Review of the Scientific Literature on Snus
(Swedish Moist Snuff)

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List of Acronyms

1-HOP	1-hydroxypyrene (urinary biomarker of exposure)
ACS	American Cancer Society
AHA	American Heart Association
ALS	amyotrophic lateral sclerosis
AUC	areas under the curve
B[b]F	benzo[b]fluoranthene
B[k]F	benzo[k]fluoranthene
BMI	body mass index
Bq	becquerel (SI derived unit of radioactivity)
B-Se	selenium in whole blood
CD	Crohn's disease
CHD	coronary heart disease
CI	confidence interval
CMM	cutaneous malignant melanoma
CO	carbon monoxide
Con A	concanavalin A (a mitogenic substance that is used to induce T-cell proliferation)
CSCC	cutaneous squamous cell carcinoma
CVD	cardiovascular disease
DNA	Deoxyribonucleic acid
ESTOC	The European Smokeless Tobacco Council
EU	European Union
g	Gram
Hb	Hemoglobin
HD	Hodgkin's disease
HDL	high-density lipoprotein
HIV	human immunodeficiency virus
Hp	*Helicobacter pylori* (an infection which increases risk of gastric cancer)
HPB	4-hydroxy-1-(3-pyridyl)-1-butanone (a type of DNA-adduct)
HPRT	hypoxanthine-guanine phosphoribosyl transferase
HPV	human papillomavirus
HR	hazard ratio
HSV	herpes simplex virus
HSV-1	herpes simplex virus-1
IARC	International Agency for Research on Cancer
IBD	inflammatory bowel disease

ICD	International Classification of Diseases
IHD	ischemic heart disease
IMM	intraocular malignant melanoma
INS-GAS	a strain of mice genetically predisposed to developing gastric cancer
IOM	Institute of Medicine
IRR	incidence rate ratio
LCMR	lung cancer mortality rate
LDL	low-density lipoprotein
LSRO	Life Sciences Research Office, Inc.
MDPH	Massachusetts Department for Public Health
MetSy	metabolic syndrome
MI	myocardial infarction
MIS	melanoma *in situ*
MM	multiple myeloma
MNBA	4-(methylnitrosamino) butyric acid (an *N*-nitrosamino acid)
MNPA	3-(methylnitrosamino) propionic acid (an *N*-nitrosamino acid)
MS	multiple sclerosis
NAB	N-nitrosoanabasine (a TSNA)
NAT	N-nitrosoanatabine (a TSNA)
NAzCA	*N*-nitrosoazetidine-4-carboxylic acid (an *N*-nitrosamino acid)
NDELA	*N*-nitrosodiethanolamine (formed from a residual contaminant in tobacco)
NDMA	*N*-nitrosodimethylamine (a volatile nitrosamine)
NHL	non-Hodgkin's lymphoma
NMOR	*N*-nitrosomorpholine (a volatile nitrosamine)
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNAL-Glucs	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol glucuronides
NNK	4-[methylnitrosamino]-1-[3-pyridyl]-1-butanone (a TSNA)
NNN	N-nitrosonornicotine (a TSNA)
NPIP	*N*-nitrosopiperidine (a volatile nitrosamine)
NPYR	*N*-nitrosopyrrolidine (a volatile nitrosamine)
NRT	Nicotine Replacement Therapy
NRU	neutral red uptake (a cytotoxicity assay)
NSAR	*N*-nitrososarcosine (an *N*-nitrosamino acid)
OOSCC	oral and oropharyngeal squamous cell carcinoma
OR	odds ratio
PAH	polycyclic aromatic hydrocarbon
PAR	population attributable risk
POB	pyridyloxobuylations (a type of DNA-adduct)

PREP	potential reduced-exposure tobacco product
RR	risk ratio
SAH	subarachnoid hemorrhage
SCD	sudden cardiac death
SCE	sister chromatid exchanges
SCENIHR	Scientific Committee on Emerging and Newly-Identified Health Risks
SES	socioeconomic status
SIL	snuff-induced lesions
S-Se	selenium in serum
STP	smokeless tobacco product
TSNA	tobacco-specific nitrosamine
UC	ulcerative colitis
US	United States
USDHHS	US Department of Health and Human Services
USEPA	US Environmental Protection Agency
UST	US Smokeless Tobacco Company
VNA	volatile nitrosamine
WHO	World Health Organization
WT	wild type mice

Executive Summary

The potential health effects of Swedish snus have been well studied, particularly in Sweden, where the product is widely used. Numerous studies, undertaken by institutions around the world over the past three decades, have resulted in a solid base of literature documenting the health effects of Swedish snus. The studies have been of great interest to the scientific and public health communities and will provide the basis for future decision-making by the US Food and Drug Administration (FDA) and other regulatory bodies.

Swedish snus is an oral smokeless tobacco product traditionally used in Sweden since the early 1800s that is manufactured using a tobacco heat-treatment process. A quality standard (GothiaTek®) for the manufacture of Swedish snus has been developed by Swedish Match, which is the market-leading snus producer in Scandinavia. A notable difference between traditional Swedish snus and other smokeless tobacco products lies in the processing of the tobacco. While during manufacturing of other products the tobacco is fermented, Swedish snus is heat-treated. This difference helps to explain the lower concentrations of certain trace components in Swedish snus, including tobacco-specific nitrosamines (TSNAs).

ENVIRON International Corporation (ENVIRON) has conducted a comprehensive review of the relevant published chemistry, epidemiology, and toxicology studies available for Swedish snus, including literature identified through systematic ongoing searches of Medline and several additional databases in Dialog® through December 31, 2009. The ENVIRON review summarizes studies of the potential health risks associated with the use of Swedish snus. The review includes sections on the chemical properties, the manufacturing process, biomarkers of exposure, and epidemiological and toxicological studies.

A principal outcome of the ENVIRON review is the presentation of information needed to conduct a quantitative product risk assessment. The review focuses on topics that are critical for a risk assessment, particularly for understanding the potential for increased health risks from use of Swedish snus. Risk assessment has become a dominant public-policy tool for informing decision-makers and the public about different policy options for protecting public health and the environment. It is particularly well suited for conducting an assessment of reduced risk from the different tobacco products. Ideally, a product risk assessment is based on credible, quality information. ENVIRON determined that generally the research is robust but there are variances for the subject areas reviewed. For example, the evidence from epidemiology studies to identify moderate to high adverse health risks in humans is particularly strong. More research is necessary, however, to determine whether the use of biomarkers of exposure for components of tobacco products will be useful for comparing and predicting health risks of various smokeless tobacco products, including Swedish snus.

ENVIRON conducted the review on behalf of Swedish Match AB, the market leading producer of Swedish snus in the Scandinavian markets, where the product has widespread use. Swedish Match was seeking an independent scientific review of the potential health effects of its product. The request is in keeping with company's commitment to research and product stewardship, as demonstrated by the development of its own quality standard, GothiaTek®.

The ENVIRON review was initially intended to be used to inform Swedish Match and to be made available to key audiences. However, with the enactment of the US Smoking Prevention Control Act, the review will be a significant part of the information Swedish Match provides to the FDA under Section 904(a)(4) of the Act that requires each tobacco product manufacturer or importer, or agent thereof, to submit all documents developed after June 22, 2009 "*that relate to health, toxicological, behavioral, or physiologic effects of current or future tobacco products, their constituents (including smoke constituents), ingredients, components, and additives*."

Ideally, the ENVIRON review will be of use to a range of regulatory bodies as well as researchers, the public health community, and other stakeholders. Certainly the review may be of use to FDA as it implements the provisions of Section 911 of the Smoking Prevention Control Act, provisions that call for the Agency to develop a process for characterizing modified risk tobacco products.

Chemical Composition

Swedish snus is a heat-treated oral moist snuff tobacco product originally developed in Sweden. Swedish snus mainly consists of air-cured tobacco, water, and salt. Other ingredients added in small quantities serve to retain moisture, stabilize the pH, and for preservation and flavoring purposes. The moisture content of traditional Swedish snus is approximately 50% and the pH close to 8.5. The manufacturing process of snus in Sweden must satisfy the hygienic requirements of the Swedish Food Act and all ingredients must comply with the Swedish Food Regulation.

Concentrations of TSNAs, traditionally the most frequently analyzed and reported trace-level components in smokeless tobacco products (STPs) due to their carcinogenic potential demonstrated in experimental animals, have significantly decreased in Swedish snus between the early 1980s and 2000. This appears to be mainly due to improvements in the Swedish snus manufacturing process that were introduced in the early 1980s, including both technical changes in the production process and the institution of more rigorous quality checks of the raw ingredients.

Published data for most other trace-level components in STPs, including Swedish snus, is limited, and only in recent years more analyses on a variety of components other than TSNAs have become available (e.g., polyaromatic hydrocarbons, aldehydes, and metals).

This limited published analytical data on the chemical composition of traditional Swedish snus does not allow distinction between different brands of snus. There are differences in portion sizes and nicotine content and delivery between snus brands. This information needs to be taken into account when conducting an exposure assessment for critical chemical substances in Swedish snus. Furthermore, for a comparison of the potential exposure to critical components in traditional Swedish snus with other oral moist snuff products, such as new products marketed as snus and traditional US-type moist snuff, other factors, such as differences in moisture content, pH and resulting nicotine delivery need to be considered, along with use patterns.

Biomarkers of Exposure to and Potential Effect from Swedish Snus and Tobacco Components

Biomarkers of exposure and biomarkers of effect are being evaluated in some studies of individuals that use various STPs. Measuring critical chemical substances or their metabolites in biological fluids or tissues ("biomarkers of internal exposure") allows for the estimation of external exposure levels that may be associated with health risks from STPs including Swedish snus. Biomarkers of exposure include specific chemical components of tobacco products or their metabolites. Biomarkers of effect may be used to evaluate the potential for the development of adverse health effects associated with exposure to tobacco or its chemical components. These biomarkers may be the products of different cellular responses following exposure that may be considered to be early indicators of the potential for subsequent adverse health effects.

To date, there is no comprehensive set of validated biomarkers of exposure or biological effects available for use to predict adverse health effects (e.g., cardiovascular, cancer) related to exposure to components in tobacco or tobacco smoke. There have been a limited number of studies conducted to evaluate exposure biomarkers such as levels of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) or its glucuronides or cotinine in humans following the use of Swedish snus. Most studies that have been conducted have not measured biomarkers in different exposure groups (e.g., Swedish snus, other STPs, cigarettes) within the same study, so it is not possible to draw conclusions regarding levels of specific biomarkers among users of different products from such studies. A few studies were identified that have evaluated biomarkers of effect (e.g., atherosclerotic changes, markers of inflammation, markers of lipid metabolism) in snus users. There were no significant differences between the biomarkers in snus users and never users of tobacco; however, there were significant differences between snus users and smokers for atherosclerotic changes (with snus users having less severe changes than smokers). Future studies may be needed to determine if biomarkers of effect will be instrumental in comparing potential early health effects associated with different tobacco-containing products to snus.

Toxicological Studies

Swedish snus has been investigated in *in vitro* assays for genotoxicity, cellular proliferation, and epithelial changes in human biopsy samples and cell cultures. The studies support the conclusion that Swedish snus is not genotoxic in mammalian cells and a recent study also reported that Swedish snus is not mutagenic, cytotoxic or clastogenic. There is limited data from a single *in vitro* assay to suggest that snus may inhibit the ability of the oral mucosa to instigate a local immune response; however, the biological relevance of this finding is not known. Three studies examined the effect of Swedish snus on markers of cellular proliferation and differentiation; the results are not entirely consistent (perhaps due to methodological differences). The results suggest that p53 mutations are not frequent in snus-induced lesions. A single study suggests that Swedish snus has effects on the growth of periodontal ligament cells, but the significance of these findings is not clear. The value of these diverse and few *in vitro* studies for evaluating the health effects of Swedish snus is limited.

There are a few oral carcinogenicity studies with Swedish snus using a surgically prepared lip canal model in rats and a dietary study with snus to evaluate gastric cancer in mice. Although the highly invasive lip canal model may not appropriately represent the types of exposure that snus users would experience, Swedish snus was not found to be tumorigenic in the lip canal studies, where the rats were exposed to relatively high doses for a long period of time. Groups such as the Life Sciences Research Office have recommended that new, less invasive, validated animal models be developed that are relevant to smokeless tobacco use in order to evaluate the safety of different products.

Epidemiological Studies

Well-controlled epidemiological evidence indicates that Swedish snus is not associated with oral cancer. Though the studies are mostly consistent showing no association between Swedish snus use and esophageal or stomach cancer, a single recent study did observe increased risks for these cancer sites. Additional research will help resolve this uncertainty. A limited number of epidemiology studies have failed to demonstrate that Swedish snus is a significant risk factor for the following cancers: kidney, bladder, lung, skin cancer, hematopoietic cancers, and all cancers combined. Two studies suggest that Scandinavian smokeless tobacco may be associated with increased risk of pancreatic cancer among specific subgroups of the population. There are inconsistencies between the two studies and the interpretation of the studies has been the topic of much scientific debate. Further research is needed to resolve the relationship between use of Swedish snus and pancreatic cancer.

Studies have reported that the use of Swedish snus is associated with a characteristic type of oral mucosal lesion which is localized to the area where the snus is placed; however, the lesions are reversible following cessation of snus use and there is no clinical evidence to suggest that they transform into malignancies. Limited evidence from uncontrolled descriptive studies suggests that Swedish snus use may also be associated with acute cardiovascular effects such as increased blood pressure and elevated heart rate almost certainly due to nicotine. A single epidemiological study observed an increased risk of death from one specific stroke type among Swedish snus users; this finding has not been replicated in other epidemiological studies.

The literature indicates that use of Swedish snus is not associated with harmful gastrointestinal effects, including peptic ulcer, heartburn, Crohn's disease or ulcerative colitis. One well-conducted analytic epidemiology study found that use of Swedish snus was not associated with increased risk of diabetes. This is in contrast to a single descriptive epidemiologic study of insulin resistance among Swedish snus users that concluded that heavy users of moist snuff have an increased risk of type 2 diabetes. However, this descriptive study, by design, cannot determine true risk, and a single experimental study found no difference in insulin action between snuffers and abstainers. A single study has suggested that heavy use of Swedish snus could be associated with increased risk of metabolic syndrome (MetSy); however, other studies have not observed this outcome, or associations with clinical markers of MetSy, such as insulin resistance or response, so further research is needed to understand whether the association observed in a single epidemiological study is real.

Multiple studies have examined weight (body mass index), weight gain, and waist-to-hip ratios, and the results are mixed, making it difficult to draw firm conclusions.

