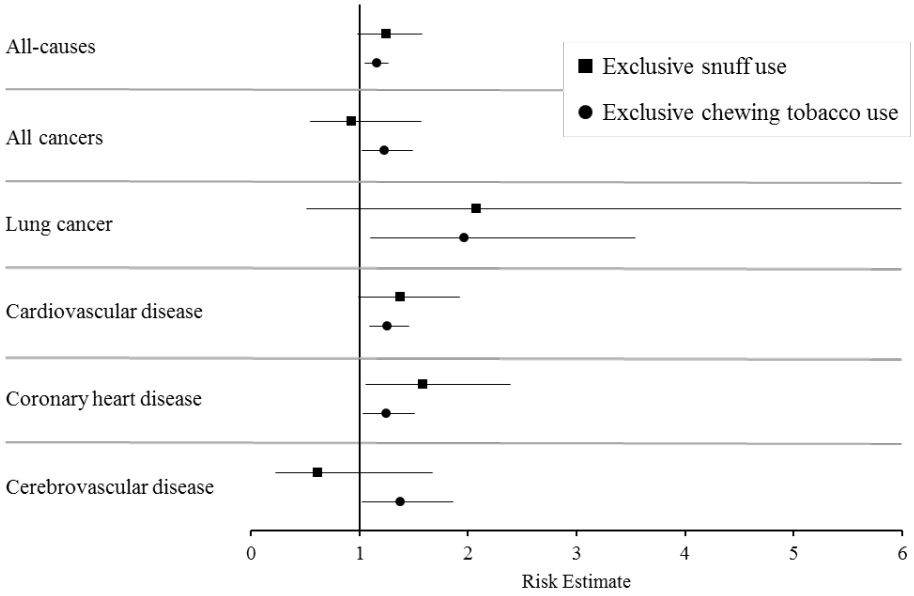
While MST and loose leaf chewing tobacco are both oral tobacco products, there are differences between the two products. For example, MST products have a pH of approximately 7 to 8.5, while chewing tobacco products have a lower pH of approximately 5 to 6. MST products, due to the inclusion of dark fire-cured tobacco, tend to have higher content of benzo(a)pyrene and other polycyclic aromatic hydrocarbons than loose leaf chewing tobacco products.

MST and chewing tobacco products currently comprise the majority of the U.S. ST market, and have done so for many years (see Section 7.5.6-1.1.1). Available epidemiology studies may combine the use of both product classes, but the health consequences for most major disease risks do not appear to be substantially different for both classes of products.

7.5.6-1.3.1. Comparison of ST Use and Chewing Tobacco: Major Health Effects

Two publications contain data assessing the differential health risk between snuff and loose leaf chewing tobacco. Henley et al. (2005) used the CPS-II cohort to compare mortality hazards for various causes of death between male current exclusive snuff users and male chewing tobacco users. As shown in Figure 7.5.6-1-4, no significant differences were found between these two groups in mortality from all causes, all cancers, lung cancer, CVD, CHD, or cerebrovascular disease.

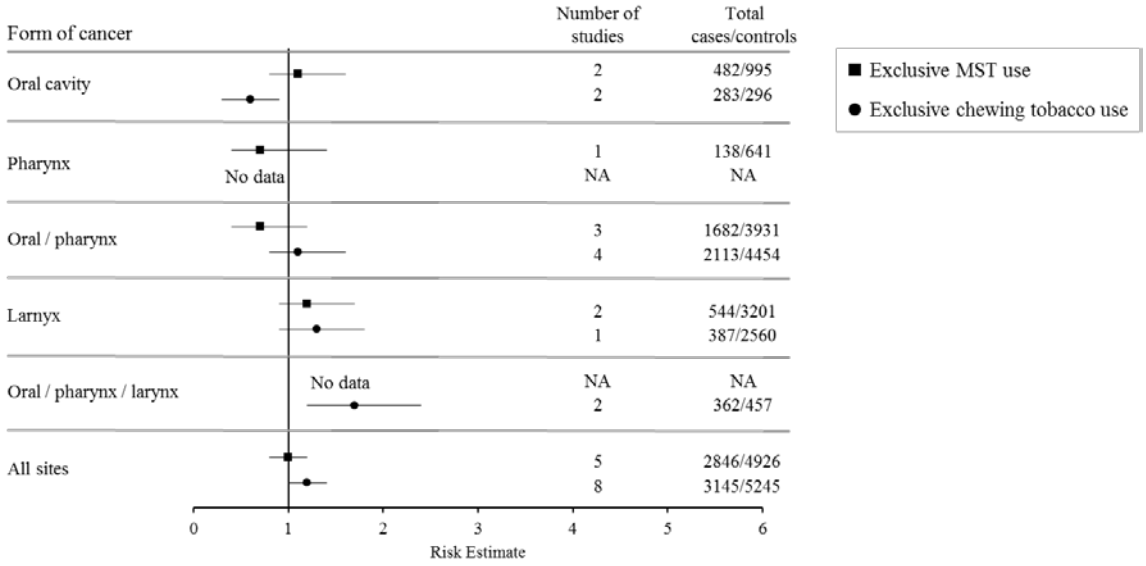
Figure 7.5.6-1-4: Mortality Hazard Ratios for Male Current Exclusive Snuff or Chewing Tobacco Users



Data adapted from Data adapted from Henley et al. (2005), Table 4

Because of the emphasis on oral cancer risk for ST products, Rodu and Cole (2002) compared oropharyngeal cancer risks between MST and loose leaf chewing tobacco by analyzing case-control studies with sufficiently precise exposure assessment to differentiate users of each product class. As shown in Figure 7.5.6-1-5, no significant differences are apparent in the RR of upper respiratory tract cancers between users of MST and users of loose leaf chewing tobacco.

Figure 7.5.6-1-5: Relative Risks for Upper Respiratory Tract Cancers among Moist Smokeless Tobacco and Loose Leaf Chewing Tobacco Users



Data adapted from Rodu and Cole (2002), Table 2

The published literature suggests that there are no differences in health risks between the use of MST and chewing tobacco.

While much of the epidemiology literature does not distinguish ST product classes, we do not conclude that this is a major limitation in using the data for review of the candidate MRTPs. Our rationale is based primarily on the findings from this analysis that indicate a lack of differences in health risks between the use of MST and chewing tobacco.

7.5.6-1.3.2. Comparison of ST Use and Cigarette Smoking: Major Health Effects

Cigarette smoking remains the most prevalent form of tobacco use and presents the greatest risk for the user (Jamal, 2015; Surgeon General Report, 2014). The published data provide clear and compelling evidence that use of ST conveys a substantially lower risk of serious diseases and all-cause mortality, as compared with that for smoking cigarettes.

On the basis of data from the CPS-II, the Centers for Disease Control and Prevention estimate that cigarette smoking causes 440,000 premature deaths annually in the U.S. (Surgeon General Report, 2014). A recent publication provided an updated estimate for smoking-attributable mortality using data from the 1987 National Health Interview Survey linkage to the National Death Index (Rostron, 2013). Rostron calculated that, in 2004, cigarette smoking resulted in 200,000 deaths among males and 180,000 deaths among females.

U.S. public health authorities have not provided comparable estimates of mortality attributable to ST use; however, published all-cause risk data for ST use can help illustrate the potential for substantially lower tobacco-related mortality.

Table 7.5.6-1-29 summarizes the age- and gender-stratified, all-cause mortality hazards associated with cigarette smoking assessed in the 2014 Surgeon General's report using a pooled analysis of five cohort studies followed from 2000 to 2010 (Surgeon General Report, 2014). Cigarette smokers have approximately a twofold to threefold excess risk of mortality from all-causes when compared with that for never tobacco users, depending largely on the intensity and duration of smoking as well as age.

Table 7.5.6-1-29: Risk for Mortality from All Causes among Current Cigarette Smokers Stratified by Gender and Age

Gender	Age	Risk Estimate[1]	95% Confidence Interval
Males	55-64	RR= 2.92	2.69-3.18
	65-74	RR = 3.00	2.89-3.13
	75+	RR = 2.36	2.24-2.48
Females	55-64	RR = 2.64	2.43-2.86
	65-74	RR = 2.87	2.76-2.99
	75+	RR = 2.47	2.37-2.58

Source: Data extracted from The Health Consequences of Smoking – 50 Years of Progress: A Report of the Surgeon General (Surgeon General Report, 2014), Tables 11.13 and 11.14.
[1] Adjusted for age, cohort, race, and education.

In addition to the estimates from the Surgeon General's report, Shavelle et al. (2008) conducted a meta-analysis using 11 published risk estimates for all-cause mortality among current and former smokers. The publications included sufficiently recent studies ranging from 1998 to 2006. Results were stratified by light, medium, and heavy smoking intensity, although the authors noted that there was variation between studies in the definitions of these groups (Light: less than 10 cigarettes per day in 5 studies, less than 15 cigarettes per day in 5 studies and less than 21 in 1 study, Medium: most often defined as 10-25 cigarettes per day, Heavy: generally 21-25+ cigarettes per day). Among males, the weighted, average, all-cause mortality hazard ratios were 1.47 for light smokers, 2.02 for medium smokers, and 2.38 for heavy smokers (CIs were not provided). For females, the weighted, average, all-cause mortality hazards were similar: light smokers, 1.50; medium smokers, 2.02; and heavy smokers, 2.66.

Two publications (Accortt, 2002; Henley, 2005) provided all-cause mortality hazard ratio estimates for ST users (Table 7.5.6-1-30).

Table 7.5.6-1-30: Summary of Published All-Cause Mortality Risk Estimate for ST Users

Study	Group	ST Exposure	Risk Estimate	95% Confidence Interval
Henley et al. (2005)	Males: CPS-I	Current	HR = 1.17	1.11-1.23
	Males: CPS-II	Current	HR = 1.18	1.08-1.29
Accortt et al. (2002)	Males	Ever	HR = 1.0	0.8-1.3
	Females	Ever	HR = 1.3	0.9-1.7

CPS-I = Cancer Prevention Study I; CPS-II = Cancer Prevention Study II; ST = smokeless tobacco. HR = Hazard ratio

From an overall perspective, comparing the RR estimates of all-cause mortality for cigarette smoking (Table 7.5.6-1-29) with HR estimates for ST use (Table 7.5.6-1-30), it is unmistakable that the risk of mortality from smoking has been shown to be greater than the mortality risk from ST use. While there are some limitations to direct comparison of the risk estimate due to inconsistency between data sources, variability in time of measurement, and differences in statistical calculation technique, one can estimate that ST use in the U.S. is associated with, at most, an excess all-cause mortality risk of less than 20 percent, as compared with that for never tobacco users, whereas cigarette smoking conveys greater than a 200 percent excess risk. Therefore, theoretically, ST use is likely associated with, at most, one-tenth the overall mortality risk of cigarette smoking.

We draw additional comparison between the health risk of ST use and cigarette smoking by matching risk estimates for selected major health diseases with ST use as identified in the previous section (Section 7.5.6-1.2). We relied on published literature or information from authoritative public health reports for the health risks of cigarette smoking. Health risk estimates for ST use were derived from two meta-analysis reviews that focused on ST product use in the U.S. (Lee, 2007; Lee, 2009b) and an evaluation of smoking-attributable disease risk using data from the CPS-II data set as shown by Rostron (2013). Table 7.5.6-1-31 provides the comparative risk estimates from these investigations, as well as conclusions from the U.S. Surgeon General about the health risks of ST and cigarette smoking (Surgeon General Report, 2014).

On the basis of our review of the published scientific literature, the use of ST in the U.S. has substantially lower risk of serious fatal diseases than continued cigarette smoking. ST use is not without some potential health risk. Nonetheless, we conclude that the evidence is clear that ST use is a viable alternative for cigarette smokers who want to use tobacco but also want to reduce their risk to major cigarette smoking associated health risks.

Table 7.5.6-1-31: Comparison of Selected Major Health Risks of ST Use and Cigarette Smoking

Disease	Quantitative Risk estimate (95% CI)		Surgeon General's Findings	
	Smokeless Tobacco[62] Meta-analysis RE RR/OR (95 % CI)	**Current Cigarette Smoking[63] CPS-II RR**	**Smokeless Tobacco Use[64]**	**Cigarette Smoking[65]**
Bladder cancer	Overall data: 1.11 (0.85-1.45) Smoking-adjusted data: 1.24 (0.83-1.85)	Males: 3.27 Females: 2.22	"…risk of bladder cancer is not altered to any large extent in users of smokeless tobacco products,…"	Sufficient to infer a causal relationship
Esophageal cancer	Overall data: 1.56 (1.11-2.19) Smoking-adjusted data: 1.89 (0.84-4.25)	Males: 6.76 Females: 7.75	"Inconclusive" (upper aerodigestive tract)	Sufficient to infer a causal relationship
Kidney cancer	Overall data: 1.52 (0.94-2.46) Smoking-adjusted data: 1.41 (0.64-3.10)	Males: 2.72 Females: 1.29	"…results from studies of kidney cancer are inconsistent."	Sufficient to infer a causal relationship
Laryngeal cancer	Overall data: 1.56 (1.21-2.00) Smoking-adjusted data: 2.01 (1.15-3.51)	Males: 14.60 Females: 13.02	"Inconclusive" (upper aerodigestive tract)	Sufficient to infer a causal relationship
Lung cancer	Overall data: 1.22 (0.82-1.83) Smoking-adjusted data: 1.38 (0.72-2.64)	Males: 23.26 Females: 12.69	No conclusion presented	Sufficient to infer a causal relationship

[62] Data obtained from Lee & Hamling (2009b), cardiovascular disease data from Lee (2007). Meta-analysis results shown in the table represent U.S. data.
[63] Data obtained from Rostron (2013). 95% CI data were not available.
[64] The Health Consequences of Using Smokeless Tobacco, A Report of the Advisory Committee to the Surgeon General, 1986 (U.S. Dept. Health Human Services, 1986)
[65] The Health Consequences of Smoking – 50 Years of Progress, A Report of the Surgeon General, 2014 (Surgeon General Report, 2014)

Table 7.5.6–32: Comparison of Selected Major Health Risks of ST Use and Cigarette Smoking (continued)

Disease	Quantitative Risk estimate (95% CI)		Surgeon General's Findings	
	Smokeless Tobacco[62] Meta-analysis RE RR/OR (95 % CI)	**Current Cigarette Smoking[63] CPS-II RR**	**Smokeless Tobacco Use[64]**	**Cigarette Smoking[65]**
Oral cavity and pharyngeal cancer	Overall data: 2.16 (1.55-3.02) Smoking/alcohol adjusted data: 1.04 (0.80-1.35)	Males: 10.89 Females: 5.08	"Evidence is strong" (oral cavity) "Inconclusive" (upper aerodigestive tract)	Sufficient to infer a causal relationship
Pancreatic cancer	Overall data: 0.86 (0.47-1.57) Smoking-adjusted data: 0.99 (0.51-1.91)	Males: 2.31 Females: 2.25	No conclusion presented	Sufficient to infer a causal relationship
Prostate cancer	Overall data:[66] 1.20 (1.03-1.40) Smoking-adjusted data: 1.29 (1.07-1.55)	Not reported	No conclusion presented	Suggestive of no causal relationship[67]
Stomach cancer	Overall data: 1.41 (0.95-2.10) Smoking-adjusted data: 1.41 (0.93-2.12)	Males: 1.96 Females: 1.36	"Inconclusive"	Sufficient to infer a causal relationship
Cerebrovascular disease	Fixed effects 1.44 (1.30-1.60) Random effects: 1.41 (1.17-1.71)	Males (35-64 y): 3.27 Females (35-64 y): 4.00	No conclusion presented	Sufficient to infer a causal relationship

[66] Includes one study from Norway and six studies from the United States.
[67] The Surgeon General concluded that the evidence was suggestive of no causal relationship between smoking and prostate cancer incidence. However, the Surgeon General did find that the evidence was suggestive of a higher death risk from prostate cancer in smokers and a higher risk of advanced-stage disease, less well-differentiated cancer and a higher risk of disease progression.