Conclusion

This comprehensive review of the published scientific literature confirms the lack of serious adverse health effects associated with Swedish snus. The use of Swedish snus is not associated with oral cancer or cancer of any part of the respiratory tract. At this time, the most likely health risks associated with chronic use of Swedish snus appear to be acute, reversible cardiovascular effects probably due to nicotine. Overall, there is very little evidence that current use levels of snus in Sweden are associated with any significant long-term health effects, and ongoing research is hoped to provide additional information to resolve remaining areas of uncertainty. The areas where firm conclusions cannot be drawn include the relationship between Swedish snus use and pancreatic cancer, potential cardiovascular risks, and possible metabolic syndrome or weight gain issues.

1 Introduction

1.1 Background

Snus[1] is a moist tobacco product used orally in Sweden for almost 200 years. It is the smokeless tobacco product (STP) most commonly used in Sweden (Lunell and Lunell 2005). Therefore, much of the past literature refers to snus as 'Swedish moist snuff', 'Swedish snuff' or snuff or oral moist snuff from Sweden.

Snus is an air-cured, finely ground, heat pasteurized tobacco product that is regularly used by approximately one-quarter of Swedish men (Wicklin 2005). The European Smokeless Tobacco Commission (ESTOC) has developed its working definition of snus as, "**an oral smokeless tobacco product traditionally used in Sweden that is manufactured using a tobacco heat-treatment process.**"

Snus is marketed as either loose snuff, or in portion-bag packets (pouches), in a variety of flavors (Andersson et al. 1995; Lunell and Lunell 2005). In contrast to snus, traditional United States (US) STPs are either air- or fire-cured, and not heat-treated during processing and product development. Additional information on the definition of snus is presented in Chapter 2. In this report, the terms snus and Swedish moist snuff are used interchangeably, often retaining the usage from original study reports.

In recent years, most of the major multinational tobacco companies have begun test-marketing their own brands of snus, often under their leading cigarette brand names (Foulds and Furberg 2008). In newer literature, the traditional snus brands are therefore often referred to as 'Swedish snus'. Some researchers have also referred to newer brands that are sold in pouches and which frequently contain lower moisture than common in traditional snus products as "spitfree tobacco" (Hatsukami et al. 2007; Stepanov et al. 2009). More recent publications have also begun to report product brand names, while older literature often lacks such information about the studied products. Since the epidemiological research conducted in Scandinavia is based on use of traditional products, this report focuses on traditional Swedish snus. Therefore, in the following report the term 'snus' and 'Swedish snus' refers to traditional Swedish snus products. The term 'Swedish snus' will only be used for distinction from newer products and any reference made to these new products will be specifically noted. An Appendix to Chapter 2 will discuss what is known on the chemical composition of newer snus products and if and how they differ from traditional products. Furthermore, in this Appendix a distinction from US-type oral moist snuff is made, where available data allowed direct comparison.

In 2007, the International Agency for Research on Cancer (IARC) released a report concluding that smokeless tobacco is a known human carcinogen, causing cancer of the oral cavity and pancreas (IARC 2007). Even recent reports have claimed that STPs, including snus, can cause cancer, heart disease, and serious oral and dental conditions (SCENIHR 2008). However, many of these reviews have inappropriately combined data on all types of smokeless tobacco when attempting to draw conclusions about snus. Because of differences in product chemistry and use patterns, snus should be considered separately. Those scientists who have limited

[1] 'Snus' is the Swedish word for 'snuff'.

their analyses to snus have differentiated the risks from traditional US STPs and have found that risks are generally lower than for these products (e.g., Lee 2007; Lee and Hamling 2009a; Lewin et al. 1998; Rodu and Jansson 2004; Rosenquist et al. 2005; Schildt et al. 1998b; Weitkunat et al. 2007).

Consequently, the purpose of this document is to evaluate the potential health risks associated with the use of snus by performing a comprehensive systematic review of the relevant published scientific, epidemiology, and toxicology data. This analysis is specifically limited to studies that examined snus (which is defined in the Chapter 2 of this report), and not other kinds of smokeless tobacco, though often data regarding other types of smokeless tobacco products are referred to in comparison to data from snus and are presented and discussed, as necessary. Though a quantitative risk assessment using data on the potential adverse health risks from use of snus has not been performed, the intent of this comprehensive review is to present the information from scientific literature that would be necessary to perform such an assessment.

It is also not the intent of this report to present a review of the evidence for Swedish snus as a replacement for cigarette smoking and to discuss its potential role in individual and population tobacco harm reduction. Tobacco harm reduction is the goal of reducing adverse health impacts for smokers who will or can not abstain from using tobacco. Quantifying individual harm reduction would entail a comparative risk assessment of the harm to health from cigarette smoking and use of a potential reduced harm product, for example, snus. The US Family Smoking Prevention and Tobacco Control Act requires the Food and Drug Administration (FDA) to assess and characterize the risks of snus and other potential harm reduction products. Section 911 –Modified Risk Tobacco Products—states that FDA must issue regulations or guidance on the scientific evidence required for assessment and ongoing review of modified risk tobacco products. The guidance is to be used by companies when applying to FDA for modified risk status, and by FDA in determining if a product can be characterized as a modified risk product.

The FDA guidance is to establish minimum standards for scientific studies needed to determine and characterize risk. The guidance must address validated biomarkers, intermediate clinical endpoints, and other feasible outcome measures, as appropriate. In addition, it must establish minimum standards for post market studies that shall include regular and long-term assessments of health outcomes and mortality, intermediate clinical endpoints, consumer perception of harm reduction, and the impact on quitting behavior and new use of tobacco products, as appropriate. The risk-based guidance is to be applied by FDA in determining whether a product will significantly reduce harm and the risk of tobacco-related disease to individual tobacco users, and if it will benefit the health of the population as a whole taking into account both users of tobacco products and persons who do not currently use tobacco products.

1.2 Risk Assessment Process

1.2.1 Risk Assessment

Risk assessment has become a dominant public-policy tool for informing decision–makers and the public about the different policy options for protecting public health and the environment (National Research Council 2009). Risk assessment has been instrumental in fulfilling the missions of many international, national and provincial agencies in evaluating and addressing public health concerns, informing regulatory and technologic decisions, setting priorities for research and funding, and developing approaches for cost-benefit analyses. This approach is particularly well suited for conducting an assessment of potential reduced risk from the various tobacco products; indeed, the Institute of Medicine's 2001 report, *Clearing the Smoke,* presents its discussion of the science base for tobacco harm reduction using the risk assessment paradigm.

Risk assessment is an essential component of regulatory and related types of decision-making. It provides an understanding regarding what public-health and environmental goals can be achieved or have been achieved by specific actions. Whatever the decision context, the goal of risk assessment is to describe the probability that adverse health effects may occur under specified conditions of exposure to an activity or an agent, to describe the uncertainty in the probability estimate, and to describe how risk varies among populations. To be most useful in decision-making, risk assessment would consider the risks associated with existing conditions (that is, the probability of harm under the "take no action" alternative) and the risks that would remain if each of various possible actions were taken to alter conditions. There would also be a need for some commonality in the uncertainty analysis and assumptions that are applied to each of the analyses so that different policy options can be compared and considered for implementation.

Achieving useful results for decision making requires the use of the standard framework for the conduct of risk assessment, which has been adopted by numerous expert committees, regulatory agencies, and public-health institutions around the world. The framework includes three well known analytical steps—hazard identification, dose-response assessment, and exposure assessment—and a fourth step, risk characterization, in which results of the first three steps are integrated to yield information on the probability that the adverse effects described in the hazard identification will occur under the conditions described in the exposure assessment. Uncertainties in the available data identified in the first three steps are also integrated into risk characterization. Several other types of review of human health data are conducted by regulatory and public health institutions, but only those which in some way incorporate all four of the above steps can properly be termed risk assessments.

1.2.2 Hazard Identification

The hazard identification step for tobacco products should consist of a systematic review of the health effects associated with use of the products. Hazard identification typically involves the review of available toxicological studies (*in vitro* and *in vivo*), clinical studies, and epidemiological studies. The evaluation of the available studies involves a critical analysis to determine the appropriateness of the study design, study material, dose levels, mode of administration, animal model or study subjects, evaluated parameters (e.g., endpoints), and

reported results. The strengths and weaknesses of the studies should be summarized to determine the usefulness of the study for developing conclusions about the safety or risks associated with the study material of interest.

1.2.3 Dose-Response Assessment

A dose-response evaluation portion of a risk assessment would provide an evaluation and comparison of the risks associated with the varying levels of STPs. A dose-response analysis typically involves first quantitatively evaluating the responses observed at the administered doses (or measured exposures). A second step is to determine whether the dose-response relationship is linear with no-threshold or whether a threshold dose, where there are no effects below that level, can be identified. Conducting a hazard evaluation and dose-response evaluation for tobacco products is more complex than for "typical" chemicals, because of the complex mixture of components in tobacco as well as ingredients added to the final tobacco product.

1.2.4 Exposure Assessment

An exposure assessment is the process of measuring or estimating the intensity, frequency, and duration of human exposures to an agent (e.g., chemical substance) present in the environment or workplace. An exposure assessment describes the route of exposure, media and amount that is taken into the body, and the duration and frequency of exposure; the number, nature, and types of human populations exposed; and the uncertainties and assumptions used to determine exposures. Exposure assessment is often used to identify feasible prospective control options and to predict the effects of available control technologies on reducing exposure.

For STPs, including snus, exposure assessment involves an understanding of the product(s) used, as the STPs are known to vary in chemical composition, and have varied over time as well. Patterns of use are also known to differ across individual users, increasing the variability in individual exposures to tobacco components. It would be useful to conduct a thorough analysis of the use of exposure biomarkers for comparing exposure to various chemical components in STPs resulting from their use. A properly conducted exposure assessment could result in a systematic evaluation and differentiation of exposure to the putative harmful agents in the various STPs.

1.2.5 Risk Characterization

Once data about the hazard potential and exposures to an agent or chemical substance has been obtained, the associated health risks can then be estimated for individuals or populations. Risk characterization is the estimation of the probable incidence of adverse health effects under various conditions of exposure, including a description of the uncertainties involved in determining the estimates. The scientific robustness and reliability of these risk estimates will depend largely on the quality of the technical analyses conducted in the hazard identification, dose-response assessment, and exposure assessment. The utility of the risk characterization depends greatly on the ways that the health risks are characterized and whether uncertainties are addressed appropriately to ensure the limitations in the risk estimates are adequately understood by decisions-makers.

1.2.6 Uncertainty

Uncertainty refers to a lack of information, incomplete information, or incorrect information. It is important that risk assessments are conducted by incorporating the most appropriate, robust and reliable scientific information available and that any uncertainties and assumptions included in the risk assessment are clearly stated. The lack of adequate scientific information would likely result in uncertainties in determining risk estimates of STP-associated health effects. As applied in a risk assessment or similar scientific evaluation, uncertainty depends on the quantity, quality, and relevance of data and on the reliability and relevance of models and inferences used to fill data gaps. The identification of uncertainties and data gaps will likely prove extremely beneficial in determining the value of new research, or how research strategies can be assessed by considering how much research may contribute to reducing the overall uncertainty in the risk estimate and how reduction in uncertainty leads to different decision options.

1.3 Identification of Published Literature on Snus

To perform a comprehensive review of the scientific literature on the potential health risks associated with the use of snus, literature searches were performed in on-line commercial and governmental data bases, as well as the World Wide Web on a periodic basis over the past eight years. In addition to electronic data base searches, references cited in the relevant literature were examined for other studies potentially missed in the data base searches. The current report incorporates new literature identified through systematic ongoing literature searches current through December 31, 2009 (note: several references included in this report were available as electronic publications in 2009; the citation in this report will therefore reflect a 2010 journal publication date). A detailed description of the literature identification process is described in Appendix I.

References reviewed and included in this report are publications published in the scientific community available through journals or on the World Wide Web. Generally, only publications that report an original scientific study, provide comment on a specific original scientific study, or conduct a systematic review of available literature on a relevant topic are included in this report; general commentaries and opinion pieces are not included in the review. In addition, the report only considers English-language publications, or for non-English language publications, only those with English language abstracts or data tables within the report that are clear or understandable without knowledge of the non-English language.

Note: Throughout this report, the name of the snus product evaluated or tested, as reported by the investigators in the study reports, is included as written, to avoid any potential confusion or misrepresentation.

2 Chemical Properties of Snus

The chemical composition of a specific STP is dependent on the type of tobacco used as well as the distinct steps used to manufacture the end product. A review of the literature on the chemical composition of snus was conducted and the findings are summarized in this chapter. In addition, the manufacturing process for snus is described.

Because the epidemiological research conducted in Scandinavia is based on use of traditional products, i.e., Swedish snus, this chapter focuses only on traditional Swedish snus. However, much of the published literature that reports analyses of the chemical composition of Swedish snus also includes data on US-type oral moist snuff. More recent studies have also investigated newer products that are marketed as 'snus'. While it is well established that the manufacturing process for traditional US-type oral moist snuff is distinctively different from that of traditional Swedish snus, most of the literature lacks sufficient detail to be certain of the production method for newer smokeless tobacco products. To distinguish these products from traditional Swedish snus, Appendix II presents a summary of the scientific literature that contains information on the chemical composition of the new products marketed as snus and discusses if and how they differ from traditional Swedish snus. Furthermore, a distinction of Swedish snus and these new products from US-type oral moist snuff is made, where available data allowed direct comparison. Appendix II also provides tables with detailed results of concentrations of components analyzed in different products (traditional Swedish snus and new products marketed as snus) as reported in the newer literature (2004 to 2009). The more recent literature is more likely to contain brand names of STP samples analyzed in the studies, and this information has been included in the present chapter whenever available.

There are at least 8,089 different components in natural tobacco (Rodgman and Perfetti 2009). The chemical composition of tobacco depends on: (a) the genetic make-up of different tobacco plants; (b) existing environmental conditions (e.g., soil, fertilizer and pesticide use) during plant growth; and (c) the method for processing the tobacco leaves and other plant parts. The processing steps involve drying the tobacco leaves and stems, blending and treating them and the addition of other ingredients to achieve a specific nicotine content, pH, taste, flavor, and aroma (IARC 2007). Consequently, during this processing of the tobacco, the quantitative chemical composition undergoes changes (Brunnemann and Hoffmann 1992).

2.1 Manufacture of Snus

Snus is a particular type of oral moist snuff product traditionally used and manufactured in Sweden. Its production method differs from the US-type oral moist snuff products in that snus is made from mostly air-cured (and sun-cured) tobacco and heat-treated (Figure 2-1). Traditional US-type oral moist snuff is produced from dark fire-cured tobacco and undergoes controlled fermentation (IARC 2007; Rodu and Jansson 2004). These differences in the processing of tobacco are anticipated to impart unique characteristics to the products.

Snus was originally developed in Sweden in the early 1800s, when fermentation of tobacco was replaced by heat treatment to achieve specific flavor characteristics. In 1981, the major manufacturer of snus in Sweden, Swedish Match, established and implemented a new

production technology[2] - modern process techniques that allowed a more controlled production in line with techniques used in the food industry (Swedish Match, personal communication with Dr. Lars Erik Rutquist). As shown in Figure 2-2, the initial step in the manufacturing process involves drying (air- or sun-curing) and blending of the leaves (Foulds et al. 2003, ESTOC 2009)[3]. The tobacco is then ground and sieved and the resulting powder is mixed with water and salt and submitted to a processing program with different temperature phases, in which it is treated with water vapor under continuous stirring (Ramström 2000). This proprietary heat treatment process results in a product that "satisfies the hygienic requirements of the Swedish Food Act" (Swedish Match 2008[4]). It is "effective enough to kill the natural microbial flora of the tobacco to specified residual bacteria limits" (Swedish Match 2010[5]). Since the mixture is low in pH, sodium carbonate is added to adjust the pH with the intent of achieving a pH of 8.5 (Swedish Match, personal communication with Dr. Lars Erik Rutquist). If flavored snus is produced, the flavorings are added at this stage as well. The final product is stored at or below 8°C prior to packaging to slow the normal ageing process and to preserve moisture (Swedish Match, personal communication with Dr. Lars Erik Rutquist). The snus is filled in tea bag-like pouches (mini-portion or standard portion sachets) or loose in tins or boxes (ESTOC 2009[6]). In Sweden, retailers also keep the product refrigerated until sale (Foulds et al. 2003).

[2] Some authors have erroneously reported that the 1981 change in processing was a switch from the fermentation to the heat treatment method (Ramström 2000).
[3] ESTOC. 2009. http://www.estoc.org/about-smokeless-tobacco/production, accessed November 2009.
[4] Swedish Match 2008. http://www.swedishmatch.com/en/Snus-and-health/Our-quality-standard-GothiaTek/, accessed February 2010.
[5] Swedish Match 2010. http://www.swedishmatch.com/en/Snus-and-health/Our-quality-standard-GothiaTek/GothiaTek-standards/, accessed February 2010.
[6] ESTOC. 2009. http://www.estoc.org/about-smokeless-tobacco/production, accessed November 2009.

Figure 2-1. Distinction Between Snus and Other Oral Smokeless Tobacco Products (adapted from Andersson and Axell 1989)

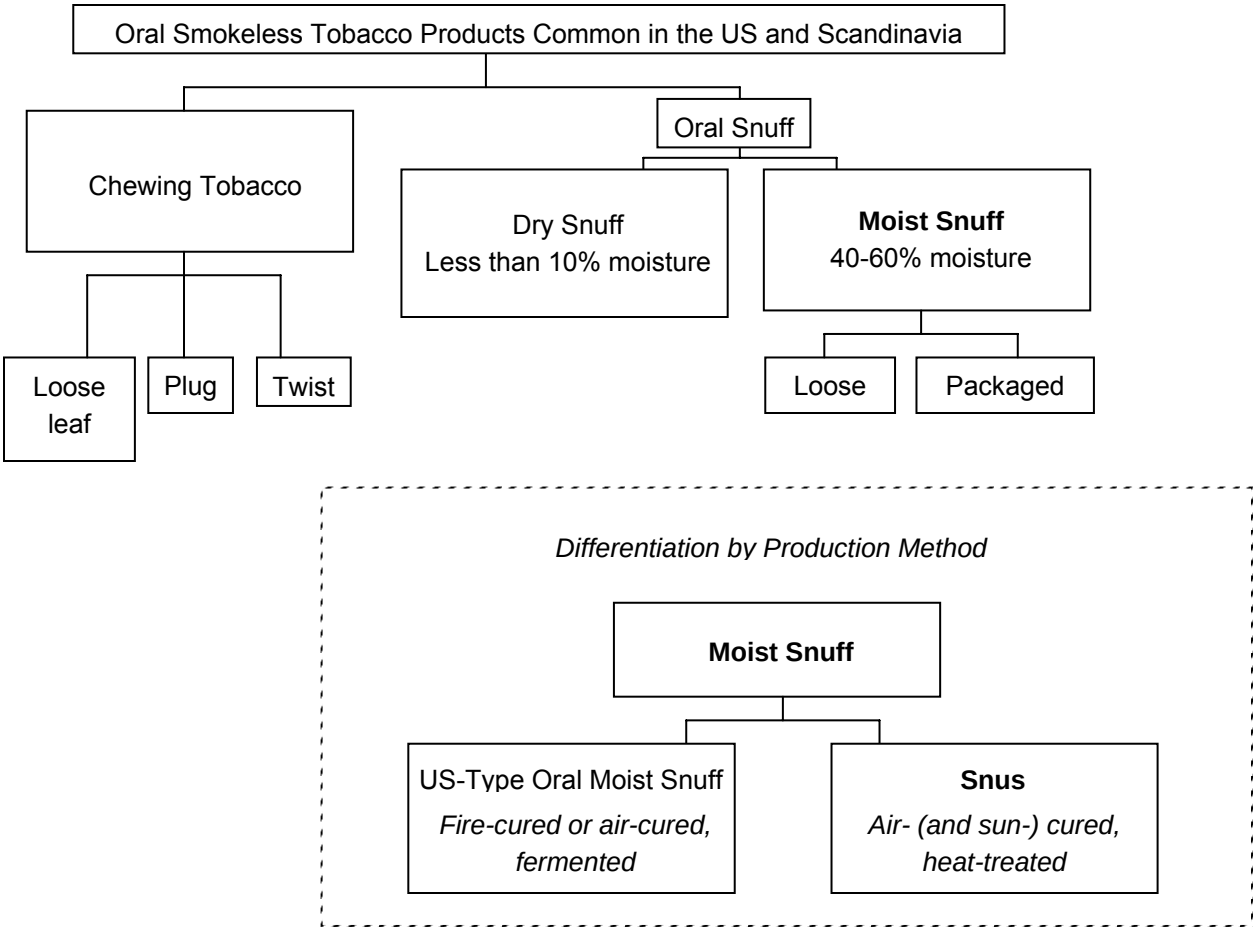
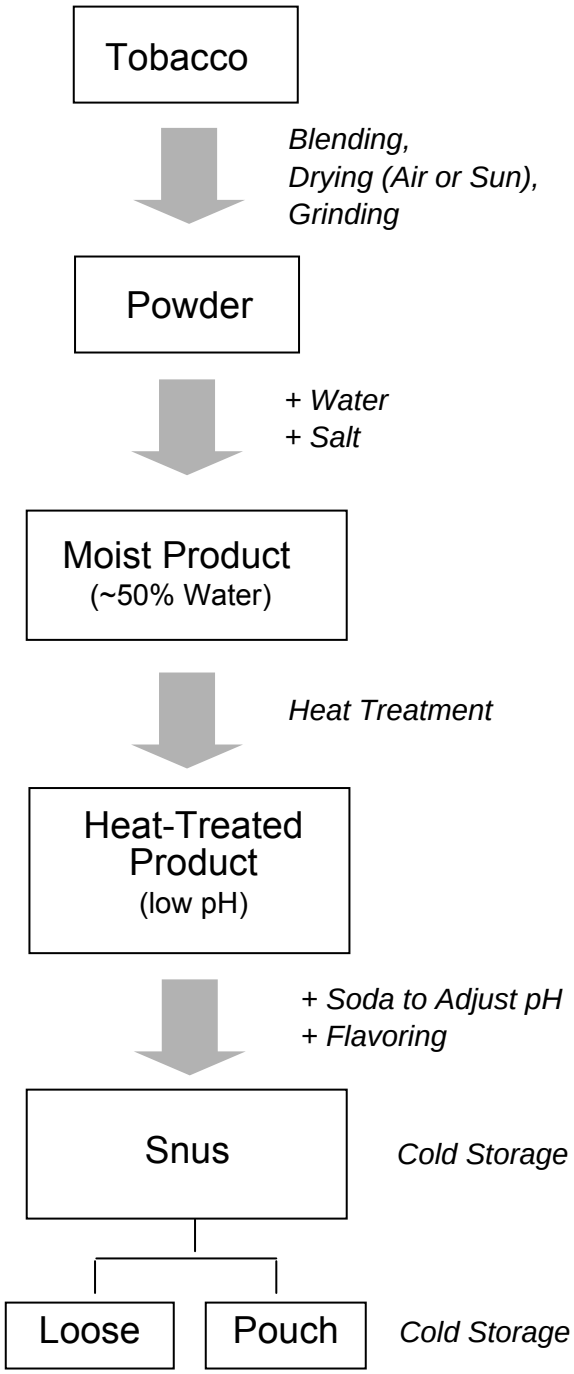


Figure 2-2. Manufacturing Process of Snus (According to ESTOC 2009[7])



[7] ESTOC. 2009. http://www.estoc.org/about-smokeless-tobacco/production, accessed November 2009.

2.2 Chemical Analysis of Snus

2.2.1 Composition of Snus

Table 2-1 summarizes the major ingredients in snus.

Table 2-1: Composition of Snus

Major Ingredients	Percentage of Total Compounds
Tobacco	40-45%
Water	45-60%
Sodium chloride (flavor enhancer and preservative)	1.5-3.5%
Moisturizer (humectants)	1.5-3.5%
Sodium carbonate (pH adjuster and stabilizer)	1.2-2.5%
Flavoring	<1%
Sources: Ramström 2000; Bolinder 1997	

The bulk of the processed tobacco leaf consists of carbohydrates (approximately 50%) and proteins. As with other plants belonging to the Solanacae family (e.g., tomatoes, potatoes, egg plants), other major classes of components in processed tobacco include: alkaloids (with nicotine as major compound in tobacco), terpenes, polyphenols, phytosterols, carboxylic acids, alkanes, aromatic hydrocarbons, aldehydes, ketones, amines, nitriles, N- and O-heterocyclic hydrocarbons, pesticide residues, alkali nitrates, and at least 30 metallic compounds (Brunnemann and Hoffmann 1992).

In addition to tobacco and water, there are various other ingredients contained in snus. Many tobacco formulations also use flavoring agents, such as plant extracts or specific flavoring chemicals. Ascorbic acid and sodium propionate are added as antimicrobial and antifungal agents, respectively. Other preservatives can be potassium sorbate, acetic acid, lactic acid, and citric acid (Swedish Match 2009a)[8]. Sodium chloride is added as taste enhancer and also serves as a preservative. Ammonia, ammonium carbonate, sodium carbonate and calcium carbonate are often used to adjust the pH (IARC 2007; Swedish Match 2009a). Ethanol may serve as a processing aid or solvent (Swedish Match 2009a). Additionally, there are a variety of different humectants (e.g., propylene glycol, glycerol), texturizers (e.g., plant fiber), thickeners (e.g., maltodextrin, gum Arabic), and sweeteners being used to modulate the properties of the final product (Swedish Match 2009a).

While the Tobacco Control Act of 2009 requires tobacco product manufacturers or importers in the US to submit a listing of all ingredients[9], there is currently no US-regulatory requirement to

[8] Swedish Match. 2009a. http://www.swedishmatch.com/en/Our-business/Snuff-and-snus/Ingredients-in-snuff/Composite-list/?intCategoryID=12, accessed November 2009.

[9] "Section 904(a)(1) of the act requires each tobacco product manufacturer or importer, or agent thereof, to submit a listing of all ingredients, including tobacco, substances, compounds, and additives that are added by the manufacturer to the tobacco, paper, filter, or other part of each tobacco product by brand and by quantity in each brand and subbrand. For tobacco products on the market as of June 22, 2009, the list of ingredients must be

list ingredients or additives on the labels of STPs. Some companies that produce snus provide composite lists of their ingredients on their website (e.g., Swedish Match). Only a few published studies have analyzed oral moist snuff for their ingredients. For example, La Voie and colleagues (1989) investigated steam distillates and aqueous extracts of commercial moist snuff for the presence of various "additives". However, snus was not investigated.

In addition to the ingredients added to the tobacco, the composition of snus will be significantly influenced by the extent to which the components in its main ingredient, tobacco, are altered by the manufacturing process.

In Sweden, the manufacturing process of snus must satisfy the hygienic requirements of the Swedish Food Act and all ingredients must comply with the Swedish Food Regulation. Additionally, the major snus-producing company in Sweden, Swedish Match, has developed a quality standard, GothiaTek® that stipulates requirements, among others, on the raw material, manufacturing process, and limits for certain components (see Section 2.2.7) (Swedish Match 2009b)[10].

In the following sections, components commonly present in STPs with potential impact on human health are identified and discussed as available from the scientific literature for traditional Swedish snus. In addition to these data, Appendix II provides detailed results of quantitative analyses of traditional Swedish snus as compared to new products marketed as snus and traditional US-type oral moist snuff where reported in more recent (2004 to 2009) published studies (Tables A II-1 to A II-5).

2.2.2 Sodium Salts

Snus contains sodium salts, i.e., sodium chloride for its flavor enhancing and preservation properties and sodium carbonate for pH adjustment (see Table 2-1). Bolinder (1997) reported the levels of each salt in snus to be up to 2.5%, whereas Ramström (2000) and Lunell and Lunell (2005) reported it to be up to 3.5%.

Sodium Extraction

The latter authors investigated the sodium extraction from different brands of snus (Table 2-2). The sodium chloride content was analyzed in the respective snus samples before and after use, and the extracted amount was determined by calculating the difference between both values. This extracted amount, i.e., the amount of oral intake by the studied snus users, varied between approximately 5 and 10 mg sodium chloride per portion.

submitted by December 22, 2009. For tobacco products not on the market as of June 22, 2009, section 904(c)(1) requires that the list of ingredients be submitted at least 90 days prior to delivery for introduction into interstate commerce. Section 904(c) of the act also requires submission of information whenever any additive, or the quantity of any additive, is changed." (FDA. 2009. Guidance for Industry: Listing of Ingredients in Tobacco Products. http://www.fda.gov/downloads/TobaccoProducts/GuidanceComplianceRegulatoryInformation/UCM192053.pdf, accessed January 2010.)

[10] Swedish Match. 2009b. http://www.swedishmatch.com/en/Snus-and-health/Our-quality-standard-GothiaTek/, accessed November 2009.

Table 2-2: Sodium Chloride Extraction from Used Snus Samples			
Brand	**Portion Size (g)**	**Extracted Amount of Sodium Chloride (mg)**	
		Per Portion (Wet Weight)	**Per Gram (Dry Weight*)**
General	1	8.13 ± 7.33	16
Catch Licorice	1	10.38 ± 6.83	21
Catch Mini	0.5	5.58 ± 4.49	22
Source: Lunell and Lunell (2005); Mean ± standard deviation *Assuming 50% moisture, values for wet weight were converted to dry weight by multiplying by 2.			