Table 7.5.6–32: Comparison of Selected Major Health Risks of ST Use and Cigarette Smoking (continued)

Disease	Quantitative Risk estimate (95% CI)		Surgeon General's Findings	
	Smokeless Tobacco[62] Meta-analysis RE RR/OR (95 % CI)	**Current Cigarette Smoking[63] CPS-II RR**	**Smokeless Tobacco Use[64]**	**Cigarette Smoking[65]**
Ischemic heart disease	Fixed effects: 1.14 (1.06-1.22) Random effects: 1.14 (0.96-1.34)	Males (35-64 y): 2.80 Females (35-64 y): 3.08	No conclusion presented	Sufficient to infer a causal relationship
COPD	1.28 (0.71-2.32)[68]	Males: 10.58 Females: 13.08	No conclusion presented	Sufficient to infer a causal relationship
Dental caries	Not established	Not established	"Combination of smokeless tobacco use in individuals with existing gingivitis may increase the prevalence of dental caries"	Sufficient to infer a causal relationship

[68] Meta-analysis has not estimated COPD risk from ST use. The data shown are from CPS-II analysis conducted by (Henley et al., 2005).

Regarding specific disease risks, cigarette smoking is causally associated with many serious diseases, most notably, COPD and cancer of the lung, larynx, or esophagus. Some studies estimated the increased risk of lung diseases in current smokers to be 10 to 20 times that of nonsmokers, depending on the extent of cigarette smoking history. Furthermore, COPD risk is elevated greater than 10-fold in current cigarette smokers. In contrast, meta-analysis of relevant U.S. epidemiology data shows that ST use conveys no substantial risk of COPD or lung cancer. Similarly, the risks for cancer of the larynx and esophagus were substantially lower in ST users than in cigarette smokers.

Oral cancer is the subject of a government-mandated warning. Current epidemiology indicates a moderately increased risk of oral cancer with ST use, which is far less then that for cigarette smokers. The impact of confounding from other oral cancer risk factors such as alcohol is a critical factor in assessing oral cancer risk in both smokers and ST users.

From the data shown in Table 7.5.6-1-31, it is evident that ST use is not without some risk to human health. Nonetheless, when comparing the individual disease-risk estimates associated with cigarette smoking with those for ST use, we see that ST use consistently presents a lower risk for each major disease.

7.5.6-1.4. The Changes in Health Risks to Users Who Switch from Another Tobacco Product to the MST Product, Including Tobacco Products within the Same Class

Studies addressing the changes in health risk related to switching among ST products are infrequently reported in the literature. Consequently, with the exception of an analysis of the CPS-II data by Henley et al. (2005) where switching between chewing tobacco and snuff was measured, there is a paucity of data in the literature directly related to the issue raised by the FDA. Further, most data relate to males only since females have not widely adopted MST use.

Henley et al. (2005) studied the association between the use of either snuff or chewing tobacco and mortality among men enrolled in the CPS-I in 1959 or the CPS-II in 1982. The CPS-II data set included cause of death information that is analyzable with respect to exclusive use of either ST product, as well as to switching between products. Table 7.5.6-1-32 presents the HRs calculated for several major causes of death according to tobacco use behavior. This analysis indicated excess mortality risk from a variety of causes in those men using chewing tobacco exclusively compared with non-users. In contrast, among male snuff users who never used chewing tobacco, death due to CHD was the only risk factor with an excess risk compared with male non-users. For tobacco chewers who switched to snuff, the only significant finding was an increased risk for lung cancer. The increased estimated risk for mortality due to lung cancer among the individuals who used chewing tobacco and then switched to snuff was substantial (9.78), but included wide CIs (95 percent CI: 3.58-26.7). In fact, lung cancer risk estimates for all groups included wide CIs. Potentially, this was due to a low incidence of lung cancer in those individuals using ST in general or due to possible misclassification of smokers within the study. None of the causes of death reported in the analysis reached statistical significance for snuff users who switched to chewing tobacco.

Table 7.5.6-1-32: Mortality HRs and 95% CI for Men Who Used ST Products Exclusively (CPS-II, 1982-2000)[1]

Cause of death	Multivariate-adjusted HR (95% CI) listed by tobacco use type[2]			
	Chewing tobacco users		Snuff users	
	Never used snuff	Switched to snuff	Never used chew	Switched to chew
All causes[3]	1.16 (1.05-1.29)	1.01 (0.69-1.47)	1.25 (0.98-1.58)	0.96 (0.61-1.50)
All cancers[4]	1.23 (1.02-1.49)	1.58 (0.87-2.87)	0.93 (0.55-1.57)	1.30 (0.58-2.89)
Lung cancer[4]	1.97 (1.10-3.54)	9.78 (3.58-26.7)	2.08 (0.51-8.46)	N/P
Cardiovascular disease[5]	1.26 (1.09-1.46)	0.64 (0.33-1.24)	1.38 (0.99-1.92)	0.87 (0.45-1.70)
Coronary heart disease[6]	1.25 (1.03-1.51)	0.80 (0.37-1.70)	1.59 (1.06-2.39)	1.02 (0.45-2.30)
Cerebrovascular disease[7]	1.38 (1.02-1.86)	0.68 (0.17-2.75)	0.62 (0.23-1.67)	1.24 (0.39-3.91)
Other causes	1.07 (0.92-1.25)	1.20 (0.73-1.97)	1.07 (0.74-1.54)	1.00 (0.53-1.87)

1 Data extracted from Henley et al. (2005).
2 Cox models adjusted for age, race, education level, body mass index, exercise, alcohol consumption, employment status and type, fat consumption, fruit/vegetable intake, and aspirin use.
3 Analysis for all causes excludes men who reported prevalent cancer, heart disease, diabetes, or stroke in 1982 (due to disease exclusions the number of all cause deaths differs from the summed total of specific causes of death).
4 Analyses for cancers exclude men who reported prevalent cancer in 1982.
5 Analysis for cardiovascular disease excludes men who reported prevalent heart disease, diabetes, or stroke in 1982.
6 Analysis for coronary heart disease excludes men who reported prevalent heart disease or diabetes in 1982.
7 Analysis for stroke excludes men who reported prevalent stroke in 1982.

One could conclude from the CPS-II data that switching to chewing tobacco from snuff conveys more risk than switching from snuff to chewing tobacco. However, we think that this is probably not the case since most of the risk estimates from the CPS-II are based on a few cases. Additionally, the calculated mortality risk estimates for the various endpoints were all relatively low and show general consistency between the product categories.

With regard to MST, some investigators suggested that different chemical composition could lead to differences in health risk, as compared with that for other U.S. ST products (Hatsukami, 2015). There is insufficient product-specific human epidemiological evidence in the literature comparing the health risks of individual MST products to accurately assess a possible change in health risk among users who switch between products. However, given the Henley study (2005) where switching between two fundamentally different ST product such as chewing tobacco and snuff failed to produce a substantial health risk impact, we conclude that the chemical composition differences between MST products would be largely inconsequential. Thus, switching between MST products would not substantially alter the established profile of the major tobacco-associated health outcomes associated with ST use.

We would expect that, for those ST users who switch to the candidate MRTPs, there would be no relevant or substantial change in health risk. The major change in health risk related to ST use remains with cigarette smokers who adopt MST use exclusively instead of smoking.

7.5.6-1.5. The Health Risks Associated with Switching to the ST Product as Compared with Quitting the Use of Tobacco Products

One large epidemiological study has assessed the health risks of switching to ST products as compared with those of quitting smoking (Henley, 2007). Henley et al. analyzed data from the CPS-II, which included 116,395 men who reported being former smokers at baseline in 1982 and did not use any tobacco products ("quitters"). Among these, 4,443 reported beginning use of ST when or after they quit smoking cigarettes ("switchers"). Table 7.5.6-1-33 lists the multivariate-adjusted HRs for some diseases. Henley et al. (2007) concluded "that switchers had significant higher rates of death from lung cancer statistically, coronary heart disease and stroke than men who quit using tobacco entirely." No significant increases of risk of COPD were observed in switchers compared with quitters.

Table 7.5.6-1-33: Mortality Hazards for Men Who Switched from Smoking to Smokeless Tobacco Use Compared with Those Who Quit Smoking and Did Not Use Tobacco

Cause of Death	Hazard Ratio[1]	95% Confidence Interval
All causes	1.08	1.01-1.15
Lung cancer	1.46	1.24-1.73
Coronary heart disease	1.13	1.00-1.29
Stroke	1.24	1.01-1.53
COPD	1.31	0.96-1.78

Source: Data extracted from Henley et al. (2007), Table 3.
1 Cox proportional hazards models adjusted for age, number of cigarettes formerly smoked per day, number of years smoked cigarettes, age at which they quit smoking cigarettes, race, educational level, body mass index, exercise level, alcohol consumption, employment type, employment status, fat consumption, fruit and vegetable intake, and aspirin use.

The association between smoking and lung cancer, stroke, and coronary heart disease is well established. The estimates derived by Henley et al. do indicate a possible excess risk for all-cause mortality, lung cancer, coronary heart disease and stroke among former smokers who use ST compared to former smokers who do use ST. However, the magnitude of the risk estimates derived suggest only a weak association, and any such increased risk would appear to be minimal, at best. Additionally, there are many factors beyond ST use that could impact the change in health risks upon quitting, including age and the duration and intensity of the previous smoking history (Doll, 2004).

7.5.6-1.6. The Health Risks Associated With Using the ST Product in Conjunction with Other Tobacco Products

The issue raised by the MRTPA Guidance in the context of ST relates to the activity often described as "dual use." It is apparent from our review of the published literature that, despite

the number of papers that attempt to assess dual use, there are many uncertainties related to measuring or assessing the health risk or behavioral aspects of dual use.

Perhaps the biggest uncertainty in any review of the health effects and behavioral characteristics of dual use is the variation and lack of consistency in defining dual use itself. For example, in some studies a regular MST user occasionally smoking a cigarette (even once in the past 30 days) would be characterized as a dual user; as would be a regular smoker occasionally using MST (even once in the past 30 days). Further complicating our analysis was the fact that many studies do not consistently, or accurately, capture the frequency of use of either product. Some consumers, especially adolescents, are most likely experimenting with multiple tobacco products and have not reached a steady state of tobacco use, while others may be using alternative tobacco products to stop smoking. When past tobacco use practices are considered, the definition of dual use becomes even more imprecise.

The literature also generally fails to adopt a uniform measure of tobacco use among dual users, whether it applies to smoking or to ST use. For example, Tomar (2003) adopted "those who reported smoking at least 100 cigarettes in their lifetime and who smoked on at least 1 day in the 30 days preceding the interview" as the definition of a current smoker. Former smokers were defined as those who "had smoked at least 100 cigarettes but reported that they did not smoke in the past 30 days, and never smokers had not smoked 100 cigarettes." Tomar noted that these definitions were in contrast to most U.S. national surveys of young people (i.e., Youth Risk Behavior Survey, the Monitoring the Future Study, the National Youth Tobacco Survey, and the National Household Survey on Drug Abuse), where current smoking does not carry the qualifier of having smoked at least 100 cigarettes.

Investigators have used a variety of survey tools and techniques to collect tobacco use data from a distribution of age groups, including adolescents and adults. In some cases, researchers conducted small focus groups with personalized discussions, whereas other investigators utilized online surveys with extensive specific questions to gather information on tobacco use. Some investigations focused on specific at-risk groups, such as military personnel or students in middle school, high school, or college; however, others utilize nationally representative samples gleaned from large national health and behavior surveys. We noted that many of the published studies are cross-sectional in nature, and conclude that the most relevant information on a topic, such as dual use, would come from longitudinal studies that consider the social dynamics impacting a consumer's behavior over time.

The primary focus for our discussion of the literature rests on those studies that utilize larger national health surveys such as CPS-I and CPS-II which rely on self-reporting of product use; however, this could be fraught with recall bias.

Overall, the question of disease risk related to dual use of tobacco products is poorly studied, and we note that health risks of dual use are frequently unaddressed in studies of smoking and ST use. However, some available epidemiological data from cohort and case-control studies that often include assessment of dual users smoking behavior can provide some helpful information to estimate disease risk using a weight-of-evidence evaluation.

The association between cigarette smoking and various diseases is well known. As we describe ST in the form of MST is also associated with an increased risk for some of the very same

diseases arising from cigarette smoking, albeit at lower risk levels than smoking. Given this similarity, it would not be surprising when exposure risks are higher in persons who both smoke and use ST than in those who use one form of tobacco exclusively. However, what would raise additional concern would be a nonindependent elevated risk due to combined use. Depending on the interaction model, this would be the case when above additive or multiplicative effects occur under dual use. However, given the body of epidemiologic literature available to date, it would seem unlikely that synergistic effects would have gone undetected. Chances for a synergistic effect to go undetected might be higher if both types of tobacco consumption would interact on a disease outcome that is not usually associated with the use of ST.