The American Heart Association (AHA) recommends limiting salt intake to less than 2,300 mg sodium (5.8 g of sodium chloride) per day to prevent its negative impacts on blood pressure (AHA 2009)[11]. Based on the results presented in Table 2-2, Lunell and Lunell (2005) assumed an average extraction of 7 mg sodium chloride per sachet of snus and concluded that it would take daily consumption of approximately 900 sachets of snus to produce an intake of approximately 6 g of sodium chloride. This is approximately equivalent to the AHA recommended upper limit of daily sodium intake.

2.2.3 Alkaloids

Alkaloids are major components in tobacco leaves (0.5-5%), with nicotine as the predominant compound (85-95% of the total alkaloids), which is discussed separately in the next section. Other major alkaloids are nornicotine, anatabine, anabasine (Ramström 2000). As discussed in section 2.2.7.1 in more detail, nicotine and other alkaloids can result in the formation of tobacco-specific nitrosamines (TSNA). There are also some indications that nornicotine may accumulate in the brain and contribute to the addiction associated with tobacco use (Crooks and Dwoskin 1997; Crooks et al. 1995; Bardo et al. 1999; all as cited in Stepanov et al. 2008a).

Concentrations of tobacco alkaloids other than nicotine are not frequently reported in the literature. In a recent study of new and traditional smokeless tobacco products, Stepanov and colleagues (Stepanov et al. 2008a) also conducted analyses on one traditional Swedish snus brand (*General*). Nornicotine, anatabine, and anabasine concentrations were measured to be 0.223, 0.367, and 0.072 mg/g dry weight, respectively (Table A II-1a in Appendix II). Expressed as percentage of the total nicotine content, the levels were 1.3%, 2.2%, and 0.4%, respectively. In a study that included analysis of three brands of snuff imported from Sweden on the market in 1989-1991, the research group of Hoffmann and Brunnemann reported nornicotine levels to be between 0.04 and 0.06% (0.4-0.6 mg/g) of the dry weight of the products. Total alkaloid levels ranged between 1.24 and 1.41% and included nicotine, nornicotine, mysomine, anatabine, anabasine, 2,3'-dipyridyl, and cotinine (Hoffmann et al. 1991a).

[11] American Heart Association (AHA). 2009. http://www.americanheart.org/presenter.jhtml?identifier=4708, accessed November 2009.

2.2.4 Nicotine, Free Nicotine, pH and Moisture

Nicotine is considered to be a major addictive component in STPs and the nicotine delivery (as described below) of a product is a major determinant of consumer acceptance (Stepanov et al. 2008a). The total nicotine content in different STPs varies, depending on various factors, including the kind of tobacco used (Ramström 2000). The actual nicotine dose taken up (delivered) from a tobacco product is influenced by the level of non-ionized nicotine, 'free nicotine', which is absorbed rapidly through the mucosal membrane (Armitage and Turner 1970). The amount of non-ionized (unprotonated) nicotine is dependent on the pH of the product. At acidic pH, nicotine in STPs is present in protonated form as a salt with organic acids. A more basic pH results in a higher amount of free nicotine base. In snuff at a pH of 7, approximately 9% of nicotine is present in its free base form, at pH 8 approximately 50% (as reviewed in Hoffmann and Djordjevic 1997). Additional product characteristics such as packaging and moisture content appeared also to be correlated with concentrations of non-ionized nicotine as studied in US-type moist snuff brands (Richter et al. 2008). Because storage conditions have an influence on moisture levels in snus and aging of snus also results in a decrease in pH (Swedish Match 2009)[12], they may influence free nicotine content and thus nicotine uptake. Therefore, aged and inappropriately stored snus may deliver less nicotine than snus freshly manufactured or snus stored under cooling conditions.

Three newer studies have analyzed total nicotine content in a few different traditional snus brands (*General* and *Catch*) and reported nicotine concentrations in the range between approximately 14 and 18 mg/g dry weight (Table A II-1a in Appendix II) (Lunell and Lunell 2005; McNeill et al. 2006). Investigators of the work group of Brunnemann and Hoffmann reported the nicotine level in one brand of Swedish snus (*Ettan*) purchased in 2000 to be 2.01% (20.1 mg/g) (Brunnemann et al. 2001). In preceding studies, the same work group analyzed oral snuff from the US and Sweden between 1980 and 1990 (Djordjevic et al. 1993). Nicotine levels in three popular Swedish snuff brands in 1990 per dry weight of tobacco were between 1.13 and 1.25% (11.3-12.5 mg/g) and in 1980 between 1.13 and 1.81% (11.3-18.1 mg/g), respectively. In a Swedish study, Andersson and colleagues (1994) reported nicotine concentrations between 8.6 and 9.0 mg/g in three different brands of loose snus and between 9.0 and 10.3 mg/g in four different brands of portion-bag snus. These authors did not specify if the values were given as per wet or dry weight

Free nicotine of traditional snus ("general [sic] pouch", *General*) was determined in two newer studies to be 6.3 and 7.69 mg/g dry weight, respectively (Table A II-1a in Appendix II) (McNeill et al. 2006; Stepanov et al. 2008a).

The target value of the pH in traditional snus is close to 8.5 (Swedish Match 2009)[12]. It has been reported that the typical pH of snus is in the range of 7.8 and 8.5 (Anderson et al. 1994, as cited in Lunell and Lunell 2005). Consistent with this, the pH of traditional snus as measured in studies from 2005 through 2008 ranged from 7.86 to 8.5 (Table A II-1a in Appendix II) (Lunell and Lunell 2005; McNeill et al. 2006; Stepanov et al. 2008a). Anderson and colleagues (Andersson et al. 1994) measured values ranging from pH 7.9 to 8.2 and from pH 8.5 to 8.6 in

[12] Swedish Match. 2009. http://www.swedishmatch.com/en/Snus-and-health/Snus-nicotine-and-nicotine-addiction/, accessed March 2010.

samples of portion-bag and loose snus, respectively. In their analysis of three Swedish snuff brands on the market around 1990, Hoffmann and colleagues (1991a) measured values between pH 7.67 and 7.94. The same investigators reported values between pH 7.3 and 8.68 in three brands of Swedish moist snuff purchased between 1984 and 1985 (Brunnemann et al. 1985).

Moisture levels in traditional snus are approximately 50% (Table 2.1). Levels as measured in two newer studies were 45.84 and 48.5% (weight/weight) moisture ("general [sic] pouch", *General*; Table A II-1a in Appendix II) (McNeill et al. 2006; Stepanov et al. 2008a). The influence of storage temperature on moisture levels was confirmed in a study conducted for the Massachusetts Department of Public Health (MDPH). Brunnemann and colleagues (2001) compared the effect of storage conditions on several parameters in different US and Swedish moist snuff products, including moisture levels. In this study, 6 months of storage at room temperature decreased the moisture in a sample of traditional Swedish snus (*Ettan*) from approximately 56% to less than 30%. In analyses of samples from 1990, moisture levels ranged from 46.6 to 54.2%, in samples from 1984/85 levels were between 50.3 and 53.3% (Brunnemann et al. 1985; Hoffmann et al. 1991b). A study by IARC researchers reported moisture levels in the range of 21 to 55% in 12 samples of snuff from Sweden (Ohshima et al. 1985). Since this study did not specify the snuff investigated, it is possible that dry snuff was included, thus providing an explanation for the large range in moisture content. As stated above, traditional Swedish snus contains approximately 50% moisture and levels well below this could indicate non-traditional snus products or the influence of aging processes.

While most study authors report to have stored STP samples under refrigeration upon analysis, it is unclear if variations in pH and moisture levels are due to specific product characteristics or also influenced by aging processes due to insufficient storage of the samples analyzed.

Nicotine Extraction

A study by Lunell and Lunell (2005) investigated nicotine extraction and uptake from different snus brands, including the traditional snus products *General* and *Catch (*both *Licorice* and *Mini* versions*)* by 12 regular snus users. The extractable nicotine was determined by the difference in nicotine content between used and unused snus samples. The mean extraction was approximately 1.55 to 2.74 mg per portion for the different brands (approximately 3 to 8 mg/g dry weight[13]), resulting in 22 to 44% extraction of the total nicotine content.

In a study with 45 habitual snus users, Andersson and colleagues (Andersson et al. 1994) reported the degree of nicotine extraction from portion-bag snus and loose snus to be 37.4 ± 17.6% and 49.1 ± 17.2% (average ± standard deviation), respectively.

(See Section 3.4. for details on biomarkers measured in these studies).

[13] Values given were on portion basis and had to be adjusted to gram considering portion sizes (*General*: 2.74 mg nicotine/g; *Catch Licorice*: 1.55 mg nicotine/g; *Catch Mini*: 2.00 mg nicotine/0.5 g) and dry weight assuming 50% moisture (value multiplied by 2)).

2.2.5 Nitrate and Nitrite

Nitrate is another endogenous tobacco component and nitrate values alone allow differentiation of STPs into three separate classes with one of them consisting of moist snuffs, including snus, and the other two, low moisture snuff and other products (Rickert et al. 2009). Air-cured tobaccos tend to be high in nitrate (Rickert et al. 2009). The nitrate content of tobacco has potential health implications, because during curing and fermentation processes, bacteria-induced reactions reduce nitrate to nitrite. Nitrite can subsequently nitrosate tobacco alkaloids to form TSNAs (Ramström 2000). Nitrate can also be converted to nitrite in saliva (Marletta 1988, as cited in Stepanov et al. 2008a). The main concerns of nitrite exposure are methemoglobin formation and formation of nitrosamines from tobacco alkaloids or dietary amines (Stepanov et al. 2008a).

The nitrate and nitrite concentrations measured in traditional snus (*General*) by Stepanov and colleagues (2008a) were 4.62 mg/g and 0.004 mg/g (4 μg/g) dry weight, respectively (Table A II-1b in Appendix II). In another study that measured nitrite content in "general [sic] pouch", the nitrite concentrations were below the detection limit of 0.2 μg/g dry weight (McNeill et al. 2006). The limit for nitrite set by the GothiaTek® Standard is 7 μg/g dry weight (see Table 2-3). Nitrate levels reported in an early study by Brunnemann and colleagues (1985) as measured in 3 brands of Swedish moist snuff purchased in 1984/85 were between 2.13 and 2.62% (21.3-26.2 mg/g). This limited data seems to indicate a decline in nitrate content in snus since 1985.

2.2.6 Other Components

In addition to nitrate and nitrite, Stepanov and colleagues (2008a) also investigated other anions, such as chloride, formate, sulfate, and phosphate in different STPs. Chloride, the anion likely stemming from the addition of sodium chloride as an ingredient, was quantified in *General* snus to be present at 75.7 mg/g dry weight[14] (Stepanov et al. 2008a). The Canadian investigators Rickert and colleagues determined ammonia and propylene glycol (a humectant) concentrations in STPs on the Canadian market, however, traditional Swedish snus was not analyzed (Rickert et al. 2009) (For details see Table A II-1b in Appendix II).

2.2.7 Trace-Level Components

According to IARC, 28 known carcinogens of different compound classes have been identified in STPs to date (Brunnemann and Hoffmann 1992; IARC 2007). Among those, the most frequently quantified compounds are non-volatile alkaloid-derived TSNAs due to their abundance and carcinogenic potential as demonstrated in laboratory animals (IARC 2007; Stepanov et al. 2008a). Other carcinogens, as stated by IARC, include *N*-nitrosoamino acids, volatile *N*-nitrosamines, volatile aldehydes, polycyclic aromatic hydrocarbons (PAHs), lactones, hydrazine, urethane, metals, and radionuclides (IARC 2007). Most studies that have analyzed STPs have focused on a limited range of analytes and thus, except for TSNAs, there is little to

[14] *General* snus with 75.7 mg chloride per g dry weight and ~50% moisture contains approximately 38 mg chloride per portion. With a molecular weight MW 35 g/mol for chloride, a 1-g portion of *General* snus contains approximately 1.08 mmol chloride. Assuming all chloride is present as sodium salt (NaCl MW = 58 g/mol), a total amount of 62 mg sodium chloride would be present in a 1-g portion of *General* snus, equaling 6.2% of the portion. This is in agreement with the ingredient list for snus provided by Swedish Match (6.7% quantity not exceeded).

no quantitative information on many of these compounds (Rickert et al. 2009; Stepanov et al. 2008a).

One company, Swedish Match, has developed limits for certain compounds in STPs that must not be exceeded (GothiaTek® Standard Limits, see Table 2-3).

Table 2-3: GothiaTek® Standard Limits

Component	Limit (µg/g dry weight)
Nitrite	7
Tobacco-specific nitrosamines*	10
N-nitrosodimethylamine ,	0.01
Benzo[a]pyrene	0.02
Cadmium	1.0
Lead	2.0
Arsenic	0.5
Nickel	4.5
Chromium	3.0
Pesticides	According to Swedish Match pesticide policy

Source: http://www.swedishmatch.com/en/Snus-and-health/Our-quality-standard-GothiaTek/GothiaTek-standards/, accessed September, 2009
* Total TSNAs

2.2.7.1 *N*-Nitroso Compounds

STPs contain three major types of *N*-nitroso compounds: non-volatile TSNAs, non-volatile *N*-nitrosamino acids, and volatile *N*-nitrosamines (VNAs). Of these, IARC considers the first two groups to be the major and most abundant group of carcinogens in tobacco (IARC 2007). TSNAs are the most frequently analyzed and reported nitroso-compounds in STPs, but there is only limited data on current concentrations of *N*-nitrosamino acids and VNAs in STPs.

Tobacco-Specific *N*-Nitrosamines

TSNAs are present in fresh green tobacco leaves, but they are primarily formed from their alkaloid precursors and nitrite/nitrate during the production steps of tobacco curing, fermentation of the processed tobacco, as well as ageing of the processed and packaged tobacco. These production processes along with agronomic practices such as fertilizer use and irrigation are therefore important determinants of TSNA concentrations in the final products (Hoffmann and Hecht 1990; IARC 2007).

The main underlying reaction leading to TSNA formation is nitrosation of tobacco alkaloids with nitrite. Bacterial formation of nitrite from nitrate is an important step for this reaction. During the early stages of tobacco processing, *N*-nitrosonornicotine (NNN), *N*-nitrosoanabasine (NAB), and

N-nitrosoanatabine (NAT) yield from the reaction of nornicotine, anabasine, and anatabine, respectively, with nitrite. During the later stages of tobacco curing and fermentation of the processed tobacco, reaction of nicotine with nitrite can result in the formation of both NNN as well as 4-[methylnitrosamino]-1-[3-pyridyl]-1-butanone (NNK) (IARC 2007; Ramström 2000). Since snus is produced with a heat-treatment instead of a fermentation step, it is expected that the resulting elimination or reduction of bacteria leads to TSNA concentrations that are lower than those in fermented STPs.

NNK and NNN are considered to be the most important TSNAs because of their abundance and tumorigenic potency in laboratory animals (Hecht and Hoffmann 1988; Hecht and Hoffmann 1989; Stepanov et al. 2008a). Both have been consistently shown to be carcinogens in rodents, with NNK having higher activity (Hecht 1998). IARC has classified NNK and NNN as "*carcinogenic to humans (Group 1)*" (IARC 2007). While there was "*inadequate evidence* in humans for the carcinogenicity of tobacco-specific *N*-nitrosamines", for their overall evaluation, IARC took mechanistic evidence into consideration[15].

NAB was a weak esophageal carcinogen in rats and NAT showed no tumorigenic activity in rats (Hecht 1998; Österdahl et al. 2004). IARC considered both, NAB and NAT, to be "*not classifiable as to its* [their] *carcinogenicity to humans (Group 3)*" (IARC 2007).

Analyses conducted in the early 1980s showed that TSNA levels in Swedish moist snuff products ranged from 7 to 17 ppm (µg/g)[16] (Rodu and Jansson 2004).

Since that time, TSNA concentrations in moist snuff on the Swedish market have declined significantly parallel to the improvements in manufacturing processes introduced in 1981 by Swedish Match (described in Section 2.1). The company also "uses tobacco with a low nitrate content, which itself reduces TSNA levels" (IARC 2007). The tobacco is "processed in a heated closed system that resembles pasteurization of milk" (IARC 2007). These changes are intended to eliminate any "bacteria that may be indirectly responsible for the formation of the nitrosamines" (Gothia 2004, as cited in IARC 2007). It is therefore thought to be an important step to reduce TSNA formation.

The elimination of bacteria in snus and their influence on TSNA formation was indirectly confirmed by a study conducted for the Massachusetts Department for Public Health (MDPH) by Brunnemann and colleagues (2001). This study investigated the aging of oral moist snuff (sold in Massachusetts in 2000) due to storing conditions and the effect on TSNA yield in the products. While 6 months of storage at room temperature did not have any significant effect on TSNA concentrations in a sample of traditional Swedish snus (*Ettan*), it led to an increase in TSNA levels in two leading US snuff brands between 30 and 130%. While some authors have

[15] IARC took the following mechanistic evidence into consideration: NNK and NNN "are the most abundant strong carcinogens in smokeless tobacco; uptake and metabolic activation in smokeless tobacco users have been clearly observed. In rats, combined application of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK] and N′-nitrosonornicotine [NNN] induced oral tumours consistent with their induction by smokeless tobacco. One of the mechanisms of carcinogenicity is cytochrome P450-mediated α-hydroxylation, which leads to the formation of DNA and haemoglobin adducts that are commonly detected in users of tobacco." (IARC 2007)

[16] It was not specified if these values are based on wet or dry weight.

reported that refrigeration of the finished snus product was introduced to prevent further bacterial growth and thus TSNA formation, the MDPH study confirms that storing temperature has influence only on TSNA formation in fermented STPs, but not in snus (Brunnemann et al. 2001). As described in Section 2.1, cool storage of snus was introduced to prevent loss of moisture and aging of the final product.

Based on the low TSNA concentrations in the snus sample (*Ettan*; total TSNA 2.8 μg/g dry weight) compared to those detected in five brands of traditional US-type moist snuff (range, 7.5-127.9 μg/g dry weight) in the study by Brunnemann et al. (2001), the MDPH intended "to request that manufacturers who sell oral snuff in Massachusetts adopt new technologies to reduce TSNA content to the lowest possible level but at a minimum below 10 μg/g" (Connolly 2001)[17].

The decrease of TSNA concentrations in snus between the 1980s to 2000s was demonstrated by Österdahl and colleagues (2004) from the Swedish National Food Administration in their analysis of published studies from 1983 to 1992 and their own results from snus on the market in 2001 and 2002.

These investigators analyzed TSNA concentrations in 14 snus samples on the Swedish market in 2001 (all but one produced by Swedish Match) and 2002 (seven Swedish Match brands and 20 brands from smaller manufacturers) (Österdahl et al. 2004). The mean total (NNK, NNN, NAB, and NAT) TSNA content was 1.1 μg/g wet weight in 2001 and 1.0 μg/g wet weight in 2002 (approximately 2.2 and 2.0 μg/g dry weight, respectively, assuming 50% moisture content[18]). Comparing these values to a mean total TSNA concentration of 7.3 μg/g wet weight (approximately 14.6 μg/g dry weight) measured in 16 brands of Swedish moist snuff in 1983, Österdahl and colleagues (2004) concluded that TSNA concentrations in moist snuff on the Swedish market have declined by about 85% since the 1980s.

Analyses of samples of a few brands of traditional snus (including *General*) on the market since 2003 conducted by different groups of investigators yielded similar total TSNA levels in the range of 2.0 to 3.1 μg/g reported as per dry weight (Rodu and Jansson 2004; Stepanov et al. 2008a). In two additional studies, Stepanov and colleagues (Hatsukami et al. 2007; Stepanov et al. 2006) reported the total TSNA concentration in traditional snus (*General*) as 2.0 μg/g per wet weight (approximately 4 μg/g dry weight). Researchers in the UK reported the total TSNA content (NNK, NNN, and NAB only) in "snus (general [sic] pouch) from Sweden" as 0.478 μg/g dry weight (McNeill et al. 2006). (For details see Table A II-2 in Appendix II)

With respect to concentrations of the individual TSNAs, mean NNK and NNN concentrations in 2002 as reported in the review by Österdahl and colleagues (2004) were decreased from 0.80 μg/g wet weight in 1983 to 0.19 μg/g wet weight (approximately from 1.6 to 0.38 μg/g dry weight) and from 3.8 μg/g wet weight in 1983 to 0.49 μg/g wet weight (approximately from 7.6 to 0.98 μg/g dry weight), respectively. Combined NNK and NNN

[17] Massachusetts Department for Public Health (MDPH). 2001. http://fl1.findlaw.com/news.findlaw.com/hdocs/docs/tobacco/masnuffsstudy.pdf; accessed November 2009.

[18] In this report, 50% moisture is assumed for traditional Swedish snus, although Österdahl et al. (2004) stated that "the moisture content in snus is about 55%".

concentrations in traditional Swedish snus have thus declined from a mean of 9.2 μg/g dry weight in 1983 to a mean of 1.36 μg/g dry weight in 2002. By comparison, in the newer studies, NNK and NNN concentrations in traditional Swedish snus (including *General*) were in the range of 0.3 to 0.5 μg/g dry weight and 1.0 to 1.66 μg/g dry weight, respectively (Rodu and Jansson 2004; Stepanov et al. 2008a). In their earlier publications, Stepanov and colleagues reported NNK and NNN concentrations in snus (*General*) as 0.18 and 0.98 μg/g wet weight, respectively (approximately 0.36 and 1.96 μg/g dry weight, respectively) (Hatsukami et al. 2007; Stepanov et al. 2006). Thus, combined NNK and NNN concentrations in traditional Swedish snus as measured in studies by different investigators after 2003 range between 1.4 and 2.32 μg/g dry weight. These differences might be due to interlaboratory variability in analytical methods.

Mean NAB and NAT concentrations reported by Österdahl and colleagues (2004), were 0.03 and 0.32 μg/g wet weight, respectively, in snus in 2002 (approximately 0.06 and 0.62 μg/g dry weight, respectively). These levels were significantly decreased compared to those detected in snus samples in 1983 (mean NAB and NAT were 0.17 and 2.5 μg/g wet weight, respectively, translating to approximately 0.34 and 5 μg/g dry weight, respectively). By comparison, NAB and NAT contents detected ranged from 0.008 to 0.1 μg/g dry weight and 0.6 to 0.969 μg/g dry weight, respectively (Rodu and Jansson 2004; Stepanov et al. 2008a). In their earlier publications, Stepanov and colleagues reported NAB and NAT concentrations as 0.06 and 0.79 μg/g wet weight, respectively (approximately 0.12 and 1.58 μg/g dry weight, respectively) (Hatsukami et al. 2007; Stepanov et al. 2006).

In summary, the total as well as individual TSNA concentrations in traditional Swedish snus decreased considerably from the 1980s to 2002 and there does not seem to have been an additional significant change in these levels between 2002 to the present. Total TSNA concentrations reported in the limited number of available more recent studies that investigated traditional Swedish snus are below the GothiaTek® Standard limit of 10 μg/g dry weight. Recently, the WHO Study Group on Tobacco Product Regulation in their Report on the Scientific Basis of Tobacco Product Regulation has recommended that "the combined concentration of NNN plus NNK in smokeless tobacco should be limited to 2 μg/g dry weight of tobacco" (WHO 2009). As can be seen the mean combined concentrations in 2002 in traditional Swedish snus were below this value and analytical results from newer studies were below or close to the 2 μg/g dry weight value.

TSNA Extraction

One study conducted by the Swedish National Food Administration investigated the extraction of TSNAs from Swedish moist snuff and measured TSNA levels in the saliva of 4 habitual male snuff dippers during and shortly after snuff use (Österdahl and Slorach 1988). Three of the investigated snuff dippers used snuff pouches of which the total TSNA content (NNK, NNN, and NAT) was determined to be 9.2 μg/g[19]. After use the TSNA content was determined again and the extracted amount of total TSNAs in two samples measured was between 0.3 and 0.9 μg/g, which was mainly due to decreases in NNK and NNN content. The TSNA content in one used sample was slightly increased by 0.3 μg/g, in spite of the fact that high TSNA concentrations

[19] It was not specified if this value was given per wet or dry weight.

were found in the saliva of the respective snuff dipper. The authors noted that this could be due to *in vivo* formation of TSNA in the saliva.

In another study by Swedish investigators, the TSNA extraction among other parameters was compared between 23 portion-bag snus users and 22 loose snus users. The latter extracted more TSNA from snus (125.3 ±115.5 μg/24 hours) than portion-bag users (44.5 ± 25.7 μg/24 hours). The total TSNA content before use was between 3.7 and 6.0 μg/g for snus in portion-bags and between 6.1 and 7.7 μg/g in loose snus. The degree of TSNA extraction was measured to be 55.7 ± 20.5% and 64.1 ± 16.4% from portion-bag snus and from loose snus, respectively (Andersson et al. 1994).

(See Section 3.4. for details on biomarkers measured in these studies).

N-Nitrosamino Acids

Similar to the alkaloids, amino acids and proteins with secondary amino groups present in tobacco can undergo *N*-nitrosation to non-volatile *N*-nitrosamino acids (IARC 2007). Out of 11 identified *N*-nitrosamino acids, four have been established as carcinogens in experimental animals, i.e., *N*-nitrososarcosine (NSAR) (classified in 1987 by IARC as Group 2B carcinogen[20]), *N*-nitrosoazetidine-4-carboxylic acid (NAzCA), 3-(methylnitrosamino)propionic acid (MNPA), 4-(methylnitrosamino)butyric acid (MNBA) (Brunnemann and Hoffmann 1992; IARC 2007). The noncarcinogenic *N*-nitrosoproline (NPRO) was reported in several tobacco products at levels that correlate well with the levels of TSNAs and was therefore proposed as an indicator of *N*-nitrosation of amines in smokeless tobacco products (Brunnemann et al. 1983, as cited in Ohshima et al. 1985).

NAzCA was reported to be contained in heavily cured/fermented tobaccos, but was not detected in five samples of Swedish moist snuff commercially available 1987/88 (Tricker and Preussmann 1989; Tricker and Preussmann 1991). In the same study and samples, the authors detected NSAR concentrations between 0.008 and 0.031 μg/g[21]. In another study, NSAR concentrations in three brands of moist snuff from Sweden on the market 1989/90 ranged between 0.030 and 0.680 μg/g dry weight (Hoffmann et al. 1991a).

MNPA and MNBA[22] were first identified by Ohshima et al. (1985) and quantified in various tobacco products. The concentrations in snuff from Sweden with approximately 50% moisture ranged from 2.92 to 4.4 μg/g dry weight and from not detected to 0.24 μg/g dry weight, respectively. In the same samples, the investigators also measured NPRO to be between 6.21 and 29.5 μg/g dry weight. Brunnemann and colleagues (1985) detected NPRO concentrations in the range of 3.12 to 8.21 μg/g dry weight in three brands of moist snuff from Sweden on the market 1984 and 1985. In their study, Tricker and Preussmann (1989; 1991) determined MNPA and MNBA concentrations in five samples of Swedish moist snuff to range from 1.04 and

[20] Group 1: The agent is carcinogenic to humans. Group 2A: The agent is probably carcinogenic to humans. Group 2B: The agent is possibly carcinogenic to humans.
(IARC. 2009. http://monographs.iarc.fr/ENG/Classification/index.php, accessed February 2010)

[21] The authors did not specify if concentrations were given as per dry weight or wet weight.

[22] MNPA and MNBA are also called NMPA and NMBA (misspelled in Tricker and Preussmann (1991) as NPMA and NBMA).

1.82 μg/g (mean 1.34 μg/g) and from 0.053 and 0.094 μg/g (mean 0.07 μg/g), respectively. NPRO concentrations were between 0.63 and 1.82 μg/g (mean 1.10 μg/g). In the study by Hoffmann and colleagues that analyzed three Swedish brands of moist snuff, concentrations of MNPA and MNBA were between 3.10 and 3.28 μg/g dry weight and 0.19 and 0.23 μg/g dry weight, respectively (Hoffmann et al. 1991a). These authors detected NPRO concentrations between 4.91 and 8.33 μg/g dry weight.

No more recent studies were identified, and from the limited amount of data, a trend over time could not be identified. *N*-nitrosamino acids do not appear to have been included in analytical studies of STPs since the 1990s.

Volatile *N*-Nitrosamines

Volatile amines naturally present in tobacco can undergo nitrosation forming a variety of volatile *N*-nitrosamines (Tricker and Preussmann 1989). VNAs frequently measured in STPs are *N*-nitrosodimethylamine (NDMA), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosopiperidine (NPIP). These VNAs have also been detected in a variety of foods, e.g. meat products, fish, cheese, beer, tea, coffee, and chocolate (Österdahl 1991). *N*-nitrosomorpholine (NMOR) is thought to be present due to contamination with morpholine either from additives or from diffusion of containers coated with morpholine-containing wax (as reviewed in IARC 2007). These VNAs were carcinogenic in laboratory animals and classified in 1987 by IARC as Group 2A[23] (NDMA) and 2B (NPYR, NPIP, and NMOR) carcinogens (IARC 2009[24]).