On the basis of this published epidemiological evidence, we conclude that the adverse health consequences and disease risks associated with dual use are driven primarily by the level of cigarette smoking. The potential added health risk burden of ST use on a smoker appears to be minuscule.

7.5.6-1.6.1. Relationship between Dual Use of ST and Cigarettes: Health Risks

We identified and reviewed 11 studies involving U.S. products and, because of the time frame or other product description within the publications, likely illustrate the use of MST and cigarettes. We highlight a few studies that evaluated risk for specific diseases, such as cancer or oral health, and others that assessed risk in terms of biochemical analysis.

Accortt et al. (2002) used the results of the NHANES1 and a 20-year mortality follow-up of subjects who took part in the original survey. The authors investigated the relation between ST use and mortality from chronic diseases, including lung cancer, stroke, IHD, and digestive cancer. Since information on smoking status was available in the surveys, the authors also compared the effects of ST use with those of cigarette smoking and investigated the mortality associated with the combined use of these two tobacco products. Because data on ST use were only collected in a random sample of NHANES1, supplemental information on ST obtained during NHEFS conducted between 1982 and 1984 was also used to classify subjects. The analysis was restricted to 6,805 subjects who were between 45 and 75 years of age at baseline. On the basis of ST use (ever/never) and cigarette smoking (ever/never), these subjects were categorized into four groups: no tobacco (n = 2,986), exclusive ST use (n = 414), exclusive smoking (n = 2,751), and both ST use and smoking (n = 654). Analyses compared the smoking groups with respect to mortality up to 1992 (by which time almost a third of subjects had died) from major causes, with RR estimates adjusted for age, race, an index of poverty, and, in some analyses, also for alcohol, exercise, fruit/vegetable intake, systolic blood pressure, serum cholesterol, and body mass index.

Accortt et al. (2002) provided descriptive analyses comparing the four groups according to baseline characteristics. Quite a few differences appeared between exclusive ST users on one hand and no-tobacco users as well as smokers on the other (Table 1 of the original publication). With respect to findings in dual users as compared with those in single users, results were rather sparse. The proportion of males was considerably higher (92.7 percent) in dual users than in exclusive ST users (56 percent) and exclusive smokers (55.7 percent). Also, physical activity and dietary fat intake were higher in dual users, likely due to the higher proportion of males.

To investigate the combined effect of ST use and smoking on specific outcomes, Accortt et al. (2002) limited their analysis to only males since the prevalence of combined use was low in females. Combined users did not experience increased mortality for IHD, although exclusive smokers had a statistically significant increase in mortality (HR = 1.6, 95 percent CI: 1.3, 1.9). With respect to dual use, the authors found that "…the lung cancer mortality among combined users was nearly twice that of exclusive smokers (HRs = 22.6 and 13.2, respectively)." In male smokers who never used ST, the reported lung cancer HRs were, as expected (and consistent with many other studies), clearly increased both in ever smokers as well as in both subgroups of current and former smokers. HRs were about three times higher in current smokers than in former smokers and were roughly in between the two estimates in ever smokers. The same pattern of smoking-associated lung cancer HRs occurred in ever users of ST; this time, however, the ratios are increased by a factor of around 1.3 to 1.7, depending on the classification of the smoking status.

In discussing their findings, Accortt et al. (2002) rejected the notion of an interactive effect of dual use. On page 736 of their publication they state the following: "Although the mortality rate among combined users was higher than that expected from the individual rates, this result is not likely due to a synergistic effect between smokeless tobacco and cigarettes. The combined users smoked more than exclusive smokers did (42.3 and 35.1 mean pack-years, respectively). The higher cigarette smoking dose, not the use of smokeless tobacco, is likely leading to the increased lung cancer mortality among combined users."

In a subsequent analysis of the same data used in their 2002 publication, Accortt et al. (2005) reviewed cancer incidence instead of mortality of chronic diseases, including cancer. While there were some inconsistencies in sample sizes, data collection, and results reporting for similar endpoints between the two studies, with respect to the question of adverse health effects related to dual use of ST and cigarettes, the authors concluded that "No synergistic effect was observed between ST and cigarette smoking among male combined users (females were not analyzed for combined use) for the major cancers."

Together, the analyses of data from NHANES I (Accortt, 2002, 2005) collected in 1971 to 1975 suggest no synergistic effect of ST use and cigarette smoking for major health risks associated with tobacco. In both studies, the authors rejected the notion of an interactive effect of dual use on lung cancer incidence/mortality.

Our literature review identified two other publications evaluating a possible association between dual use of ST and cigarettes with cancer risk. Hassan et al. (2007) conducted an evaluation of passive smoking and pancreatic cancer risk. Specific to the issue of dual use, the authors reported "…there was no significant association between ever-use or heavy intake (>20 total times/years) of chewing tobacco, snuff, pipes, or cigars and the risk of pancreatic cancer among cigarette smokers." Zahm et al. (1992) measured the relationship between tobacco use and the risk for STS among 248,046 military veterans. Mail questionnaires collected tobacco use data in 1954 or 1957, with cancer incidence measured in 1980. Unfortunately, the publication presents neither the history of tobacco use subsequent to the baseline assessment nor the specifics of concurrent use of ST and cigarettes. Nonetheless, although the authors found an association between cigarette smoking and STS (RR = 1.8, CI = 1.1-2.9), they found no statistically significant increased risk for ST use only (RR = 1.4, CI = 0.8-2.6) or ST use with other tobacco

products (RR = 1.5, CI = 0.8 -2.7). Since the authors only described dual use as ST and other tobacco products, one cannot be totally confident that dual use represented only ST and cigarettes.

Yatsuya and colleagues (2010) assessed the possible relationship of dual use of ST and cigarettes with CVD. These investigators used data from the ARIC Study to survey tobacco use among 14,498 men and women aged 45 to 64 years at baseline (1987-1989) and incidence of CVD events (myocardial infarction, coronary revascularization, coronary death, or stroke) during a follow-up period of up to about 19 years. The description of tobacco use patterns by the study participants during the follow-up period is not well described in the publication. The authors reported an HR of 1.31 (95 percent CI: 1.06-1.61) for current ST users who did not smoke versus 1.09 (95 percent CI: 0.74-1.60) for those ST users who also reported cigarette smoking.

Regarding oral disease, we identified three publications that provided very limited data. Andrews et al. (1998) sampled over 34,000 dental patients to assess tobacco use and frequency of oral care such as flossing and brushing, and perception of oral health problems. The authors noted that, overall, non-users generally practiced better oral hygiene than tobacco users. Cigarette smokers had a greater incidence of gingival bleeding and mouth sores than nonusers. Those who reported dual use of ST and cigarette smoking had a higher incidence of gingival bleeding and mouth sores than either cigarette smokers or tobacco non-users. The subset of dual users in this study was exceedingly small, as compared with that in other prevalence reports (only 100 dual users out of >34,000 subjects). Grady et al. (1990) surveyed major and minor league baseball players during spring training on patterns of ST use. With regard to dual use, the authors found that the “Severity of leukoplakic lesions did not vary by age, race, cigarette smoking, alcohol consumption or dental hygiene practices.” Finally, Wolfe and Carlos (1987) conducted oral examinations on 226 Navajo Indians, aged 14-19 years, to investigate any association with the use of ST, cigarettes, and alcohol. Among the subjects, 75.4 percent of males and 49.0 percent of females were users of ST, with 54.0 percent of these also smoked cigarettes. Leukoplakia occurred in 25.5 percent of the users and 3.7 percent of the nonusers. While duration and frequency of use of ST were highly significant risk factors associated with leukoplakia, the concomitant use of alcohol or cigarettes did not appear to increase the prevalence of these lesions. When compared with the relationship in nonusers, there was no consistent relationship observed between the use of ST and gingival bleeding, calculus (tartar), gingival recession, or attachment loss.

Table 7.5.6-1-34 summarizes 11 publications we identified as having information assessing the health risks associated with dual use of ST and cigarette smoking.

Table 7.5.6-1-34: Summary of Relevant Published Literature – Health Risks Associated with Dual Use of ST and Cigarettes

Author	Title	Study Type and Sample	Author's Findings	Comments
(Wolfe, 1987)	Oral health effects of smokeless tobacco use in Navajo Indian adolescents	Cross-sectional study Navajo Indians enrolled in the 9th or 10th grade in a U.S. Government boarding school at Fort Wingate, New Mexico N = 226 aged 14-19	The authors found the following: "Over half (54.0%) of the subjects said they smoked, usually 1-5 cigarettes per week." "The highest prevalence of leukoplakia (36.0%) was found in subjects who used smokeless tobacco and alcohol. However, the difference between users of ST only, and ST users who also used alcohol with or without smoking tobacco was not statistically significant."	Recall bias
(Grady, 1990)	Oral mucosal lesions found in smokeless tobacco users	Cross-sectional study Major and minor league baseball players N = 1,109	The authors reported that 86.9% of the participants who used ST never smoked, 9.1% were former smokers, and 4.0% were current smokers (dual users). "Analysis of users with leukoplakia revealed a significant increase in the percent of severe lesions (degree 3 or 4) with increasing amount of use, duration of use, shorter time since last use…" "Severity of leukoplakic lesions did not vary by age, race, cigarette smoking, alcohol consumption or dental hygiene practices."	Recall bias

Table 7.5.6–1-34:Summary of Relevant Published Literature – Health Risks Associated with Dual Use of MST and Cigarettes (continued)

Author	Title	Study Type and Sample	Author's Findings	Comments
(Wennmalm, 1991)	Relation between tobacco use and urinary excretion of thromboxane A2 and prostacyclin metabolites in young men	Clinical study N = 577 randomly sampled 18- to 19-year-old tobacco users	The authors found that cigarettes only smokers used about 17.4 ±2.0 cigarettes per day, and had mean measured cotinine levels 1,560 ng/mL. Those who used both cigarettes and ST smoked about 7.8 cigarettes, used about 27 g of tobacco per day, and had a mean cotinine levels of 1,773 ng/mL. The authors also noted that "The excretion of Tx-M was elevated in smokers (and those using both cigarettes and smokeless tobacco), without any parallel change in the excretion of PGI-M....The unaffected excretion of Tx-M in the snuff-only group seems to disfavor the hypothesis that nicotine can elicit platelet activation."	The study may be subject to recall bias due to self-reported tobacco use.
(Zahm, 1992)	Soft tissue sarcoma and tobacco use: data from a prospective cohort study of United States veterans	Cohort study Veterans providing tobacco use histories on mail questionnaires in 1954 or 1957 N = 248,046	The authors reported that the RR (95% CI) of soft tissue sarcoma for smokers compared with nonsmokers was 1.8 (1.1-2.9); for smokeless only users, the RR was 0 (no cases reported); and, for those using both cigarettes and ST, the RR was 1.5 (0.8-2.7).	The authors noted that recall bias and the fact that tobacco-use data were collected only once from each participant, sometimes years before the appearance of death or disease, as study limitations. The study did not adequately differentiate types of ST.

Table 7.5.6–1-34:Summary of Relevant Published Literature – Health Risks Associated with Dual Use of MST and Cigarettes (continued)

Author	Title	Study Type and Sample	Author's Findings	Comments
(Andrews, 1998)	Relationship between tobacco use and self-reported oral hygiene habits	Cross-sectional study Dental patients, Oregon N = 632 ST users, 100 dual users	Among men in this sample, the prevalence of a combination of both ST and cigarette use was 0.67% (100 out of 34,897 subjects). Cigarette only smokers were significantly ($p<0.05$) less likely to have bleeding gingivae and mouth sores than ST users or dual users.	The authors considered this to be a large study with a high participation rate (81%), but noted that the education demographics of their sample did not fit national samples. Furthermore, the participants were recruited from those seeking oral hygiene treatments.
(Accortt, 2002)	Chronic disease mortality in a cohort of smokeless tobacco users	Cohort study First National Health and Nutrition Examination Survey Epidemiologic Followup Study (1971-1975) N = 1,068 ST users N = 5,737 non-ST users	The authors reported the following: "...lung cancer mortality rate among combined users (smokeless tobacco and cigarettes), based on the rates for exclusive smokeless tobacco users and exclusive smokers, was higher than expected..." Regarding this mortality rate, the authors noted that "...this result is not likely due to a synergistic effect between smokeless tobacco and cigarettes. The combined users smoked more than exclusive smokers did (42.3 and 35.1 mean pack years, respectively). The higher cigarette smoking dose, not the use of smokeless tobacco, is likely leading to the increased lung cancer mortality among combined users."	Large sample size Unknown and uncontrolled confounders, residual or uncontrolled confounding (e.g., other tobacco habits or factors relating to survival) may contribute to the results found in this study. Approach may have resulted in some nondifferential misclassification of tobacco use since data from 1982-1984 may not be as accurate as those collected from 1971-1975 (because of recall error or the use of proxies for subjects who died between the baseline interview and the initial follow-up).

Table 7.5.6–1-34:Summary of Relevant Published Literature – Health Risks Associated with Dual Use of MST and Cigarettes (continued)

Author	Title	Study Type and Sample	Author's Findings	Comments
(Accortt, 2005)	Cancer incidence among a cohort of smokeless tobacco users (United States).	Cohort study First National Health and Nutrition Examination Survey Epidemiologic Followup Study (1971-1975) N = 414 ST users N = 2,979 non-ST users	The authors reported the following: "In contrast to the well-known deleterious effects of cigarette smoking, ST use did not substantially increase the risk for cancer incidence above that of non-tobacco users, particularly among males." "…our data suggests that cancer risks are much lower from ST use than from cigarette smoking." "Though the sample of ST users was small, this research demonstrates that ST users may not experience the same cancer risk as users of other tobacco products. Of the five cancers studied, none was found to have a statistically significant positive association with ST use for both males and females." Regarding dual use, the authors noted that "…higher rates of lung cancer (HR=22.3, 95% CI: 7.5, 66.3) were observed among combined users of ST and cigarettes than would have been expected based on the rates for exclusive ST use and for exclusive cigarette smoking." This result is consistent with a 2002 paper by the same group.	The authors noted that the study relies on self-reported data. Additionally, there is some inconsistency in the collection of tobacco use data between the various surveys analyzed.