In a review for the IARC in 1991, Österdahl from the Swedish National Food Administration stated that levels of VNA in Swedish snuff decreased considerably since 1979 (Österdahl 1991). This investigator reported mean concentrations of NDMA and NPYR in 67 samples of snuff on the Swedish market 1983-86 to be 0.7 and 5.1 ng/g wet weight, respectively (approximately 1.4 and 10.2 ng/g dry weight, respectively, assuming 50% moisture content). Both, NPIP and NMOR were only detected at trace levels. A study by Brunnemann and colleagues (1985) in STPs on the market in 1984/85 reported concentrations of NDMA below the detection limit of 0.2 ng/g dry weight in three brands of moist snuff from Sweden. Concentrations of NPYR and NMOR ranged between 12.2 and 22.1 ng/g dry weight and from below the detection limit to 9.1 ng/g dry weight, respectively. A study by investigators from the German Cancer Research Center, that analyzed *N*-nitroso compounds in STPs commercially available 1987/88, detected NDMA concentrations of 1.0 to 2.5 ng/g (mean 1.5 ng/g) in five samples of Swedish moist snuff (Tricker and Preussmann 1989; 1991). NPYR and NMOR concentrations ranged from 4.5 to 6.0 ng/g and from non-detectable up to 1.0 ng/g, respectively. NPIP was not detected. In a study that investigated *N*-nitroso compounds in different snuff brands, the work group of Brunnemann and Hoffmann also analyzed three brands of moist snuff from Sweden on the market in 1989/90 and detected NDMA and NPYR concentrations in the range of 51 and 63 ng/g dry weight and below the detection limit of 0.01 and 155 ng/g dry weight, respectively

[23] Group 1: The agent is carcinogenic to humans. Group 2A: The agent is probably carcinogenic to humans. Group 2B: The agent is possibly carcinogenic to humans.
(IARC. 2009. http://monographs.iarc.fr/ENG/Classification/index.php, accessed February 2010)

[24] IARC 2009. http://monographs.iarc.fr/ENG/Classification/Listagentsalphorder.pdf, accessed February 2010.

(Hoffmann et al. 1991a; Hoffmann et al. 1991b). The authors did no comment on the discrepancy in these concentrations compared to their earlier study.

As seen for *N*-nitrosamino acids, more recent studies did not focus on these components in STPs and only limited data on their presence in snus is available. Only NDMA has been mentioned recently. One study by McNeill and colleagues (2006) investigated oral STPs on the market in the UK and measured NDMA as a marker for VNAs. The concentration in snus ("general [sic] pouch") was below the detection limit of 5 ng/g dry weight. The GothiaTek® standard limit for NDMA is set to 10 ng/g dry weight (Table 2-3).

N-Nitrosodiethanolamine

N-Nitrosodiethanolamine (NDELA), a non-volatile nitrosamine, is formed from diethanolamine, a residual contaminant in tobacco, but concentrations have decreased with the gradual agronomic reduction of maleic hydrazide-diethanolamine as a sucker growth-controlling agent (IARC 2007). This reduction is reflected in analytical results from Swedish moist snuff products in studies from 1982, 1985 and 1991. While studies by Brunnemann and colleagues (1982; 1985) showed concentrations of NDELA in several Swedish moist snuff brands on the market in 1981 and 1984/85 to be in the range of 225 to 390 and 230 to 300 ng/g dry weight, respectively, a later study by Tricker and Preussmann (1991) detected a mean concentration of 19 ng/g (range 8-31 ng/g) NDELA in five samples of Swedish moist snuff. The latter authors did not specify if the values were based on dry or wet weight of the tobacco product. More recent publications that presented NDELA concentrations in snus were not identified.

2.2.7.2 Polycyclic Aromatic Hydrocarbons

PAHs are formed during the incomplete burning of organic substances. PAHs detected in STPs originate primarily from exposure of the tobacco leaves to polluted air (IARC 2007). In particular the fire-curing process, i.e., wood smoke, is associated with the formation of PAHs (Hoffmann et al. 1986). Therefore, tobaccos cured by other methods are expected to have lower PAH content. The source of PAHs, such as benzo[a]pyrene (B[a]P), for non-fire-cured STPs may be from such sources as environmental contamination of the leaf surfaces or inadvertent exposure to combustion fumes during processing (Rickert et al. 2009).

Many PAHs have been shown to produce tumors in experimental animals and genotoxicity or DNA damage in *in vivo* and *in vitro* tests (EPA IRIS 2010). PAHs are normally present as a mixture and B[a]P is often used as an indicator chemical for their presence. B[a]P is classified as a known human carcinogen (Group 1) by IARC (IARC 2006; 2009)[25].

Few studies have quantified PAHs in snus (Table A II-3 in Appendix II). Stepanov and colleagues (2008a) analyzed eight different PAHs in new and traditional STPs. These

[25] The overall evaluation of B[a]P was upgraded from 2B (possibly carcinogenic to humans) to 1 (carcinogenic to humans) based on mechanistic and other relevant data (IARC 2006: http://monographs.iarc.fr/ENG/Meetings/92-pahs.pdf; IARC 2009: http://monographs.iarc.fr/ENG/Classification/crthgr01.php). According to IARC, by inhalation, B[a]P is associated with both urinary bladder and lung cancer. By oral exposure, B[a]P has not been associated with any specific cancer type and IARC considers the available information is at present too limited to draw definitive conclusions. B[a]P has recently been used as an index chemical for carcinogenic PAHs and the derivation of the relative potency of PAHs (relative potency factors, RFPs) compared to B[a]P was proposed (US EPA 2010).

investigators did not detect B[a]P in traditional Swedish snus (*General*). McNeill and colleagues (2006) reported a B[a]P concentration of 1.99 ng/g dry weight in "snus (general [sic] pouch) from Sweden". These concentrations are considerably lower than the GothiaTek® Standard limit for B[a]P of 20 ng/g dry weight (see Table 2-3), and lower than the recent WHO Study Group on Tobacco Product Regulation recommendation that "the concentration of benzo[a]pyrene in smokeless tobacco should be limited to 5 ng/g dry weight of tobacco" (WHO 2009). These more recent B[a]P concentrations are also lower than those reported secondarily by Ramström (2000). In his review, Ramström reported B[a]P in "snuff without fire-cured tobacco has concentrations around 10 ppb" (10 ng/g), citing a presentation on the chemical composition of Swedish snuff given by Wahlberg in 1996. Ramström did not describe additional details of the Wahlberg analysis and did not specify if these concentrations were based on wet or dry weight.

Of the seven other PAHs that Stepanov and colleagues (2008a) quantified in their study in addition to B[a]P, benzo[b]fluoranthene (B[b]F) and benzo[k]fluoranthene (B[k]F) are considered to be Group 2B[26] carcinogens by IARC in 2009. The remaining five PAHs analyzed by Stepanov and colleagues (2008a), including fluoranthene that has shown co-carcinogenic activity in animal experiments (as cited in EPA IRIS 2010)[27], were declared as *not classifiable as to carcinogenicity to humans* by IARC (2009). In a recent evaluation for the "Development of a relative potency factor approach for polycyclic aromatic hydrocarbon (PAH) mixtures", the US Environmental Protection Agency (US EPA 2010) considered anthracene, phenanthrene, and pyrene to be not carcinogenic.

B[b]F and B[k]F were not detected in the traditional snus sample (*General*) investigated by Stepanov and colleagues (2008a). The concentrations of fluoranthene and acenaphthylene were reported to be 31.1 and 1.70 ng/g dry weight, respectively. Anthracene was not detected and concentrations of phenanthrene and pyrene were 55.3 and 29.7 ng/g dry weight, respectively.

In a recent study, Stepanov and colleagues (2010) expanded the list of PAHs analyzed in STPs to include priority environmental PAH pollutants identified by the US EPA, as well as those PAHs that, according to IARC, are carcinogenic and present in cigarette smoke. In their study, Stepanov and colleagues (2010) analyzed different oral moist snuff products for 23 PAHs[28], but did not include a traditional Swedish snus product. Twenty-two PAHs were detected, of which, in addition to B[a]P, eight are classified by IARC as carcinogens based on data in experimental animals and mechanistic studies[29].

[26] Group 1: The agent is carcinogenic to humans. Group 2A: The agent is probably carcinogenic to humans. Group 2B: The agent is possibly carcinogenic to humans. (http://monographs.iarc.fr/ENG/Classification/index.php)

[27] US EPA. 2010. http://www.epa.gov/ncea/iris/subst/0444.htm, accessed in February 2010

[28] Acenaphthene, acenaphthylene, anthracene, benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[e]pyrene, benzo[g,h,i]perylene, chrysene, dibenz[a,h]anthracene, fluorene, fluoranthene, indeno[1,2,3-cd]pyrene, methylchrysene isomers, naphthalene, phenanthrene, pyrene

[29] Relative potency factors (RFPs) have been proposed by US EPA (2010) for 7 of the 8 PAHs. The RFPs ranged from 10 for dibenz[a,h]anthracene (the only IARC Group 2A carcinogen of these PAHs) to 0.03 for benzo[k]fluoranthene (IARC Group 2B). Napthalene (Group 2B) was not included the RFP approach by USEPA (2010).

2.2.7.3 Aldehydes

Volatile aldehydes are widely present in the human environment (Stepanov et al. 2008a). Similar to in foods, they can be detected in STPs, but have not been widely quantified in those products (IARC 2007; Stepanov et al. 2008a). The investigators Stepanov and colleagues (2008a) analyzed four aldehydes in different STPs: formaldehyde and acetaldehyde, which are classified by IARC as known and probable human carcinogens, respectively, as well as acrolein and crotonaldehyde, both classified by IARC "*as not classifiable as to its [their] carcinogenicity to humans*" (IARC 2009[30]). The concentrations of formaldehyde, acetaldehyde, acrolein, and crotonaldehyde in traditional snus (*General*) detected in this study were 8.49, 31.7, 1.01, and 1.05 μg/g dry weight, respectively (Table A II-4 in Appendix II). The authors concluded that the overall levels in the STPs studied were relatively low compared to other sources of exposure such as diet and alcoholic beverages (Stepanov et al. 2008a).

2.2.7.4 Heavy Metals

Tobacco plants, like most plants, readily accumulate a variety of heavy metals from soils (Pappas et al. 2008). Additionally, trace amounts of nickel and chromium can originate from processing equipment used in cutting and grinding the tobacco (Rickert et al. 2009). Several heavy metals are considered known human carcinogens, e.g., arsenic, beryllium, cadmium, as well as chromium (VI) compounds and nickel compounds; probable human carcinogens, e.g., lead compounds; and possible human carcinogens, e.g., cobalt compounds (IARC 2009[28]). Additionally, in a recent study, Pappas and colleagues (2008) considered barium, an alkaline earth metal, as an important toxic element to be investigated in STPs. These authors analyzed commercial moist snuff, but not snus, and detected barium levels significantly higher than those of the other metals examined. To mimic human use and uptake, Pappas and colleagues (2008) also investigated the extractable amount of these metals. Only cadmium, cobalt, and nickel were more efficiently extracted, i.e., 20-65% of the metal in the product could be detected in artificial saliva. Metal contents and extractability from snus were not studied by Pappas and colleagues (2008).

While the GothiaTek® Standard limits were established for certain heavy metals (cadmium, lead, arsenic, nickel and chromium; see Table 2-3), other analytes, such as cobalt and barium are not included.

One newer study has reported levels of heavy metals in oral tobacco products in the UK, including snus (Table A II-5 in Appendix II) (McNeill et al. 2006). These investigators detected 1.54, 2.59, 0.5, and 0.3 μg/g per dry weight chromium, nickel, lead, and arsenic, respectively, in "snus (general [sic] pouch) from Sweden". Cadmium was not investigated. All metal levels determined were below the GothiaTek® Standard limits.

2.2.7.5 Radioisotopes

All tobacco products contain relatively low levels of radioactive substances, in particular polonium-210 (Samuelsson 1989). Polonium-210 in tobacco and other plants can originate from certain fertilizers and it also occurs naturally in soil and air in small amounts. As reported

[30] IARC. 2009. http://monographs.iarc.fr/ENG/Classification/Listagentsalphorder.pdf, accessed in February 2010.

in a Swedish review and risk assessment in 1989, levels of polonium-210 in snus ranged from 11 to 60 becquerels (Bq) per kg wet weight (0.022-0.120 Bq/g dry weight) (Samuelsson 1989). Polonium-210 emits alpha-particles, which have a range of approximately 0.04 mm in tissue and therefore their radioactive effects are limited to the immediate area of exposure (Samuelsson 1989). According to Samuelsson (1989), the polonium-210 is thought to not be absorbed into the body from snus use, but rather remains in the snus product, where it subjects the oral mucous membrane in closest proximity to a localized radiation dose. In his risk assessment, the author suggested that habitual snus users are exposed to a radiation dose per year similar to the exposure from three single dental x-rays. Analyses of polonium-210 in the five most popular moist snuff brands on the market in the US in 1985/1986 showed that levels ranged from 0.006 to 0.045 Bq/g dry weight (Hoffmann et al. 1987).

2.2.7.6 Other Trace-Level Components

Other carcinogenic compounds, including urethane and hydrazine can also be present in STPs at trace concentrations. Urethane is formed during fermentation processes. Hydrazines can be found in both air- and fire-cured tobaccos (IARC 2007). Recent studies have not investigated these compounds in snus.

2.2.8 Potentially Protective Compounds

Like most other plant products, tobacco also contains substances that are potentially antimutagenic and anticarcinogenic (Nyren 2001). Rodu and Jansson (2004) list two classes of compounds that may inhibit carcinogenesis and have antioxidant properties: carotenoids, such as β-carotene and phenolic compounds, e.g., flavonoids. Other examples of potentially protective compounds are ubiquinone, α-tocopherol, isoprenoids, and certain fatty acids, as well as nicotine itself (Brown et al. 2001; Nyren 2001). To date, it is uncertain whether the concentrations of these compounds in snus are sufficient to provide any protective effects (Nyren 2001).

2.3 Summary and Discussion of Chemical Properties

Swedish snus is a heat-treated oral moist snuff tobacco product originally developed in Sweden. Swedish snus mainly consists of air-cured tobacco, water, and salt. Other ingredients added in small quantities serve to retain moisture, stabilize the pH, and for preservation and flavoring purposes. The moisture content of traditional Swedish snus is approximately 50% and the pH close to 8.5. The manufacturing process of snus in Sweden must satisfy the hygienic requirements of the Swedish Food Act and all ingredients must comply with the Swedish Food Regulation.

The major producer of traditional Swedish snus, Swedish Match, established and adheres to quality control limits for certain trace-level components in snus that are described in the GothiaTek® standards.

Concentrations of TSNAs, traditionally the most frequently analyzed and reported trace-level components in STPs due to their carcinogenic potential in experimental animals, have significantly decreased in Swedish snus since the early 1980s. This appears to be mainly due to improvements in the snus manufacturing process that were introduced in the early 1980s,

including both technical changes in the production process and the institution of more rigorous quality checks of the raw ingredients.

Published data for most other trace-level components in STPs, including snus, is limited, and only in recent years more analyses on a variety of components other than TSNAs have become available (e.g., PAHs, aldehydes, and metals).

This limited published analytical data on the chemical composition of traditional Swedish snus does not allow distinction between different brands of snus. It should be noted that there are differences in portion sizes and nicotine content and delivery between snus brands. These characteristics need to be taken into account when conducting an exposure assessment for critical chemical substances in snus. It should also be noted that in the present report quantitative data for components was given as per dry weight of tobacco, which by itself does not allow an estimate of exposure to these agents.

Furthermore, for a comparison of the potential exposure to critical components in traditional Swedish snus with other oral moist snuff products, such as new products marketed as snus and traditional US-type moist snuff, other factors, such as moisture content, pH and resulting free nicotine need to be considered (along with use patterns). More details on these variables in other products are provided in Appendix II.

For a risk assessment, patterns of use of these products might differ depending on their nicotine delivery; this may affect individual users' exposure to components and therefore associated potential health risks. One approach suggested by Rickert and colleagues (2009) is to take these variabilities into account by basing comparisons between products on ratios of levels of components to a product's nicotine yield.

3 Biomarkers of Exposure to and Effect from Snus and Tobacco Components

3.1 Overview of Biomarkers for Components in Tobacco

3.1.1 Introduction

This section provides an overview of biomarkers of exposure and biomarkers of effect that have been evaluated for tobacco components from various tobacco-containing products. Therefore, the studies described herein are not limited to snus as in other sections of this report. A biomarker is any substance, structure, or process that can be measured in the body to quantify internal exposure or predict an outcome or disease and is a measurable endpoint in a continuum of events leading from exposure to toxic agents to adverse effects or diseases. Different types of biomarkers are used to: 1) assess exposure, 2) identify early changes or effects of this exposure, 3) identify the initiation of pathological changes prior to development of a disease state, and 4) predict underlying susceptibility of individuals to disease (Santamaria et al. 2006).

There are three main classes of biomarkers, commonly referred to as biomarkers of exposure, effect, and host susceptibility. The National Research Council classified biomarkers into three categories based on their relation to the exposure-disease continuum (Committee on Biological Markers of the National Research Council 1987). Biomarkers of exposure were defined as the identification of an exogenous substance within the biologic system, the interactive product between a xenobiotic compound and the endogenous components, or other events in the biologic system related to exposure. Biomarkers of effect were defined as any changes that are qualitatively or quantitatively predictive of health impairment or potential impairment resulting from exposure. Biomarkers of susceptibility were defined as indicators that the health of an organism is especially sensitive to the challenge of exposure to a xenobiotic compound. The Life Sciences Research Office (LSRO) defines a biologic marker of exposure as a component or metabolite that is measured in biological fluid or tissue or that is measured after it has interacted with critical subcellular or target tissues, “biologically effective dose” (LSRO 2007). Biomarkers of effect are measured effects such as early subclinical biological effects, alterations in morphology, structure, or function, or clinical symptom(s) consistent with the development of health impairment or disease (LSRO 2007). Although not defined by LSRO, biomarkers of susceptibility provide a means of assessing the variability of response by individuals to environmental stress, depending on the genetic makeup of the individual. In some instances, a biomarker may have the potential to serve as a measure of uptake as well as metabolic activation, thus serving as both a biomarker of exposure and effect (e.g., adducts).

There is controversy regarding the biological significance of DNA and protein adducts. The presence of DNA or protein adducts may only indicate exposure and not necessarily adverse health effects or early effects in the carcinogenic process. While DNA adducts are likely to be a first step in the carcinogenic process, the mere presence of DNA adducts alone is not evidence of mutations and no specific threshold or quantitative correlation exists between DNA adducts and the production of tumors. If a cell is unsuccessful in repairing DNA damage such as DNA adducts, it may continue to survive without effect, die, or mutate. Available evidence suggests that there may not be a threshold for DNA adduct formation, but there is a threshold for tumor formation because of the existence of repair mechanisms for many adducts (Swenberg et al.

2008). Therefore, DNA and protein adducts are often defined as biomarkers of exposure and not biomarkers of effect (Swenberg et al. 2008), and for the purposes of this report, DNA and protein adducts measured in biological fluids and tissues will be considered biomarkers of exposure. Many DNA adducts can result in mutations if DNA replication takes place before repair. Mutations, at either the gene or the chromosome level, are irreversible changes in DNA structure that alter its genetic information content, and unlike DNA adducts, mutations cannot be repaired and are heritable in the progeny of the originally mutated cell and therefore may be considered to be biomarkers of effect (Swenberg et al. 2008).

The evaluation of health risks associated with the use of tobacco-containing products such as Swedish snus will have to consider external exposure, markers of internal exposure, estimates of the biologically effective dose, and biomarkers of potential harm; this is particularly true when attempting to compare health risks among different STPs and/or cigarettes.

The Institute of Medicine (IOM) (2001) also developed definitions for four types of markers of external exposure and biomarkers of internal exposure/effect to evaluate the toxicity and harm reduction potential of products such as STPs. These include: 1) External exposure marker[31], 2) biomarker of exposure[32], 3) biologically effective dose[33], and 4) biomarkers of potential harm[34]. With respect to the use of biomarkers to evaluate exposure and effects from various tobacco-containing products, IOM (2001) stated, "Currently there is sufficient evidence to show that biomarkers can provide better estimates of risk in the context of exposure, and therefore they will likely be able to provide improved assessments for harm reduction products." IOM (2001) also stated, "different types of biomarkers along the pathway from internal exposure, biologically effective dose, and potential harm are needed, and additional research is necessary to identify the best combination of markers to be used."

3.1.2 Biomarkers of Exposure for Components in Tobacco

Exposure to chemical components present in tobacco-containing products, tobacco smoke, or nicotine replacement products is influenced by the concentration of the components in the tobacco product or smoke, the metabolism and absorption of these substances, physicochemical properties of the product (e.g., pH, size of tobacco), and the patterns of product use. There are a variety of potentially toxic substances in tobacco and tobacco smoke, and there are several ways to account for differences in human exposure to these components. One way to estimate exposure to the chemical compounds in tobacco-containing products is based on the chemical composition of the product (e.g., level of component per dry weight of tobacco) and use patterns. However, such measures may not accurately reflect internal dose, may be insensitive to changes in risk or behavior, are difficult to assess over time, and may be subject to recall bias regarding product use. A potentially more accurate way to measure exposure and internal dose of the substance(s) of interest in a tobacco-containing product such

[31] A tobacco component or product that may reach or is at the portal of entry to the body.
[32] A tobacco component or metabolite that is measured in a biological fluid or tissue that has the potential to interact with a biological macromolecule; sometimes considered a measure of internal dose.
[33] The amount that a tobacco component or metabolite binds to or alters a macromolecule; estimate of the biologically effective dose might be performed in surrogate tissue.
[34] A measurement of an effect due to exposure; these include early biological effects, alterations in morphology, structure, or function, and clinical symptoms consistent with harm; also includes "preclinical changes".

as snus is through the use of biomarkers. Biomarkers of exposure measured in a body fluid, tissue, or in exhaled air, represent an internal dose of tobacco smoke or a tobacco product component that is either the parent compound or its metabolite (IOM 2001). Several studies have been conducted to measure biomarkers of exposure and/or effect in the serum, urine, or tissues of nonsmokers, smokers, STP users and/or cancer patients that may provide useful information for comparing the health risks of various STPs (Gray et al. 2008; Hecht et al. 2007b; Hecht et al. 2008a; Hecht et al. 2008b; Stepanov et al. 2008b). Where available, biomarkers of exposure (e.g., chemical, metabolites, DNA or protein adducts adducts[35]) may be useful to determine systemic or tissue dose of the chemical substance(s) of interest, which may be a more reliable indicator of actual exposure/dose than typical external measures of exposure for a product such as snus (e.g., concentration of the chemical in the product and amount of product used). Some exposure biomarkers have been researched extensively (e.g. urinary cotinine) and are more representative of actual human exposures to tobacco-containing products than external measures of exposure. Their measurement expresses an individual's overall exposure, including the intake from each possible route by which the chemical can enter the body (ingestion, inhalation, or skin contact) (Dor et al. 1999). Measuring a chemical or metabolite in biological fluids or tissues allows for the scientific estimation of external exposure levels that is necessary for characterizing risks associated with a product such as snus. However, one potential source of error regarding exposure biomarkers may be when intake results from a source other than the product being studied (e.g., cotinine measurements among STP users who are also exposed to environmental tobacco smoke). Biomarkers of exposure are assayed in a bodily fluid and/or tissue and are a measure of a component or metabolite of tobacco smoke, tobacco-related products, or metabolites, where the component is not bound to a biomolecule (IOM 2001). This definition for a biomarker of exposure focuses on the measurement of a chemical or metabolite that is present in a bodily fluid or tissue (e.g., urine, saliva, blood, hair, toenail, exhaled air), and does not require interaction with subcellular or tissue targets (e.g., adducts), as included in the LSRO definition.

Exposure biomarkers for tobacco components may include specific chemical components in tobacco or products of tobacco combustion or their metabolites, for example, carbon monoxide (CO), caboxyhemoglobin (COHb); nicotine and metabolites of nicotine (cotinine); and 1-hydroxypyrene [1-HOP]). Other biomarkers of exposure for tobacco components and combustion products include mutagens and metabolites of chemical compounds in urine, including benzene, acrolein, 1,3-butadiene, acrylonitrile, acetonitrile, benzo[a]pyrene, and benz[a]anthracene. Biomarkers of exposure including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol glucuronides (NNAL-Glucs) in urine and aminobiphenyl/aromatic amine hemoglobin (Hb) adducts in blood have been studied extensively by a group of investigators from the University of Minnesota Cancer Center for studies evaluating new "potential reduced exposure products (PREP)", including snus. NNAL and NNAL-Glucs are metabolites of the TSNAs NNK and NNN.

[35] Some tobacco-related carcinogens bind directly to DNA whereas most require enzymatic activation. The resulting covalent binding products, called DNA adducts, are believed to be involved in the carcinogenic process and may cause mutations in growth control genes in tumors (Hatsukami et al. 2006).

LSRO (2007) placed biomarkers to assess exposure to cigarette smoke into three classes, including: 1) Category A – biomarkers that have been sufficiently studied and provide reliable exposure measurements; 2) Category B – biomarkers that have sufficient data to support their use in exposure studies but also have limitations related to one or more desirable biomarker characteristics; and 3) Category C – biomarkers that are not considered sufficiently reliable for routine use in exposure studies. Most of the studies that have been conducted with snus users evaluated biomarkers of exposure that fall into Category A.

Some exposure biomarkers may be associated with smoking and not STPs (e.g., exhaled carbon monoxide), whereas others may be associated with both smoking and the use of STPs (e.g., cotinine and NNAL and NNAL-glucuronides). A recent study was conducted by Stepanov and Hecht (2008) to evaluate the feasibility of a biomarker of exposure using toenail NNN and NNAL levels in smokers. The authors reported a strong positive correlation of toenail NNN with toenail NNAL and cotinine in smokers, similar to that reported for urinary biomarkers (Stepanov and Hecht 2008). However, low but detectable levels of NNN and cotinine were also found in toenail samples from nonsmokers. It is not clear whether a biomarker of exposure using toenails will be a useful biomarker for an STP such as snus, particularly since this is the only study to evaluate toenails and it only included individuals exposed to smoking. Only a few studies have been conducted to evaluate biomarkers of exposure such as cotinine or NNAL in the urine or saliva of snus users, as described later in this report (Andersson et al. 1995; Gray et al. 2008; Hatsukami et al. 2004a; Post et al. 2005).

The uptake of polycyclic aromatic hydrocarbons (PAHs) by smokers has been clearly demonstrated using the urinary biomarker of exposure, 1-hydroxypyrene (1-HOP), and there is evidence for the presence of DNA adducts derived from benzo(a)pyrene (B[a]P) in lung tissue from some smokers (Beland et al. 2005; Boysen and Hecht 2003; Hecht 2002). However, there are other potential sources of 1-HOP, as diet may contribute significantly to the levels reported in nonsmokers (Hatsukami et al. 2007). In addition, STPs have been reported to contain fewer and/or lower levels of PAHs than cigarette smoke (Stepanov et al. 2010), particularly because most are formed during combustion (e.g., during smoking itself and fire-curing of tobacco), limiting the usefulness of substances such as 1-HOP as a biomarker of exposure for smokeless tobacco products such as snus produced from non-fire-cured tobacco. The benzene metabolite *trans,trans*-muconic acid is a widely used biomarker for benzene and a quantitatively significant component of cigarette smoke, however, like 1-HOP, this biomarker is often elevated in smokers but lacks specificity to tobacco products (Hatsukami et al. 2007).

B[a]P is an indicator of PAH exposure, however, in comparison with NNK and NNN, the levels of PAHs classified as carcinogens in snus are very low (see Section 2.2.7.2) . Mutagens in urine have also been measured as a biomarker of exposure in several studies with smokers, and some studies have reported differences in groups exposed to different types of cigarettes (LSRO 2007). However, urine mutagenicity is not specific to tobacco components, so there may be other sources of mutagens such as diet and occupational exposures, limiting the usefulness of this measurement as a biomarker of exposure to a product like snus.

The most well-studied biomarkers of exposure in individuals exposed to tobacco-containing products include metabolites in serum, urine, or saliva, DNA adducts, protein adducts, and in

several studies, the levels were increased in tobacco-exposed individuals (several studies cited in Hecht 2003). Examples include 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB)-releasing DNA adducts, which may be formed by the interaction of NNK and NNN with DNA, or protein adducts such as hemoglobin-aromatic amine adducts. Although studies have been conducted in an attempt to evaluate the role of adducts in the carcinogenic process (reviewed in Hecht 2003), there is controversy regarding the biological significance of DNA and protein adducts. As previously mentioned, the presence of DNA or protein adducts may only indicate exposure and not necessarily adverse health effects or early effects in the carcinogenic process. Some studies have attempted to correlate the presence and magnitude of adducts with tumors in humans exposed to tobacco-containing products. For example, a meta-analysis by Veglia and colleagues (2003) reported that DNA adduct levels were significantly higher (83%) in lung, oral, and bladder tumor tissue samples from current smokers than in non-smoking controls. Other studies have reported that adducts such as 4-aminobiphenyl–hemoglobin adducts were higher in smokers than in non-smokers, were related to dose, and decreased upon smoking cessation (WHO 2008). A 2-year chronic bioassay was conducted by Stinn et al. (2005), in which rats were exposed to a surrogate for environmental tobacco smoke, diesel engine exhaust, or fresh air for 6 hr/day, 7 days/wk and evaluated for lung tumors. The investigators reported a significant dose-related *increase* in DNA adducts in sidestream smoke treated rats, with no increased tumor rates, while in diesel exhaust treated groups, increased tumor rates were observed with no significantly increased DNA adduct levels in spite of substantial exposure to carcinogenic substances. However, DNA adducts are difficult to detect even with highly sensitive methods, and many active smokers will not have detectable adduct levels (Hatsukami et al. 2007).