Table 7.5.6–1-34:Summary of Relevant Published Literature – Health Risks Associated with Dual Use of MST and Cigarettes (continued)

Author	Title	Study Type and Sample	Author's Findings	Comments
(Ferketich, 2007b)	Smokeless tobacco use and salivary cotinine concentration	Cross-sectional study Tobacco users in the Ohio Appalachian region N = 256 males	The authors reported that cotinine levels for the 40 participants using both ST and smoking cigarettes were 460 ±332 ng/mL (mean ±SD), as compared with 560 ±370 ng/mL for the 216 exclusive ST users.	The authors cautioned that generalizability of the findings could be limited since the study participants were male volunteers interested in joining a dental clinic tobacco-cessation study. Furthermore, the participants were all from the Appalachian region in one state, and there was some question whether cotinine was a valid marker of dependence among ST users.
(Ferketich, 2007a)	A measure of nicotine dependence for smokeless tobacco users.	Cross-sectional study This study attempted to correlate a modification of the Fagerström Test of Nicotine Dependence in a large sample of ST users with salivary cotinine. N = 256 males	The correlation between the total score and salivary cotinine was moderate among the ST only users (r = 0.34), whereas it was lower (r = 0.19) among the ST + cigarette users.	The authors noted that the ST users were male volunteers who were interested in joining a tobacco-cessation study and that they were all from the Appalachian region in one state. Additionally, some of the items used in the scale had to be modified.
(Hassan, 2007)	Passive smoking and the use of non-cigarette tobacco products in association with risk for pancreatic cancer: a case-control study	Case-control study Pancreatic cancer patients N = 808 patients with pancreatic adenocarcinoma N = 808 controls	The authors reported that these was "no significant association between ever-use or heavy intake (>20 total time-years) of chewing tobacco, snuff, pipes, or cigars and the risk of pancreatic cancer among cigarette smokers."	The authors' list of limitations includes reliance on a questionnaire to collect information about passive smoking (misclassification of exposure is possible) and potential selection bias related to the use of hospital visitors as a control group.

Table 7.5.6–1-34:Summary of Relevant Published Literature – Health Risks Associated with Dual Use of MST and Cigarettes (continued)

Author	Title	Study Type and Sample	Author's Findings	Comments
(Yatsuya, 2010)	Risk of incident cardiovascular disease among users of smokeless tobacco in the Atherosclerosis Risk in Communities (ARIC) study.	Cohort study ARIC Study (1987-1989) N = 14,498 men and women aged 45-64 years	The authors noted the following: Out of 3,744 current cigarette smokers in the AIRC Study, 102 (2.7%) reported concurrent use of ST. "At baseline (1987-1989) in the ARIC Study, the overall prevalence of current smokeless tobacco use among cigarette nonsmokers was 3.1% (n = 456). The prevalence was higher in black and white men (5.9% and 5.3%, respectively) and black women (4.0%) and lower in white women (0.4%)." "Although CVD incidence rates in current smokeless tobacco users were higher than those in nonusers among both cigarette nonsmokers and current smokers, the association was statistically significant and independent of confounding factors only in cigarette nonsmokers."	Noted limitations of the study included no assessment of the quantity or duration of ST use, misclassification of tobacco use, and a relatively small number of ST consumers.

7.5.6-1.7. The Health Risks Associated with Switching to the MST Product as Compared with Using an FDA-Approved Tobacco-Cessation Medication

Meaningful comparisons of the risks to persons who may use ST products, as described in the previous sections, compared to FDA-approved tobacco-cessation medications present a significant challenge due to the vastly different contexts and circumstances associated with their respective uses.

Tobacco-cessation medications can help some people quit tobacco use altogether, and the potential health risks of these products have been extensively assessed by the FDA. In general, these medications are only used for a relatively short time period (e.g., the current label indication on these products is limited to use for 12 weeks), often in conjunction with behavioral modifications. ST, however, is not a product specifically indicated for smoking cessation. Rather, it is a consumer product often used for enjoyment and intended for adult use ad libitum. Assessing the health risks of ST products could include individuals who have used ST products for 40 years or more.

Although tobacco-cessation medications may convey some associated health risk, the benefit of smoking cessation is considered to outweigh any identified health risks. For those who succeed in smoking cessation, the major health risk to the individual former smoker appears to be a result of the residual and lasting effects of smoking. For those who do not succeed in quitting, their health risk logically reverts to that of continued smoking. For those who use MST exclusively, the health risks are detailed throughout Section 6.1.

7.5.6-1.7.1. Health Risks of FDA-Approved Tobacco-Cessation Medication

A full review of the entire literature regarding the health risks of cessation therapies is beyond the scope of this application. We do attempt to discuss here some review articles for three FDA-approved tobacco-cessation medications; nicotine replacement therapies (NRTs), bupropion, and varenicline.

7.5.6-1.7.2. Health Risks of Nicotine Replacement Therapies

Nicotine has been implicated in a number of potential health effects. These include acute toxicity, carcinogenicity, CVD, immune function, reproductive health outcomes, lung development, and cognitive function (Surgeon General Report, 2014). Within the context of NRT use under prescribed conditions, the health effects have focused primarily on adverse events, CV effects, and reproductive health outcomes.

Mills et al. (2010) conducted a systematic review and meta-analysis of 120 studies involving 177,390 individuals to evaluate adverse events associated with NRT used for smoking cessation. The investigators concluded that use of NRTs is associated with a variety of adverse effects that may be discomforting but that are not life-threatening. There was no statistically significant increase in anxiety or depressive symptoms associated with NRT use.

The longest documented use of NRT is in the Lung Health study, in which participants used nicotine gum for up to 5 years (Murray, 1996). This was a multicentered, randomized,

controlled trial of early intervention for the prevention of COPD. Ten university medical centers in the U.S. and Canada participated in the study. The subjects included adult smoking volunteers with evidence of early COPD: n = 3,923 in the intervention group and n = 1,964 the control group. The intervention included a smoking-cessation program and using nicotine gum. According to the investigators, "NP (nicotine polacrilex), as used in the Lung Health Study, appears to be safe and unrelated to any cardiovascular illnesses or other serious side effects."

Beyond the 5-year study period in the Lung Health Study, there are little data on the chronic use of NRTs. As noted by the Royal College of Physicians (Royal College of Physicians, 2007), "evidence on the safety of long term use of NRT is lacking, but there are no grounds to suspect appreciable long term adverse effects on health."

Mills et al. (2014) conducted a review of 63 randomized clinical trials involving 30,508 patients using smoking-cessation aids, including NRTs, to assess possible associations with CV events. On the basis of findings from 21 randomized clinical trials of NRTs, the investigators reported that there was an elevated risk of less serious events (RR = 2.29, 95 percent CI, 1.39-3.82) associated with NRT but no increase in serious CV events. The authors concluded that "Smoking cessation therapies do not appear to raise the risk of serious cardiovascular disease events."

Greenland et al. (1998) conducted a meta-analysis of adverse event data from 47 reports of 35 clinical trials of subjects using the nicotine patch. The collective studies involved nicotine patch recipients, totaling 5,501 subjects who used the nicotine patch and 3,752 subjects who were controls. There were no statistically significant increases in myocardial infarction, stroke, tachycardia, arrhythmia, or angina. The authors noted, however, that this outcome may have been influenced by studies included in the analyses.

In the 2011 review, Coleman et al. (2011) conducted a systematic search of the literature in several electronic databases as well as the Cochrane Pregnancy and Childbirth Group Trial Register. The search resulted in five studies meeting eligibility criteria, which collectively included a total of 695 pregnant smokers. In addition to smoking-cessation efficacy, a number of pregnancy outcomes were evaluated. These included birth weight, low birth weight (<2,500 g), preterm birth (<37 weeks gestation), neonatal intensive care unit admissions, and fetal demise. The investigators reported that five of the seven safety outcomes were more positive among infants born to women who had used NRT; however, none of the observed differences between trial groups reached statistical significance. The investigators concluded the following: "We found that there is currently insufficient evidence to demonstrate that NRT, used by pregnant women for smoking cessation, is either effective or safe."

In 2012, the same research group published a Cochrane Review of the safety of NRT related to pregnancy outcomes (Coleman, 2012). The review included findings from trials that evaluated the efficacy of smoking-cessation aids in pregnant women. The authors conducted a systematic search of the literature in several electronic databases including the Cochrane Pregnancy and Childbirth Group Trial Register. The search resulted in six studies meeting eligibility criteria; in total, 1,745 pregnant smokers were included. In addition to smoking-cessation efficacy, a number of pregnancy outcomes were evaluated. These included birth weight, low birth weight (<2,500 g), preterm birth (<37 weeks gestation), neonatal intensive care unit admissions

miscarriage/spontaneous abortion, neonatal death, and caesarean section. As with the previous review, the authors of this review concluded: "There is currently insufficient evidence to support either the efficacy or safety of nicotine replacement therapy (NRT) used with behavioural support by pregnant women for smoking cessation."

7.5.6-1.7.3. Health Risks of Bupropion Hydrochloride

Several systematic reviews have been published that summarized tolerability and adverse events associated with use of bupropion as a smoking-cessation aid (Aubin, 2002; Cahill, 2013; Ferry, 2003). These reviews have reported that bupropion is generally well tolerated. The most commonly reported adverse events are insomnia, headache, dry mouth, nausea, and anxiety. Some trials have report the occurrence of allergic reactions (Cahill, 2013).

Mills et al. (2014) conducted a review of 63 randomized clinical trials involving 30,508 patients using smoking-cessation aids, including bupropion, to assess possible association with CV events. On the basis of findings from 28 randomized clinical trials of bupropion, the investigators reported no association between use of bupropion and all CV events assessed (RR, 0.98; 95 percent CI, 0.54-1.73). With respect to major CV events, the investigators reported a protective effect with bupropion (RR, 0.45; 95 percent CI, 0.21-0.85).

According to Coleman et al. (2012), studies of pregnancy outcomes have not been conducted on varenicline or bupropion. These investigators state that "There are no studies of either varenicline or bupropion and neither can be recommended for use in pregnancy."

7.5.6-1.7.4. Health Risks of Varenicline

It has been reported that serious neuropsychiatric events, such as depression, suicidal ideation, suicide attempt, and completed suicide can occur in patients taking Chantix, a commercially marketed varenicline smoking-cessation aid. Accordingly, the FDA requires the manufacturer to provide a black box warning to the consumer.[69] However, Hughes (2016) recently reviewed data from several placebo-controlled trials and uncontrolled observational studies and concluded that "…there is consistent evidence that varenicline either does not cause increased suicide outcomes, or if it does, the effect is very small."

A recent literature review, covering 12 Cochrane reviews, summarized efficacy and adverse event findings related to several smoking-cessation pharmacotherapies (Cahill, 2013). The primary focus of the review was on NRT, bupropion, and varenicline. With respect to adverse events associated with varenicline, the review concluded that the main adverse event was mild-to-moderate nausea. Other events included insomnia, abnormal dreams, and headache.

Singh et al. (2011) conducted a systematic review and meta-analysis of data from 14 double-blind, randomized, controlled trials (8,216 participants) involving varenicline. The investigators reported that varenicline was associated with a significantly increased risk of adverse CV events, as compared with that for placebo.

[69] http://www.chantix.com/important-safety-information

Mills et al. (2014) conducted a review of 63 randomized clinical trials involving 30,508 patients using smoking-cessation aids, including varenicline, to assess possible association with CV events. On the basis of 18 randomized clinical trials of varenicline, the investigators reported no association between use of varenicline for smoking cessation and CVD events (RR, 1.30; 95 percent CI, 0.79-2.23).

According to Coleman et al. (2012), studies of pregnancy outcomes have not been conducted on varenicline or bupropion. These investigators state that "There are no studies of either varenicline or bupropion and neither can be recommended for use in pregnancy."

7.5.6-1.7.5. Summary

We are aware of no specific appropriate comparison study that measures the long-term epidemiological outcomes for smokers switching to either ST or cessation medications. We described the known health risks of ST throughout Section 6.1. It is plausible that ST presents a higher risk for some diseases than cessation therapies due to the differences in product formulation or the period of use encountered with the different product categories (a few weeks for cessation medications vs. potentially years for ST use).

7.5.6-1.8.Summary

This comprehensive literature review summarizes the published scientific literature related to the health risks of using ST in the U.S. The diverse data set of epidemiology studies presented in this section should inform the potential risks of the candidate MRTP by itself and in comparison to other more risky products like cigarettes. Below, we briefly summarize the main points addressed in this comprehensive literature review section of the MRTPA.

- The health risks associated with initiating use of the candidate MRTP as compared with never using tobacco products

We used existing epidemiology studies to address the health risks of the candidate MRTP, with a caveat that interpretations, however, are not always clear. The epidemiology literature indicates ambiguous results in the association between ST use and all-cause mortality and risk of all cancers (in particular; oropharyngeal, lung, esophageal, digestive, kidney and prostate cancers). No association appears to exist between ST use and cancers of the bladder, cancers of the pancreas, as well as hematopoietic or lymphoid cancers. There is mixed or equivocal evidence regarding the association between ST use and various cardiovascular disease (CVD) endpoints. On the other hand, there is clear evidence of a temporal association between ST use and oral lesions, gingival recession, and tooth loss.

We also reviewed published nonclinical data for potential mechanistic aspects in disease development that are relevant to the candidate MRTP. The studies were limited to those from the U.S. investigating the potential adverse effect of exposing oral mucosa, oral-derived tissue, or cells to MST products. Overall, findings from laboratory animal studies provided conflicting results and indicated a minimal potential for an adverse developmental effect of MST exposure.

- The health risks associated with use of the candidate MRTP as compared with those associated with using other tobacco products on the market, including tobacco products within the same class of products

MST and chewing tobacco products comprise the majority of the ST market in the U.S. Some epidemiology studies combine the use of both products since the health risks do not appear to be substantially different. For example, studies (Rodu, 2002) that looked at differential health risk between snuff, MST, and loose leaf chewing tobacco did not find substantial differences in mortality from all causes, all cancers, lung cancer, CVD, CHD, or cerebrovascular disease. No significant differences were also apparent in the RR of upper respiratory tract cancers between users of MST and users of loose leaf chewing tobacco.

Cigarette smoking remains the most prevalent form of tobacco use and presents the greatest risk for the user. ST use is not without some potential health risk. On the basis of the literature analysis, we believe that ST use is a viable alternative for cigarette smokers who want to use tobacco but also want to reduce their risk to major cigarette smoking–associated health risks.

- The changes in health risks to users who switch from using another tobacco product to using the candidate MRTP, including tobacco products within the same class of products

Studies addressing the changes in health risk related to switching among ST products are scarce in the literature. Henley et al. (2005) looked at the association between the use of snuff or chewing tobacco and mortality among men. The analysis indicated excess mortality risk from a variety of causes (all-causes, all-cancers, lung cancer, CVD, CHD, cerebrovascular disease, and other causes) in chewing tobacco users compared with non-users. Snuff users who never used chewing tobacco, compared with non-users, show an excess risk of mortality due to CHD. Tobacco chewers who switched to snuff, however, show a significantly increased risk for lung cancer. One could conclude that switching to chewing tobacco from snuff conveys more risk than switching from snuff to chewing tobacco; more importantly, for those who switched between chewing tobacco and snuff, there was no substantial difference in mortality risk estimates.

There are suggestions that the differences in chemical composition between MST and other ST products could lead to differences in health risk. Although epidemiology evidence directly comparing the health risks of MST use with other ST products is limited, current data suggests little reason to believe a remarkable difference in health outcomes. We expect that, for ST users who switch to the candidate MRTP, there would be no substantial change in health risk. The major change in health risk related to ST use remains with cigarette smokers who adopt MST use instead of smoking.

- The health risks associated with switching to the candidate MRTP as compared with quitting the use of tobacco products

Comparisons of the health risks between individuals who switch and individuals who quit MST products are complicated due to potential unaccounted confounding factors (e.g., age, and previous smoking history). Nevertheless, Henley et al. (2007) investigated this comparison and found that switchers had significantly higher rates of mortality from lung cancer, CHD, and

stroke than those who quit using tobacco entirely. The evidence suggests that, while switching to ST from cigarette smoking is not as safe as quitting all tobacco products, this behavior poses significantly lower risks than continuing to smoke.

- The health risks associated with using the candidate MRTP in conjunction with other tobacco products

The question of health risk related to dual use is poorly studied and frequently unaddressed in publications of smoking and ST use. The association between cigarette smoking and various diseases is well known; likewise, MST is also associated with an increased risk for some of the same diseases. Therefore, synergistic effects would be expected with health risks and dual use of both products. This assumption was examined by Accortt et al. (2002). The results of this study found that dual use of ST and smoking resulted in lung cancer mortality that was nearly twice that of exclusive smokers, although exclusive smokers who did not use ST also showed an increased risk as well. The authors concluded that the results were not likely due to a synergistic effect because dual users smoked more than exclusive smokers. The authors also looked at adverse health effects (cancer incidence) and concluded that there was no synergistic effect between ST and cigarette smoking. A similar result was shown in a subsequent study, where Accortt et al. (2005) found no statistically significant increase in cancer risk for dual users. Regarding oral disease, Andrews et al. (1998) reported that dual users had higher incidences of gingival bleeding and mouth sores than either cigarette smokers or tobacco non-users. Grady et al. (1990), however, found no consistent relationship between dual users and gingival bleeding, calculus (tartar), gingival recession, or attachment loss, when compared with nonusers. Overall, on the basis of this published epidemiological evidence, we conclude that the health effects associated with dual use are driven primarily by the level of cigarette smoking.

- The health risks associated with switching to the candidate MRTP as compared with using an FDA-approved tobacco-cessation medication

Tobacco-cessation medications can help some individuals quit tobacco use altogether, and the potential health risks of these medicines have been extensively assessed by the FDA. ST, however, is not a product specifically indicated for smoking cessation. Rather, it is a consumer product intended for adult use ad libitum. We are aware of no specific, appropriately-designed, comparison study that directly measures the long-term epidemiological outcomes for smokers switching to either ST or cessation medications.

7.5.6-1.9.Literature Cited

Accortt, N. A., Waterbor, J. W., Beall, C., & Howard, G. (2002). Chronic disease mortality in a cohort of smokeless tobacco users. *American Journal of Epidemiology, 156*(8), 730-737.

Accortt, N. A., Waterbor, J. W., Beall, C., & Howard, G. (2005). Cancer incidence among a cohort of smokeless tobacco users (United States). *Cancer Causes and Control, 16*(9), 1107-1115.

Agarwal, S. (2009). The association of active and passive smoking with peripheral arterial disease: Results from NHANES 1999-2004. *Angiology, 60*(3), 335-345.

Alguacil, J., & Silverman, D. T. (2004). Smokeless and other noncigarette tobacco use and pancreatic cancer: a case-control study based on direct interviews. *Cancer Epidemiology, 13*(1), 55-58.

Alonge, O. K., Ashrafi, S. H., & Colvard, M. D. (2003). Mitochondrial volume densities in the smokeless tobacco-treated hamster cheek pouch epithelium. *Oral Diseases, 9*(3), 138-143.

Andersson, G., Wahlin, A., & Bratthall, G. (2006). The effect of Swedish and American smokeless tobacco extract on periodontal ligament fibroblasts in vitro. *Swedish Dental Journal, 30*(3), 89-97.

Andrews, J. A., Severson, H. H., Lichtenstein, E., & Gordon, J. S. (1998). Relationship between tobacco use and self-reported oral hygiene habits. *Journal of the American Dental Association, 129*(3), 313-320.

Antoniades, D., Niukian, K., Schwartz, J., & Shklar, G. (1984). Effects of smokeless tobacco on the immune system of Syrian hamsters. *Journal of Oral Medicine, 39*(3), 136-141.

Arimilli, S., Damratoski, B. E., Bombick, B., Borgerding, M. F., & Prasad, G. L. (2012). Evaluation of cytotoxicity of different tobacco product preparations. *Regulatory Toxicology and Pharmacology, 64*(3), 350-360.

Arimilli, S., Damratoski, B. E., & Prasad, G. L. (2013). Combustible and non-combustible tobacco product preparations differentially regulate human peripheral blood mononuclear cell functions. *Toxicology in Vitro, 27*(6), 1992-2004.

Arredondo, J., Nguyen, V. T., Chernyavsky, A. I., Jolkovsky, D. L., Pinkerton, K. E., & Grando, S. A. (2001). A receptor-mediated mechanism of nicotine toxicity in oral keratinocytes. *Laboratory Investigation, 81*(12), 1653-1668.

Asal, N. R., Risser, D. R., Kadamani, S., Geyer, J. R., Lee, E. T., & Cherng, N. (1988). Risk factors in renal cell carcinoma: I. Methodology, demographics, tobacco, beverage use, and obesity. *Cancer Detection and Prevention, 11*(3-6), 359-377.

Ashrafi, S. H., Das, A., Worowongvasu, R., Mehdinejad, B., & Waterhouse, J. P. (1992). A light, transmission and scanning electron microscope study of snuff-treated hamster cheek pouch epithelium. *Scanning Microscopy, 6*(1), 183-193.

Aubin, H. J. (2002). Tolerability and safety of sustained-release bupropion in the management of smoking cessation. *Drugs, 62* (Suppl 2), 45-52.

Bagchi, D., Hassoun, E. A., Bagchi, M., & Stohs, S. J. (1995a). Protective effects of free radical scavengers and antioxidants against smokeless tobacco extract (STE)-induced oxidative stress in macrophage J774A.1 cell cultures. *Archives of Environmental Contamination and Toxicology, 29*(3), 424-428.

Bagchi, M., Bagchi, D., Adickes, E., & Stohs, S. J. (1995b). Chronic effects of smokeless tobacco extract on rat liver histopathology and production of HSP-90. *Journal of Environmental Pathology and Toxicology, 14*(2), 61-68.

Bagchi, M., Bagchi, D., Hassoun, E. A., & Stohs, S. J. (1994). Smokeless tobacco induced increases in hepatic lipid peroxidation, DNA damage and excretion of urinary lipid metabolites. *International Journal of Experimental Pathology, 75*(3), 197-202.

Bagchi, M., Bagchi, D., Hassoun, E. A., & Stohs, S. J. (1998). Subchronic effects of smokeless tobacco extract (STE) on hepatic lipid peroxidation, DNA damage and excretion of urinary metabolites in rats. *Toxicology, 127*(1-3), 29-38.

Bagchi, M., Bagchi, D., & Stohs, S. J. (1996). In vitro effects of a smokeless tobacco extract on the production of reactive oxygen species by human oral epidermal cells and rat hepatic mitochondria and microsomes, and peritoneal macrophages. *Archives of Environmental Contamination and Toxicology, 30*(3), 418-422.

Bagchi, M., Balmoori, J., Bagchi, D., Ray, S. D., Kuszynski, C., & Stohs, S. J. (1999). Smokeless tobacco, oxidative stress, apoptosis, and antioxidants in human oral keratinocytes. *Free Radical Biology and Medicine, 26*(7-8), 992-1000.

Bagchi, M., Kuszynski, C., Patterson, E. B., Tang, L., Bagchi, D., Saani, T., & Stohs, S. J. (1997). In vitro free radical production in human oral keratinocytes induced by smokeless tobacco extract. *In Vitro Toxicology, 10*(2), 263-274.

Bagchi, M., Kuszynski, C. A., Balmoori, J., Joshi, S. S., Stohs, S. J., & Bagchi, D. (2001). Protective effects of antioxidants against smokeless tobacco-induced oxidative stress and modulation of Bcl-2 and p53 genes in human oral keratinocytes. *Free Radical Research, 35*(2), 181-194.

Barley, R. D. C., Pollock, S., Shallow, M. C., Peters, E., & Lam, E. W. N. (2004). Tobacco-related-compound-induced nitrosative stress injury in the hamster cheek pouch. *Journal of Dental Research, 83*(12), 903-908.

Benedetti, M. D., Bower, J. H., Maraganore, D. M., McDonnell, S. K., Peterson, B. J., Ahlskog, J. E., &Rocca, W. A. (2000). Smoking, alcohol, and coffee consumption preceding Parkinson's disease: a case-control study. *Neurology, 55*(9), 1350-1358.

Bennington, J. L., & Laubscher, F. A. (1968). Epidemiologic studies on carcinoma of the kidney. I. Association of renal adenocarcinoma with smoking. *Cancer, 21*(6), 1069-1071.

Bernzweig, E., Payne, J. B., Reinhardt, R. A., Dyer, J. K., & Patil, K. D. (1998). Nicotine and smokeless tobacco effects on gingival and peripheral blood mononuclear cells. *Journal of Clinical Periodontology, 25*(3), 246-252.

Bertuccio, P., La, V. C., Silverman, D. T., Petersen, G. M., Bracci, P. M., Negri, E., &Boffetta, P. (2011). Cigar and pipe smoking, smokeless tobacco use and pancreatic cancer: an analysis from the International Pancreatic Cancer Case-Control Consortium (PanC4). *Annals of Oncology, 22*(6), 1420-1426.

Blot, W. J., McLaughlin, J. K., Winn, D. M., Austin, D. F., Greenberg, R. S., Preston-Martin, S., &Fraumeni, J. F., Jr. (1988). Smoking and drinking in relation to oral and pharyngeal cancer. *Cancer Research, 48*(11), 3282-3287.

Boffetta, P., & Straif, K. (2009). Use of smokeless tobacco and risk of myocardial infarction and stroke: systematic review with meta-analysis. *BMJ, 339*, b3060.

Bracci, P. M., & Holly, E. A. (2005). Tobacco use and non-Hodgkin lymphoma: results from a population-based case-control study in the San Francisco Bay Area, California. *Cancer Causes and Control, 16*(4), 333-346.

Brinton, L. A., Blot, W. J., Becker, J. A., Winn, D. M., Browder, J. P., Farmer, J. C., Jr., & Fraumeni, J. F., Jr. (1984). A case-control study of cancers of the nasal cavity and paranasal sinuses. *American Journal of Epidemiology, 119*(6), 896-906.

Broders, A. C. (1920). Squamous-cell epithelioma of the lip: A study of five hundred and thirty-seven cases. *Journal of the American Medical Association, 74*(10), 656-664.

Brown, L. M., Blot, W. J., Schuman, S. H., Smith, V. M., Ershow, A. G., Marks, R. D., & Fraumeni, J. F., Jr. (1988). Environmental factors and high risk of esophageal cancer among men in coastal South Carolina. *Journal of the National Cancer Institute, 80*(20), 1620-1625.

Brown, L. M., Everett, G. D., Gibson, R., Burmeister, L. F., Schuman, L. M., & Blair, A. (1992a). Smoking and risk of non-Hodgkin's lymphoma and multiple myeloma. *Cancer Causes and Control, 3*(1), 49-55.

Brown, L. M., Gibson, R., Blair, A., Burmeister, L. F., Schuman, L. M., Cantor, K. P., & Fraumeni, J. F. (1992b). Smoking and risk of leukemia. *American Journal of Epidemiology, 135*(7), 763-768.

Brunnemann, K. D., Rivenson, A., Adams, J. D., Hecht, S. S., & Hoffmann, D. (1987). A study of snuff carcinogenesis. *IARC Scientific Publications*(84), 456-459.

Burch, J. D., Rohan, T. E., Howe, G. R., Risch, H. A., Hill, G. B., Steele, R., & Miller, A. B. (1989). Risk of bladder cancer by source and type of tobacco exposure: a case-control study. *International Journal of Cancer, 44*(4), 622-628.

Burkey, M. D., Feirman, S., Wang, H., Choudhury, S. R., Grover, S., & Johnston, F. M. (2014). The association between smokeless tobacco use and pancreatic adenocarcinoma: A systematic review. *Cancer Epidemiology, 38*(6), 647-653.

Cahill, K., Stevens, S., Perera, R., & Lancaster, T. (2013). Pharmacological interventions for smoking cessation: an overview and network meta-analysis. *The Cochrane Database of Systematic Reviews*(5), CD009329.

Castelao, J. E., Yuan, J. M., Skipper, P. L., Tannenbaum, S. R., Gago-Dominguez, M., Crowder, J. S., &Yu, M. C. (2001). Gender- and smoking-related bladder cancer risk. *Journal of the National Cancer Institute, 93*(7), 538-545.

Chan, W. S., Chowdhry, S., Chang, T., & Kew, R. R. (1999). Initial characterization of the complement activating compounds in extracts of smokeless tobacco. *Immunobiology, 201*(1), 64-73.

Chang, T., Chowdhry, S., Budhu, P., & Kew, R. R. (1998). Smokeless tobacco extracts activate complement in vitro: a potential pathogenic mechanism for initiating inflammation of the oral mucosa. *Clinical Immunology and Immunopathology, 87*(3), 223-229.

Chen, S. Y. (1989). Effects of smokeless tobacco on the buccal mucosa of HMT rats. *Journal of Oral Pathology and Medicine, 18*(2), 108-112.

Coggon, D., Barker, D., & Rose, G. (2003). *Epidemiology of the Unitiated* (5th ed.). London: BMJ Books.

Cole, P., Monson, R. R., Haning, H., & Friedell, G. H. (1971). Smoking and Cancer of the Lower Urinary Tract. *The New England Journal of Medicine, 284*(3), 129-134.

Coleman, T., Chamberlain, C., Cooper, S., & Leonardi-Bee, J. (2011). Efficacy and safety of nicotine replacement therapy for smoking cessation in pregnancy: systematic review and meta-analysis. *Addiction (Abingdon, England), 106*(1), 52-61.

Coleman, T., Chamberlain, C., Davey, M. A., Cooper, S. E., & Leonardi-Bee, J. (2012). Pharmacological interventions for promoting smoking cessation during pregnancy. *The Cochrane Database of Systematic Reviews, 9*, CD010078.

Colvard, M. D., Ashrafi, S. H., Alonge, O. K., & Cordell, G. A. (2006). Smokeless tobacco-induced lamellar body abnormalities. *Oral Diseases, 12*(3), 343-348.

Coppe, J., Boysen, M., Sun, C. H., Wong Brian, J. F., Kang, M. K., Park, N., &Krtolica, A. (2008). A role for fibroblasts in mediating the effects of tobacco-induced epithelial cell growth and invasion. *Molecular Cancer Research, 6*(7), 1085-1098.

Craun, G. F., & Calderon, R. L. (2005). How to interpret epidemiological associations. In World Health Organization (Ed.), *Nutrients in Drinking Water* (pp. 108-115). Geneva, Switzerland: WHO Press.

Demirci, C. S., Miller, D. R., Baillargeon, J., Moyer, M. P., & Jenson, H. B. (2000). Smokeless tobacco extracts modulate exogenous gene expression in early passage cultured human oral epithelial cells: an in vitro system to study chemical and viral enhancer/promoter interaction. *Journal of Pharmacological and Toxicological Methods, 44*(3), 527-532.

Doll, R., Peto, R., Boreham, J., & Sutherland, I. (2004). Mortality in relation to smoking: 50 years' observations on male British doctors. *BMJ, 328*(7455), 1519.

Falk, R. T., Pickle, L. W., Fontham, E. T., Correa, P., & Fraumeni, J. F., Jr. (1988). Life-style risk factors for pancreatic cancer in Louisiana: a case-control study. *American Journal of Epidemiology, 128*(2), 324-336.

Falkler, W. A., Jr., Zimmerman, M. L., Martin, S. A., & Hall, E. R. (1987). The effect of smokeless-tobacco extracts on the growth of oral bacteria of the genus Streptococcus. *Archives of Oral Biology, 32*(3), 221-223.

Farrow, D. C., & Davis, S. (1990). Risk of pancreatic cancer in relation to medical history and the use of tobacco, alcohol and coffee. *International Journal of Cancer, 45*(5), 816-820.

Ferketich, A. K., Wee, A. G., Shultz, J., & Wewers, M. E. (2007a). A measure of nicotine dependence for smokeless tobacco users. *Addictive Behaviors, 32*(9), 1970-1975.

Ferketich, A. K., Wee, A. G., Shultz, J., & Wewers, M. E. (2007b). Smokeless tobacco use and salivary cotinine concentration. *Addictive Behaviors, 32*(12), 2953-2962.

Ferry, L., & Johnston, J. A. (2003). Efficacy and safety of bupropion SR for smoking cessation: data from clinical trials and five years of postmarketing experience. *International Journal of Clinical Practice, 57*(3), 224-230.

Fisher, M. T., Bennett, C. B., Hayes, A., Kargalioglu, Y., Knox, B. L., Xu, D., &Gaworski, C. L. (2012). Sources of and technical approaches for the abatement of tobacco specific nitrosamine formation in moist smokeless tobacco products. *Food and Chemical Toxicology, 50*(3-4), 942-948.

Food and Drug Administration. (2012). Modified Risk Tobacco Product Applications Draft Guidance. Retrieved from http://www.fda.gov/downloads/TobaccoProducts/GuidanceComplianceRegulatoryInformation/UCM297751.pdf

Fox, J. W., Gyorfi, T., & Dragulev, B. (1995). Effect of smokeless tobacco extract on HT 1080 cell adhesion, pp125(FAK) phosphorylation and apoptosis. *In Vitro Toxicology, 8*(4), 339-347.

Furie, M. B., Raffanello, J. A., Gergel, E. I., Lisinski, T. J., & Horb, L. D. (2000). Extracts of smokeless tobacco induce pro-inflammatory changes in cultured human vascular endothelial cells. *Immunopharmacology, 47*(1), 13-23.

Galvin, R. J., Ramp, W. K., & Lenz, L. G. (1988). Smokeless tobacco contains a nonnicotine inhibitor of bone metabolism. *Toxicology and Applied Pharmacology, 95*(2), 292-300.

Galvin, R. J., Ramp, W. K., & Lenz, L. G. (1991). Comparison of the effects of smokeless tobacco extract with the effects of prolyl hydroxylase inhibitors on collagenous and noncollagenous protein synthesis by osteoblasts. *Connective Tissue Research, 26*(3), 217-229.

Galvin, R. J., Ramp, W. K., Lenz, L. G., & Pierce, W. M., Jr. (1992). Smokeless tobacco contains an inhibitor of prolyl hydroxylase activity. *Toxicology Letters, 62*(2-3), 301-310.

Gao, H., Prasad, G. L., & Zacharias, W. (2013). Differential cell-specific cytotoxic responses of oral cavity cells to tobacco preparations. *Toxicology in Vitro, 27*(1), 282-291.

Gao, H., Prasad, G. L., & Zacharias, W. (2014). Combusted but not smokeless tobacco products cause DNA damage in oral cavity cells. *Environmental Toxicology and Pharmacology, 37*(3), 1079-1089.

Gao, X. P., Akhter, S. R., Ikezaki, H., Hong, D., & Rubinstein, I. (1999). Dexamethasone attenuates acute macromolecular efflux increase evoked by smokeless tobacco extract. *Journal of Applied Physiology, 87*(2), 619-625.

Gao, X. P., Suzuki, H., Olopade, C. O., Pakhlevaniants, S., & Rubinstein, I. (1997a). Angiotensin-converting enzyme and neutral endopeptidase modulate smokeless tobacco-induced increase in macromolecular efflux from the oral mucosa in vivo. *The Journal of Laboratory and Clinical Medicine, 130*(4), 395-400.

Gao, X. P., Suzuki, H., Olopade, C. O., Pakhlevaniants, S., & Rubinstein, I. (1997b). Purified ACE attenuates smokeless tobacco-induced increase in macromolecular efflux from the oral mucosa. *Journal of Applied Physiology, 83*(1), 74-81.

Gao, X. P., Vishwanatha, J. K., Conlon, J. M., Olopade, C. O., & Rubinstein, I. (1997c). Mechanisms of smokeless tobacco-induced oral mucosa inflammation: role of bradykinin. *Journal of Immunology, 157*(10), 4624-4633.

Gatehouse, D. (1987). Critical features of bacterial mutation assays. *Mutagenesis, 2*(5), 397-409.

Goodman, M. T., Morgenstern, H., & Wynder, E. L. (1986). A case-control study of factors affecting the development of renal cell cancer. *American Journal of Epidemiology, 124*(6), 926-941.

Goud, S. N., Zhang, L., & Kaplan, A. M. (1993). Immunostimulatory potential of smokeless tobacco extract in in vitro cultures of murine lymphoid tissues. *Immunopharmacology, 25*(2), 95-105.

Grady, D., Greene, J., Daniels, T. E., Ernster, V. L., Robertson, P. B., Hauck, W., &Silverman, S., Jr. (1990). Oral mucosal lesions found in smokeless tobacco users. *Journal of the American Dental Association, 121*(1), 117-123.

Greenland, S., Satterfield, M. H., & Lanes, S. F. (1998). A meta-analysis to assess the incidence of adverse effects associated with the transdermal nicotine patch. *Drug Safety, 18*(4), 297-308.

Guttenplan, J. B. (1987). Mutagenic activity in smokeless tobacco products sold in the USA. *Carcinogenesis, 8*(5), 741-743.

Hartge, P., Hoover, R., & Kantor, A. (1985). Bladder cancer risk and pipes, cigars, and smokeless tobacco. *Cancer, 55*(4), 901-906.

Hassan, M. M., Abbruzzese, J. L., Bondy, M. L., Wolff, R. A., Vauthey, J., Pisters, P. W., &Li, D. (2007). Passive smoking and the use of noncigarette tobacco products in association with risk for pancreatic cancer: a case-control study. *Cancer*, *109*(12), 2547-2556.

Hassoun, E. A., Bagchi, D., Bagchi, M., & Stohs, S. J. (1995). Effect of vitamin E succinate on smokeless tobacco-induced production of nitric oxide by rat peritoneal macrophages and J774A.1 macrophage cells in culture. *Free Radical Biology and Medicine*, *18*(3), 577-583.

Hatsukami, D., Stepanov, I., Severson, H., Jensen, J. A., Lindgren, B. R., Horn, K., &Hecht, S. S. (2015). Evidence supporting product standards for carcinogens in smokeless tobacco products. *Cancer Prevention Research (Philadelphia, Pa.)*, *8*(1), 20-26.

Hayes, R. B., Pottern, L. M., Swanson, G. M., Liff, J. M., Schoenberg, J. B., Greenberg, R. S., &Hoover, R. N. (1994). Tobacco use and prostate cancer in blacks and whites in the United States. *Cancer Causes and Control*, *5*(3), 221-226.

Hecht, S. S., & Hoffmann, D. (1988). Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. *Carcinogenesis*, *9*(6), 875-884.

Hecht, S. S., Rivenson, A., Braley, J., DiBello, J., Adams, J. D., & Hoffmann, D. (1986). Induction of oral cavity tumors in F344 rats by tobacco-specific nitrosamines and snuff. *Cancer Research*, *46*(8), 4162-4166.

Heineman, E. F., Zahm, S. H., McLaughlin, J. K., & Vaught, J. B. (1994). Increased risk of colorectal cancer among smokers: results of a 26-year follow-up of US veterans and a review. *International Journal of Cancer*, *59*(6), 728-738.

Heineman, E. F., Zahm, S. H., McLaughlin, J. K., Vaught, J. B., & Hrubec, Z. (1992). A prospective study of tobacco use and multiple myeloma: evidence against an association. *Cancer Causes and Control*, *3*(1), 31-36.

Henderson, J. S., & Johnson, R. B. (1995). The effects of smokeless tobacco extract on bone nodule formation and mineralization by chick osteoblasts in vitro. *Archives of Oral Biology*, *40*(7), 615-621.

Henley, J., Connell, C. J., Richter, P., Husten, C., Pechacek, T., Calle, E. E., & Thun, M. J. (2007). Tobacco-related disease mortality among men who switched from cigarettes to spit tobacco. *Tobacco Control*, *16*(1), 22-28.

Henley, J., Thun, M. J., Connell, C., & Calle, E. E. (2005). Two large prospective studies of mortality among men who use snuff or chewing tobacco (United States). *Cancer Causes and Control*, *16*(4), 347-358.

Hill, A. B. (1965). The Environment and Disease: Association or Causation? *Proceedings of the Royal Society of Medicine*, *58*, 295-300.

Homburger, F. (1971). Mechanical irritation, polycyclic hydrocarbons, and snuff. Effects on facial skin, cheek pouch, and oral mucosa in Syrian hamsters. *Archives of Pathology*, *91*(5), 411-417.

Homburger, F., Hsueh, S. S., Russfield, A. B., Laird, C. W., & Van Dongen, C. G. (1976). Absence of carcinogenic effects of chronic feeding of snuff in inbred syrian hamsters. *Toxicology and Applied Pharmacology*, *35*(3), 515-521.

Hsing, A. W., McLaughlin, J. K., Hrubec, Z., Blot, W. J., & Fraumeni, J. F., Jr. (1991). Tobacco use and prostate cancer: 26-year follow-up of US veterans. *American Journal of Epidemiology*, *133*(5), 437-441.

Hsing, A. W., McLaughlin, J. K., Schuman, L. M., Bjelke, E., Gridley, G., Wacholder, S., &Blot, W. J. (1990). Diet, tobacco use, and fatal prostate cancer: results from the Lutheran Brotherhood Cohort Study. *Cancer Research, 50*(21), 6836-6840.

Huckabee, K. D., Barnes, R. T., Williams, A. G., Jr., Fan, W. L., & Downey, H. F. (1993). Effects of snuff on regional blood flow to the cheek and tongue of anesthetized dogs. *Oral Surgery, Oral Medicine, and Oral Pathology, 76*(6), 729-735.

Hughes, J. R. (2016). Varenicline as a cause of suicidal outcomes. *Nicotine & Tobacco Research, 18*(1), 2-9.

Institute of Medicine. (2012). *Scientific Standards for Studies on Modified Risk Tobacco Products*. Washington, DC: The National Academies Press.

International Agency on Research for Cancer (IARC). (2007). *Smokeless Tobacco and Some Tobacco-specific N-Nitrosamines* (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans Ed. Vol. 89). Lyon, France: International Agency for Research on Cancer.

Jamal, A., Homa, D. M., O-Connor, E., Babb, S. D., Caraballo, R. S., Singh, T., &King, B. A. (2015). Current Cigarette Smoking Among Adults-United States, 2005-2014. *MMWR. Morbidity and Mortality Weekly Report, 64*(44), 1233-1240.

Jenson, H. B., Baillargeon, J., Heard, P., & Moyer, M. P. (1999a). Effects of smokeless tobacco and tumor promoters on cell population growth and apoptosis of B lymphocytes infected with epstein-barr virus types 1 and 2. *Toxicology and Applied Pharmacology, 160*(2), 171-182.

Jenson, H. B., Heard, P., & Moyer, M. P. (1999b). Evaluation of the effect of smokeless tobacco purified products and extracts on latent Epstein-Barr virus. *Toxicology, 133*(1), 35-42.

Johansson, S. L., Hirsch, J. M., & Johnson, D. R. (1991a). Effect of repeated oral administration of tobacco snuff on natural killer-cell activity in the rat. *Archives of Oral Biology, 36*(6), 473-476.

Johansson, S. L., Hirsch, J. M., Larsson, P. A., Saidi, J., & Osterdahl, B. G. (1989). Snuff-induced carcinogenesis: effect of snuff in rats initiated with 4-nitroquinoline N-oxide. *Cancer Research, 49*(11), 3063-3069.

Johansson, S. L., Hirsch, J. M., Larsson, P. A., Saidi, J., & Osterdahl, B. G. (1991b). Lack of promoting ability of snuff in rats initiated with 4-nitroquinoline-N-oxide. *IARC Scientific Publications*(105), 507-509.

Johansson, S. L., Saidi, J., Osterdahl, B. G., & Smith, R. A. (1991c). Promoting effect of snuff in rats initiated by 4-nitroquinoline-N-oxide or 7,12-dimethylbenz(a)anthracene. *Cancer Research, 51*(16), 4388-4394.

Johnson, G. K., Poore, T. K., Payne, J. B., & Organ, C. C. (1996). Effect of smokeless tobacco extract on human gingival keratinocyte levels of prostaglandin E2 and interleukin-1. *Journal of Periodontology, 67*(2), 116-124.

Joyce, A. R., Hawkins, W., Fariss, M. W., & Sengupta, T. K. (2010). Role of plasma membrane disruption in reference moist smokeless tobacco-induced cell death. *Toxicology Letters, 198*(2), 191-199.

Kabat, G. C., Chang, C. J., & Wynder, E. L. (1994). The role of tobacco, alcohol use, and body mass index in oral and pharyngeal cancer. *International Journal of Epidemiology, 23*(6), 1137-1144.

Kabat, G. C., Dieck, G. S., & Wynder, E. L. (1986). Bladder cancer in nonsmokers. *Cancer, 57*(2), 362-367.

Kallischnigg, G., Weitkunat, R., & Lee, P. N. (2008). Systematic review of the relation between smokeless tobacco and non-neoplastic oral diseases in Europe and the United States. *BMC Oral Health, 8*, 13.

Keller, A. Z. (1970). Cellular types, survival, race, nativity, occupations, habits and associated diseases in the pathogenesis of lip cancers. *American Journal of Epidemiology, 91*(5), 486-499.

Kneller, R. W., McLaughlin, J. K., Bjelke, E., Schuman, L. M., Blot, W. J., Wacholder, S., &Fraumeni, J. F., Jr. (1991). A cohort study of stomach cancer in a high-risk American population. *Cancer, 68*(3), 672-678.

Lee, P. N. (2007). Circulatory disease and smokeless tobacco in Western populations: a review of the evidence. *International Journal of Epidemiology, 36*(4), 789-804.

Lee, P. N., & Hamling, J. (2009a). The relation between smokeless tobacco and cancer in Northern Europe and North America. A commentary on differences between the conclusions reached by two recent reviews. *BMC Cancer, 9*, 256.

Lee, P. N., & Hamling, J. (2009b). Systematic review of the relation between smokeless tobacco and cancer in Europe and North America. *BMC Medicine, 7*, 36.

Lenz, L. G., Ramp, W. K., Galvin, R. J., & Pierce, W. M., Jr. (1992). Inhibition of cell metabolism by a smokeless tobacco extract: tissue and species specificity. *Proceedings of the Society for Experimental Biology and Medicine, 199*(2), 211-217.

Lindemann, R. A., & Park, N. H. (1988). Inhibition of human lymphokine-activated killer activity by smokeless tobacco (snuff) extract. *Archives of Oral Biology, 33*(5), 317-321.

Ljungberg, L. U., Persson, K., Eriksson, A. C., Green, H., & Whiss, P. A. (2013). Effects of nicotine, its metabolites and tobacco extracts on human platelet function in vitro. *Toxicology in Vitro, 27*(2), 932-938.

Lombard, C., Farthing, D., Sun, J., Fariss, M. W., & McKallip, R. J. (2010). Reference moist smokeless tobacco-induced apoptosis in human monocytes/macrophages cell line MM6. *International Immunopharmacology, 10*(9), 1029-1040.

Maden, C., Beckmann, A. M., Thomas, D. B., McKnight, B., Sherman, K. J., Ashley, R. L., &Daling, J. R. (1992). Human papillomaviruses, herpes simplex viruses, and the risk of oral cancer in men. *American Journal of Epidemiology, 135*(10), 1093-1102.

Malpass, G. E., Howlett, A. C., Arimilli, S., & Prasad, G. L. (2014). Regulation of gene expression by tobacco product preparations in cultured human dermal fibroblasts. *Toxicology and Applied Pharmacology, 279*(2), 211-219.

Mangipudy, R. S., & Vishwanatha, J. K. (1999). Role of nitric oxide in the induction of apoptosis by smokeless tobacco extract. *Molecular and Cellular Biochemistry, 200*(1-2), 51-57.

Martinez, I. (1969). Factors associated with cancer of the esophagus, mouth, and pharynx in Puerto Rico. *Journal of the National Cancer Institute, 42*(6), 1069-1094.

Mashberg, A., Boffetta, P., Winkelman, R., & Garfinkel, L. (1993). Tobacco smoking, alcohol drinking, and cancer of the oral cavity and oropharynx among U.S. veterans. *Cancer, 72*(4), 1369-1375.

Maxwell, J. C., Jr. (1986). Smokeless Sales Drop in US. Retrieved from https://industrydocuments.library.ucsf.edu/tobacco/docs/ntdh0199

Maxwell, J. C., Jr. (1992). INDUSTRY REPORT THE MAXWELL CONSUMER REPORT THE SMOKELESS TOBACCO INDUSTRY IN 910000. Retrieved from https://industrydocuments.library.ucsf.edu/tobacco/docs/klpw0007

Maxwell, J. C., Jr. (2006). *The Maxwell Report: The Smokeless Tobacco Industry in 2005*.

Maxwell, J. C., Jr. (2007). *The Maxwell Report: The Smokeless Tobacco Industry in 2006*.

Maxwell, J. C., Jr. (2008). *The Maxwell Report: The Smokeless Tobacco Industry in 2007*.

Maxwell, J. C., Jr. (2009). *The Maxwell Report: The Smokeless Tobacco Industry in 2008*.

Maxwell, J. C., Jr. (2011). *The Maxwell Report: The Smokeless Tobacco Industry in 2010*.

Maxwell, J. C., Jr. (2012). *The Maxwell Report: The Smokeless Tobacco Industry in 2011*.

McLaughlin, J. K., Mandel, J. S., Blot, W. J., Schuman, L. M., Mehl, E. S., & Fraumeni, J. F., Jr. (1984). A population--based case--control study of renal cell carcinoma. *Journal of the National Cancer Institute, 72*(2), 275-284.

Mills, E. J., Thorlund, K., Eapen, S., Wu, P., & Prochaska, J. J. (2014). Cardiovascular events associated with smoking cessation pharmacotherapies: a network meta-analysis. *Circulation, 129*(1), 28-41.

Mills, E. J., Wu, P., Lockhart, I., Wilson, K., & Ebbert, J. O. (2010). Adverse events associated with nicotine replacement therapy (NRT) for smoking cessation. A systematic review and meta-analysis of one hundred and twenty studies involving 177,390 individuals. *Tobacco Induced Diseases, 8*, 8.

Mitchell, C., Joyce, A. R., Piper, J. T., McKallip, R. J., & Fariss, M. W. (2010). Role of oxidative stress and MAPK signaling in reference moist smokeless tobacco-induced HOK-16B cell death. *Toxicology Letters, 195*(1), 23-30.

Monson, R. R. (1990). *Occupational epidemiology* (2nd ed.). Boca Raton, FL: CRC press.

Moore, G. E., Bissinger, L. L., & Proehl, E. C. (1953). Intraoral cancer and the use of chewing tobacco. *Journal of the American Geriatrics Society, 1*(7), 497-506.

Muns, G., Vishwanatha, J. K., & Rubinstein, I. (1994). Effects of smokeless tobacco on chemically transformed hamster oral keratinocytes: role of angiotensin I-converting enzyme. *Carcinogenesis, 15*(7), 1325-1327.

Murrah, V. A., Gilchrist, E. P., & Moyer, M. P. (1993). Morphologic and growth effects of tobacco-associated chemical carcinogens and smokeless tobacco extracts on human oral epithelial cells in culture. *Oral Surgery, Oral Medicine, and Oral Pathology, 75*(3), 323-332.

Murray, R. P., Bailey, W. C., Daniels, K., Bjornson, W. M., Kurnow, K., Connett, J. E., &Kiley, J. P. (1996). Safety of nicotine polacrilex gum used by 3,094 participants in the Lung Health Study. Lung Health Study Research Group. *Chest, 109*(2), 438-445.

Muscat, J. E., Hoffmann, D., & Wynder, E. L. (1995). The epidemiology of renal cell carcinoma. A second look. *Cancer, 75*(10), 2552-2557.

Muscat, J. E., Stellman, S. D., Hoffmann, D., & Wynder, E. L. (1997). Smoking and pancreatic cancer in men and women. *Cancer Epidemiology, Biomarkers and Prevention, 6*(1), 15-19.

Muscat, J. E., & Wynder, E. L. (1998). A case/control study of risk factors for major salivary gland cancer. *Otolaryngology and Head and Neck Surgery, 118*(2), 195-198.

Nilsson, R. (2006). De minimus non curat lex--virtual thresholds for cancer initiation by tobacco specific nitrosamines--prospects for harm reduction by smokeless tobacco. *International Journal of Occupational Medicine and Environmental Health, 19*(1), 6-35.

O'Berst, B. B., & McIntyre, R. A. (1953). Acute nicotine poisoning; case report. *Pediatrics, 11*(4), 338-340.

Oh, J. S., Cherrick, H. M., & Park, N. H. (1990). Effect of snuff extract on the replication and synthesis of viral DNA and proteins in cells infected with herpes simplex virus. *Journal of Oral and Maxillofacial Surgery, 48*(4), 373-379.

Park, N. H., Akoto-Amanfu, E., & Paik, D. I. (1988). Smokeless tobacco carcinogenesis: the role of viral and other factors. *CA: A Cancer Journal for Clinicians, 38*(4), 248-256.

Park, N. H., Herbosa, E. G., Niukian, K., & Shklar, G. (1985). Combined effect of herpes simplex virus and tobacco on the histopathologic changes in lips of mice. *Oral Surgery, Oral Medicine, and Oral Pathology, 59*(2), 154-158.

Park, N. H., Herbosa, E. G., & Sapp, J. P. (1987). Effect of tar condensate from smoking tobacco and water-extract of snuff on the oral mucosa of mice with latent herpes simplex virus. *Archives of Oral Biology, 32*(1), 47-53.

Park, N. H., Sapp, J. P., & Herbosa, E. G. (1986). Oral cancer induced in hamsters with herpes simplex infection and simulated snuff dipping. *Oral surgery, 62*(2), 164-168.

Paulson, R., Shanfeld, J., Mullet, D., Cole, J., & Paulson, J. O. (1994a). Prenatal smokeless tobacco effects on the rat fetus. *Journal of Craniofacial Genetics and Developmental Biology, 14*(1), 16-25.

Paulson, R., Shanfeld, J., Sachs, L., Price, T., & Paulson, J. (1989). Effect of smokeless tobacco on the development of the CD-1 mouse fetus. *Teratology, 40*(5), 483-494.

Paulson, R., Shanfeld, J., Vorhees, C. V., Cole, J., Sweazy, A., & Paulson, J. O. (1994b). Behavioral effects of smokeless tobacco on the neonate and young Sprague Dawley rat. *Teratology, 49*(4), 293-305.

Paulson, R. B., Shanfeld, J., Dean, J., Mullet, D., Fernandez, M., & Paulson, J. O. (1992). Alcohol and smokeless tobacco effects on the CD-1 mouse fetus. *Journal of Craniofacial Genetics and Developmental Biology, 12*(2), 107-117.

Paulson, R. B., Shanfeld, J., Prause, L., Iranpour, S., & Paulson, J. O. (1991). Pre- and post-conceptional tobacco effects on the CD-1 mouse fetus. *Journal of Craniofacial Genetics and Developmental Biology, 11*(1), 48-58.

Paulson, R. B., Shanfeld, J., Vorhees, C. V., Sweazy, A., Gagni, S., Smith, A. R., & Paulson, J. O. (1993). Behavioral effects of prenatally administered smokeless tobacco on rat offspring. *Neurotoxicology and Teratology, 15*(3), 183-192.

Payne, J. B., Johnson, G. K., Reinhardt, R. A., Maze, C. R., Dyer, J. K., & Patil, K. D. (1994). Smokeless tobacco effects on monocyte secretion of PGE2 and IL-1 beta. *Journal of Periodontology, 65*(10), 937-941.

Peacock, E. E., Jr., & Brawley, B. W. (1959). An evaluation of snuff and tobacco in the production of mouth cancer. *Plastic and Reconstructive Surgery, 23*(6), 628-635.

Peacock, E. E., Jr., Greenberg, B. G., & Brawley, B. W. (1960). The effect of snuff and tobacco on the production of oral carcinoma: an experimental and epidemiological study. *Annals of Surgery, 151*, 542-550.

Perry, H., Rosenbaum, W. L., Sterling, T. D., & Weinkam, J. J. (1993). *Attributable oral cancer risk due to smokeless tobacco use based on a case-control study at Sinai Hospital in Detroit*. cited by Gross et al (*Environment International* 21: 391-394; 1995).

Petro, T. M. (2003). Modulation of IL-12 p35 and p40 promoter activity by smokeless tobacco extract is associated with an effect upon activation of NF-kappaB but not IRF transcription factors. *International Immunopharmacology, 3*(5), 735-745.

Petro, T. M., Anderson, L. L., Gowler, J. S., Liu, X. J., & Schwartzbach, S. D. (2002). Smokeless tobacco extract decreases IL-12 production from LPS-stimulated but increases IL-12 from IFN-gamma-stimulated macrophages. *International Immunopharmacology, 2*(2-3), 345-355.

Petro, T. M., Schwartzbach, S. D., & Zhang, S. (1999). Smokeless tobacco and nicotine bring about excessive cytokine responses of murine memory T-cells. *International Journal of Immunopharmacology, 21*(2), 103-114.

Petro, T. M., & Zhang, S. (1997). The effect of smokeless tobacco extract on murine T cell cytokine production. *Immunopharmacology, 36*(1), 17-26.

Pottern, L. M., Morris, L. E., Blot, W. J., Ziegler, R. G., & Fraumeni, J. F., Jr. (1981). Esophageal cancer among black men in Washington, D.C. I. Alcohol, tobacco, and other risk factors. *Journal of the National Cancer Institute, 67*(4), 777-783.

Prokopczyk, G., Adams, J. D., LaVoie, E. J., & Hoffmann, D. (1987). Effect of snuff and nicotine on DNA methylation by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Carcinogenesis, 8*(10), 1395-1397.

Rickert, W. S., Joza, P. J., Trivedi, A. H., Momin, R. A., Wagstaff, W. G., & Lauterbach, J. H. (2009). Chemical and toxicological characterization of commercial smokeless tobacco products available on the Canadian market. *Regulatory Toxicology and Pharmacology, 53*(2), 121-133.

Rickert, W. S., Wright, W. G., Trivedi, A. H., Momin, R. A., & Lauterbach, J. H. (2007). A comparative study of the mutagenicity of various types of tobacco products. *Regulatory Toxicology and Pharmacology, 48*(3), 320-330.

Rodu, B., & Cole, P. (2002). Smokeless tobacco use and cancer of the upper respiratory tract. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics, 93*(5), 511-515.

Rostron, B. (2013). Smoking-attributable mortality by cause in the United States: revising the CDC's data and estimates. *Nicotine & Tobacco Research, 15*(1), 238-246.

Royal College of Physicians. (2007). *Harm reduction in nicotine addiction: Helping People Who Can't Quit*. London.

Rubinstein, I. (2000). Smokeless tobacco potentiates VIP-induced DNA synthesis and inactivates NEP 24.11 in oral keratinocytes. *American Journal of Physiology: Cell Physiology, 278*(2), C391-C396.

Rubinstein, I., Gao, X. P., Pakhlevaniants, S., & Oda, D. (1998). Smokeless tobacco-exposed oral keratinocytes increase macromolecular efflux from the in situ oral mucosa. *American Journal of Physiology, 274*(1 Pt 2), R104-R111.

Rubinstein, I., & Pedersen, G. W. (2002). Bacillus species are present in chewing tobacco sold in the United States and evoke plasma exudation from the oral mucosa. *Clinical and Diagnostic Laboratory Immunology, 9*(5), 1057-1060.

Schroeder, J. C., Olshan, A. F., Baric, R., Dent, G. A., Weinberg, C. R., Yount, B., &Blair, A. (2002). A case-control study of tobacco use and other non-occupational risk factors for t(14;18) subtypes of non-Hodgkin's lymphoma (United States). *Cancer Causes and Control, 13*(2), 159-168.

Schwartz, J. L., Brunnemann, K. D., Adami, A. J., Panda, S., Gordon, S. C., Hoffmann, D., & Adami, G. R. (2010). Brand specific responses to smokeless tobacco in a rat lip canal model. *Journal of Oral Pathology and Medicine, 39*(6), 453-459.

Schwartz, S. M., Daling, J. R., Doody, D. R., Wipf, G. C., Carter, J. J., Madeleine, M. M., &Galloway, D. A. (1998). Oral cancer risk in relation to sexual history and evidence of human papillomavirus infection. *Journal of the National Cancer Institute, 90*(21), 1626-1636.

Shavelle, R. M., Paculdo, D. R., Strauss, D. J., & Kush, S. J. (2008). Smoking habit and mortality: a meta-analysis. *Journal of Insurance Medicine, 40*(3-4), 170-178.

Shirname-More, L. (1991a). Forward mutation of S. typhimurium by smokeless tobacco extracts. *Mutation Research, 259*(1), 37-42.

Shirname-More, L. (1991b). Smokeless tobacco extracts mutate human cells. *Carcinogenesis, 12*(5), 927-930.

Shklar, G., Niukian, K., Hassan, M., & Herbosa, E. G. (1985). Effects of smokeless tobacco and snuff on oral mucosa of experimental animals. *Journal of Oral and Maxillofacial Surgery, 43*(2), 80-86.

Singh, S., Loke, Y. K., Spangler, J. G., & Furberg, C. D. (2011). Risk of serious adverse cardiovascular events associated with varenicline: a systematic review and meta-analysis. *Canadian Medical Association Journal, 183*(12), 1359-1366.

Slattery, M. L., Schumacher, M. C., West, D. W., & Robison, L. M. (1988). Smoking and bladder cancer. The modifying effect of cigarettes on other factors. *Cancer, 61*(2), 402-408.

Smith, R. A., Lawson, T., & Johansson, S. L. (1997). Detection of DNA adducts by 32P postlabeling following chronic exposure of rats to snuff. *Cancer Letters, 121*(1), 11-17.

Spangler, J. G., Michielutte, R., Bell, R. A., & Dignan, M. B. (2001). Association between smokeless tobacco use and breast cancer among Native-American women in North Carolina. *Ethnicity and Disease, 11*(1), 36-43.

Spitz, M. R., Fueger, J. J., Goepfert, H., Hong, W. K., & Newell, G. R. (1988). Squamous cell carcinoma of the upper aerodigestive tract. A case comparison analysis. *Cancer, 61*(1), 203-208.

Sponsiello-Wang, Z., Weitkunat, R., & Lee, P. N. (2008). Systematic review of the relation between smokeless tobacco and cancer of the pancreas in Europe and North America. *BMC Cancer, 8*, 356.

Squires, W. G., Jr., Brandon, T. A., Zinkgraf, S., Bonds, D., Hartung, G. H., Murray, T., &Miller, R. R. (1984). Hemodynamic effects of oral smokeless tobacco in dogs and young adults. *Preventive Medicine, 13*(2), 195-206.

Stamm, S. C., Zhong, B. Z., Whong, W. Z., & Ong, T. (1994). Mutagenicity of coal-dust and smokeless-tobacco extracts in Salmonella typhimurium strains with differing levels of O-acetyltransferase activities. *Mutation Research, 321*(4), 253-264.

Sterling, T. D., Rosenbaum, W. L., & Weinkam, J. J. (1992). Analysis of the relationship between smokeless tobacco and cancer based on data from the National Mortality Followback Survey. *Journal of Clinical Epidemiology, 45*(3), 223-231.

Stich, J. E., Li, K. K., Chun, Y. S., Weiss, R., & Park, N. H. (1987). Effect of smokeless tobacco on the replication of herpes simplex virus in vitro and on production of viral lesions in hamster cheek pouch. *Archives of Oral Biology, 32*(4), 291-296.

Stockwell, H. G., & Lyman, G. H. (1986). Impact of smoking and smokeless tobacco on the risk of cancer of the head and neck. *Head and Neck Surgery*, *9*(2), 104-110.

Summerlin, D. J., Dunipace, A., & Potter, R. (1992). Histologic effects of smokeless tobacco and alcohol on the pouch mucosa and organs of the Syrian hamster. *Journal of Oral Pathology and Medicine*, *21*(3), 105-108.

Surgeon General Report, & U.S. Department of Health and Human Services. (2014). The Health Consequences of Smoking—50 Years of Progress. A Report of the Surgeon General. Retrieved from https://www.surgeongeneral.gov/library/reports/50-years-of-progress/exec-summary.pdf

Suzuki, H., Gao, X. P., Olopade, C. O., Jaffe, H. A., Pakhlevaniants, S., & Rubinstein, I. (1996). Aqueous smokeless tobacco extract impairs endothelium-dependent vasodilation in the oral mucosa. *Journal of Applied Physiology*, *81*(1), 225-231.

Theophilus, E. H., Hayes, J. R., Potts, R. J., Ayres, P. H., Williams, C. D., & Garner, C. D. (2012). Toxicological evaluation of smokeless tobacco: 90-day rodent feeding studies. *Experimental and Toxicologic Pathology*, *64*(1-2), 15-24.

Timberlake, D. S. (2016). Advertising Receptivity and Youth Initiation of Smokeless Tobacco. *Substance Use and Misuse*, *51*(9), 1077-1082.

Tobey, N. A., Schreiner, V. J., Readling, R. D., & Orlando, R. C. (1988). The acute effects of smokeless tobacco on transport and barrier function of buccal mucosa. *Journal of Dental Research*, *67*(11), 1414-1421.

Tomar, S. L. (2003). Is use of smokeless tobacco a risk factor for cigarette smoking? The U.S. experience. *Nicotine & Tobacco Research*, *5*(4), 561-569.

Tucker, J. D., & Ong, T. (1985). Induction of sister chromatid exchanges by coal dust and tobacco snuff extracts in human peripheral lymphocytes. *Environmental Mutagenesis*, *7*(3), 313-324.

U.S. Dept. Health Human Services. (1986). *The Health Consequences of Using Smokeless Tobacco: A report of the Advisory Committee to the Surgeon General* (Vol. NIH Publication No.86-2874). Bethesda, Maryland: USDHHS, Public Health Service.

Vincent, R. G., & Marchetta, F. (1963). The Relationship of the Use of Tobacco and Alcohol to Cancer of the Oral Cavity, Pharynx, or Larynx. *American Journal of Surgery*, *106*, 501-505.

Vishwanatha, J. K., Swinney, R., & Banerjee, A. G. (2003). Modulation of annexin I and cyclooxygenase-2 in smokeless tobacco-induced inflammation and oral cancer. *Molecular and Cellular Biochemistry*, *248*(1-2), 67-75.

Vogler, W. R., Lloyd, J. W., & Milmore, B. K. (1962). A retrospective study of etiological factors in cancer of the mouth, pharynx, and larynx. *Cancer*, *15*(2), 246-258.

Wahlberg, I., & Ringberger, T. (1999). Smokeless Tobacco. In D. L. Davis & M. T. Nielsen (Eds.), *Tobacco: Production, Chemistry and Technology*. Oxford: Blackwell Publishing.

Wang, Y., Rotem, E., Andriani, F., & Garlick, J. A. (2001). Smokeless tobacco extracts modulate keratinocyte and fibroblast growth in organotypic culture. *Journal of Dental Research*, *80*(9), 1862-1866.

Wennmalm, A., Benthin, G., Granstrom, E. F., Persson, L., Petersson, A. S., & Winell, S. (1991). Relation between tobacco use and urinary excretion of thromboxane A_2 and prostacyclin metabolites in young men. *Circulation*, *83*(5), 1698-1704.

Westbrook, K. C., Suen, J. Y., Hawkins, J. M., & McKinney, D. C. (1980). Snuff dipper's carcinoma: Fact or fiction. In H. E. Nieburg (Ed.), *Prevention and Detection of Cancer* (pp. 1367-1371). New York: Marcel Dekker.

Whong, W. Z., Ames, R. G., & Ong, T. M. (1984). Mutagenicity of tobacco snuff: possible health implications for coal miners. *Journal of Toxicology and Environmental Health, 14*(4), 491-496.

Williams, R. R., & Horm, J. W. (1977). Association of cancer sites with tobacco and alcohol consumption and socioeconomic status of patients: interview study from the Third National Cancer Survey. *Journal of the National Cancer Institute, 58*(3), 525-547.

Winn, D. M., Blot, W. J., Shy, C. M., Pickle, L. W., Toledo, A., & Fraumeni, J. F., Jr. (1981). Snuff dipping and oral cancer among women in the southern United States. *New England Journal of Medicine, 304*(13), 745-749.

Wolfe, M. D., & Carlos, J. P. (1987). Oral health effects of smokeless tobacco use in Navajo Indian adolescents. *Community Dentistry and Oral Epidemiology, 15*(4), 230-235.

Wolk, R., Shamsuzzaman Abu, S. M., Svatikova, A., Huyber, C. M., Huck, C., Narkiewicz, K., & Somers, V. K. (2005). Hemodynamic and autonomic effects of smokeless tobacco in healthy young men. *Journal of the American College of Cardiology, 45*(6), 910-914.

Wynder, E. L., & Bross, I. J. (1957). Aetiological factors in mouth cancer; an approach to its prevention. *Br. Med. J, 1*(5028), 1137-1143.

Wynder, E. L., & Bross, I. J. (1961). A study of etiological factors in cancer of the esophagus. *Cancer, 14*, 389-413.

Wynder, E. L., Kabat, G., Rosenberg, S., & Levenstein, M. (1983). Oral cancer and mouthwash use. *Journal of the National Cancer Institute, 70*(2), 255-260.

Wynder, E. L., & Stellman, S. D. (1977). Comparative epidemiology of tobacco-related cancers. *Cancer Research, 37*(12), 4608-4622.

Yatsuya, H., & Folsom Aaron, R. A. G. A. (2010). Risk of incident cardiovascular disease among users of smokeless tobacco in the Atherosclerosis Risk in Communities (ARIC) study. *American Journal of Epidemiology, 172*(5), 600-605.

Yuan, J. M., Castelao, J. E., Gago-Dominguez, M., Yu, M. C., & Ross, R. K. (1998). Tobacco use in relation to renal cell carcinoma. *Cancer Epidemiology, Biomarkers and Prevention, 7*(5), 429-433.

Zahm, S. H., Blair, A., Holmes, F. F., Boysen, C. D., Robel, R. J., & Fraumeni, J. F., Jr. (1989). A case-control study of soft-tissue sarcoma. *American Journal of Epidemiology, 130*(4), 665-674.

Zahm, S. H., Heineman, E. F., & Vaught, J. B. (1992). Soft tissue sarcoma and tobacco use: data from a prospective cohort study of United States veterans. *Cancer Causes and Control, 3*(4), 371-376.

Zeller, M., & Hatsukami, D. (2009). The Strategic Dialogue on Tobacco Harm Reduction: a vision and blueprint for action in the US. *Tobacco Control, 18*(4), 324-332.

Zheng, T., Cantor, K. P., Zhang, Y., Chiu, B. C., & Lynch, C. F. (2001). Risk of brain glioma not associated with cigarette smoking or use of other tobacco products in Iowa. *Cancer Epidemiology, 10*(4), 413-414.

Zheng, W., McLaughlin, J. K., Gridley, G., Bjelke, E., Schuman, L. M., Silverman, D. T., &Fraumeni, J. F., Jr. (1993). A cohort study of smoking, alcohol consumption, and dietary factors for pancreatic cancer (United States). *Cancer Causes and Control, 4*(5), 477-482.