3.1.3 Biomarkers of Effect for Components in Tobacco

Biomarkers of effect may be used to evaluate the potential for the development of adverse health effects associated with exposure to a substance of interest such as tobacco. These biomarkers may be the products of different cellular responses following exposure to the toxic substance, leading to the production of a variety of biomolecules or cellular or metabolic alterations. Molecular, cellular, tissue, and organ events associated with the development of lung cancer, chronic pulmonary disease, and cardiovascular disease have been evaluated in studies to assess differences in adverse health effects associated with cigarettes and potential reduced risk tobacco products (LSRO 2007). Biomarkers of effect for tobacco components include DNA mutations, genetic damage, injury such as early biochemical or histological effects (e.g., cytopathological changes, oxidative stress, inflammation, lipoproteins, white cells, C-reactive protein, fibrinogen, F2 isoprostanes, platelet aggregation), or early health effects (e.g., epithelial injury, mucous production, airway obstruction, electrical cardiac activity, hypertension, deterioration of lung function) (Hatsukami et al. 2007). The biomarker may be the net effect of metabolic activation, decreased rate of detoxification, decreased repair capacity, loss of cell-cycle checkpoint control, or decreased rates of cell death, however, not all of these alterations lead to an adverse health effect, limiting the usefulness of some biomarkers for evaluating health risks. DNA damage may also be evaluated by measuring sister chromatid exchanges (SCE) in peripheral lymphocytes; however, these tests are not necessarily specific to smoking or tobacco products, as substances in the diet or other exposures may contribute to the formation of SCE and other biomarkers of effects.

3.1.3.1 Biomarkers of Susceptibility for Components in Tobacco

Biomarkers of susceptibility provide a means of assessing the variability of response by individuals to environmental stress, depending on the genetic makeup of the individual. There are large interindividual differences in the metabolic response to chemical exposure and some individuals may be at an increased risk of developing adverse effects because of these differences. For example, sequence variations in the genes encoding certain enzymes and other proteins may accumulate in a population and if the frequency of a specific variant reaches 1% or more in a population, it is referred to as polymorphism. A number of enzymes or other molecules exhibit polymorphisms, which may play a role in determining the extent and type of response to a chemical substance. For example, a 16-fold difference in the rate of metabolic activation of nicotine to cotinine has been reported for human subjects that seems to be related to genetic polymorphisms of members of the P450 gene superfamily (Cholerton et al. 1994, as reported in Nilsson 1998). Researchers are also exploring individual differences and associated genotypes in the activation and detoxification of carcinogens (Hecht 2006). For example, Hecht (2006) reported that activation-to-detoxification ratios of the representative PAH phenanthrene correlated with polymorphisms in the CYP1A1 and CYP1B1 genes, although in different directions. Hecht (2006) also observed that high ratios — presumably indicating higher cancer risk — cannot be predicted by a combination of 11 different polymorphisms in PAH-metabolizing genes, demonstrating some of the limitations of genotyping individuals to determine their response to carcinogens because of differences in metabolism. No studies were identified that have evaluated biomarkers of susceptibility in snus users. The following sections of the report will provide an overview about what is known about biomarkers of exposure in users of tobacco-containing products, including snus.

3.2 Biomarkers of Exposure for Tobacco-Specific Nitrosamines

NNK induces pulmonary and other tumors in rats, mice, and hamsters independent of the route of administration (IARC 2007). Apparently most, if not all, of the carcinogenic action of certain types of fermented tobacco products can be ascribed to the presence of TSNAs (Nilsson 1998). NNK has been causally associated with the induction of lung cancer in smokers, and along with the related nitrosamine NNN, may be associated with the induction of oral cancer (Carmella et al. 2002). NNK is metabolized to NNAL, which can be further metabolized to NNAL-*O*-Gluc and NNAL-*N*-Gluc. These metabolites have been identified at different levels in the urine of rodents, monkeys, and humans exposed to NNK or various tobacco-containing products (Carmella et al. 2002; Hecht et al. 1993; Hecht et al. 2008a; Morse et al. 1990; Murphy et al. 1994). Several tobacco product manufacturers are continuing to develop and use methods to reduce TSNA formation in green plants and during tobacco processing. For example, the TSNA concentrations in snus have significantly decreased between the early 1980s and today (see Section 2.2.7.1). It is important to determine if the variations in TSNA concentrations in products result in a measurable difference in the internal dose levels of NNAL and NNAL-glucs by evaluating these biomarkers. Such measures will be useful in evaluating and comparing health risks associated with various types of tobacco products.

The sum of the urinary metabolites of NNAL and its glucuronides have been used to estimate NNK uptake in humans, and as new STPs with lower levels of NNK are being introduced, the application of the assay to quantify levels of NNK uptake in humans will become more important

for comparing health risks among different STPs. Measurement of NNAL and its glucuronides has been conducted to demonstrate and quantify the uptake of NNK in smokers, STP users, and nonsmokers exposed to environmental tobacco smoke (Hecht 1998; Hecht et al. 1999; Hecht et al. 2008b). These studies have reported that total NNAL levels vary predictably with the amount of NNK in smokeless tobacco, with duration of use of smokeless tobacco, or with the number of cigarettes smoked per day (Hatsukami et al. 2007; Hecht et al. 2008a; Joseph et al. 2005). Total NNAL levels decrease on cessation of tobacco use, and are significantly higher in tobacco users than in nonusers, in whom total NNAL is generally not detected unless there has been exposure to secondhand smoke.

Unchanged NNK is not detected in urine (Hecht et al. 1999); however, studies have quantified the metabolites of NNK in 24-h urine samples. Baseline levels of excreted NNAL and NNAL-Gluc are typically about 1 nmol NNAL/24 h and 2.2 nmol NNAL-Gluc/24 h (Hecht et al. 1999); levels of total NNAL reported were 6.6 nmol/24 h in smokeless tobacco users, 3–4 nmol/24 h in smokers and 0.03–0.13 nmol/24 h in nonsmokers exposed to secondhand tobacco smoke (IARC 2007). Occasionally, however, as in the case of Sudanese toombak users, far higher levels of total NNAL in urine have been observed in some individuals (IARC 2007). Advantages of the NNAL and NNAL-Gluc biomarkers include tobacco specificity, direct relevance to carcinogen uptake, and consistent detection in exposed individuals (Carmella et al. 2003).

Snuff dippers/tobacco chewers in the USA excreted 6.6 nmol/24 h total NNAL (NNAL plus NNAL-Gluc) in urine (Hecht et al. 2002). In one study of U.S. snuff dippers and tobacco chewers, urinary excretion of total NNAL (NNAL and NNAL-Gluc) averaged 4.4 pmol/mg creatinine (Kresty et al. 1996). The authors stated that the mean levels of were not significantly different from those measured in a previous study of smokers by the same investigators (3.8 pmol/mg creatinine). Levels of NNAL-Gluc in 23 snuff-dippers (4.7 pmol/mg creatinine) were significantly higher than those in 13 tobacco chewers (1.6 pmol/mg creatinine) and the levels of NNAL-Gluc were significantly higher in 61 smokers (2.81 pmol/mg creatinine) than the 13 tobacco chewers (Kresty et al. 1996). The investigators also reported that the urinary biomarkers NNAL-Gluc, NNAL, and cotinine were associated with the presence of oral leukoplakia observed in 16 out of 39 STP users. In a study by Murphy et al. 1994, seven toombak users excreted an average of 1,276 pmol/mL urine total NNAL (mean daily excretion was 270 µg, as compared to a 24-hr average excretion of 4 µg in smokers). Among snuff dippers in the USA, total daily dip duration, total daily dipping time and number of dips per day were significantly correlated with levels of total NNAL (Lemmonds et al. 2005). Levels of total NNAL correlated with the number of tins used per week in one study (Hecht et al. 2002) but not in another (Lemmonds et al. 2005). Total levels of NNAL were significantly lower in users of smokeless tobacco after they switched to Swedish snuff or to nicotine patch; the overall mean level of total NNAL among participants who used a nicotine patch was significantly lower than that among those who used snuff (Hatsukami et al. 2004b). Levels of NNAL and NNAL-Gluc were quantified in the urine of 420 smokers (25.8 mean cigarettes/day) and 182 smokeless tobacco users (4.2 mean tins/wk) (Hecht et al. 2007a). The smokeless tobacco users used traditional US-type moist snuff, such as *Copenhagen* (31.5%), *Skoal* (12.7%), *Kodiak* (47.0%), and other brands (8.8%). Levels of total NNAL/mL urine and levels of total NNAL/mg creatinine at baseline, adjusted for age and sex, were significantly higher in smokeless tobacco users than in smokers ($p < 0.001$). Levels of cotinine/mL urine and cotinine/mg creatinine were also

significantly higher in smokeless tobacco users than in smokers ($p < 0.001$). The pharmacokinetics of nicotine have been compared in smokeless tobacco users and smokers (Benowitz et al. 1989), and although similar, the ratio of cotinine to nicotine area under the curve was significantly greater while using smokeless tobacco compared with smoking, possibly due to first pass clearance of swallowed nicotine (Hecht et al. 2007a). Pharmacokinetic data on NNK and NNAL in smokers and smokeless tobacco users are limited and the investigators cited one study that showed that the distribution half-lives of NNAL and its glucuronides were significantly less in smokeless tobacco users than in smokers, whereas the terminal half lives were the same (Hecht et al. 2002). In the Hecht et al. (2007a) study, the authors concluded, "Nevertheless, the results of the present study indicate that exposure to NNK is at least comparable in smokeless tobacco users and smokers."

Studies have been conducted to characterize and quantify biomarkers of exposure (e.g., DNA or protein adducts) formed as a result of exposure to tobacco components such as NNK and their role in the carcinogenic process (Lao et al. 2007; Stepanov and Hecht 2009; Upadhyaya et al. 2008). NNK and NNN form two primary types of DNA lesions, including nucleotide methylations and pyridyloxobuylations (known as POB or HPB adducts). Mechanistic studies may contribute to the understanding of the dose-related biological significance and toxicity of exposure to TSNAs. For example, Lao and colleagues (2007) hypothesized that the lung carcinogenicity of NNK in the rat is due in part to the preferential retention of (*S*)-NNAL in the lung, the reconversion to NNK, and then the metabolic activation of NNK to form pyridyloxobutyl (POB)-DNA adducts. Lao and colleagues (2007) treated male F344 rats with 10 ppm of NNK, (*R*)-NNAL, or (*S*)-NNAL in drinking water and measured POB-DNA adducts in the liver and lung after 1, 2, 5, 10, 16, or 20 weeks of treatment. The authors reported the accumulation and persistence of specific POB-DNA adducts in the rat lung and liver during chronic treatment with NNK, (*R*)-NNAL, and (*S*)-NNAL and provides support for the hypothesis that the preferential retention of (*S*)-NNAL in the lung, followed by reconversion to NNK and then the metabolic activation of NNK is critical for lung carcinogenesis by NNK and NNAL (Lao et al. 2007).

3.3 Biomarkers of Exposure for Nicotine

Nicotine is a highly specific biomarker of tobacco product or tobacco smoke exposure and may be measured in blood or urine. There are a few additional potential sources of nicotine, including food sources (e.g., from other plants belonging to the Solanacae family such as tomatoes, eggplant, and potatoes); however, dietary sources only contribute a negligible amount of nicotine (Davis et al. 1991; Siegmund et al. 1999). Nicotine is extensively metabolized to six primary metabolites by the liver and about 90% of a systemic dose of nicotine can be accounted for as nicotine and metabolites in urine, with approximately 5-10% excreted unmetabolized. Nicotine has a very short half-life (1-2 hrs), and blood levels fluctuate significantly throughout the day. Metabolites of nicotine (e.g., cotinine, *trans*-3'hydroxycotinine and their glucuronides) have been measured as biomarkers of exposure for tobacco-containing and nicotine replacement products, and together with nicotine, they account for 80-85% of the total nicotine taken into the body (LSRO 2007). In humans, about 70 to 80% is converted to cotinine, a transformation that occurs in two steps, first by cytochrome P450, thereafter by aldehyde dehydrogenase. Cotinine may be measured in blood, serum, saliva, urine, hair, and other fluids. The half-life of cotinine is approximately 16 hours and approximately 10-15% is

excreted unchanged in urine, which contributes to its usefulness as a biomarker (LSRO 2007). Several studies have been conducted to measure cotinine and other nicotine metabolites in smokers and non-smokers (several were reviewed by LSRO 2007), whereas few studies have evaluated these substances in STP users (Gray et al. 2008; Stepanov and Hecht 2008).

Cotinine is present in the blood of tobacco product users in much higher concentrations than nicotine because of its longer half-life. Cotinine blood concentrations average about 250 to 300 ng/ml in groups of cigarette smokers, and in some smokers, levels up to 900 ng/ml have been reported (Benowitz et al. 1983; Gori and Lynch 1985). After exposure to the last cigarette, levels of cotinine in plasma decline in a log linear fashion with an average half-life of about 16 hours and with a range of 12.8-18.8 hours.

Nicotine absorption from moist snuff is rapid and reaches a maximum at 30 minutes, however, absorption from cigarette smoke is more rapid (Benowitz et al. 1988). Smoking is a highly efficient form of nicotine administration, as it enters the circulation rapidly through the lungs and moves into the brain within seconds and escapes first-pass intestinal and hepatic metabolism (Benowitz 2009). Blood levels of nicotine fall more slowly after removing exposure to a STP compared to after smoking a cigarette, presumably due to absorption of nicotine that has been swallowed and the nicotine remaining in the buccal epithelium. Individuals who smoke cigarettes are primarily exposed to nicotine through inhalation, whereby the nicotine is directly absorbed into the bloodstream. Swallowing the juice from STPs is prevalent in users and nearly 80% of nicotine that is absorbed from the intestine is metabolized to cotinine in the first pass through the liver and never reaches the systemic circulation (LSRO 2007). In one study with 10 male cigarette smokers that switched to American snuff, chewing tobacco, or nicotine gum, the absorbed dose of nicotine was found to be at least twice as great from STPs compared to cigarettes, with estimated absorbed doses of nicotine of 1.8, 3.6 4.5, and 1.9 mg from cigarette, snuff, chewing tobacco, and gum, respectively (Benowitz et al. 1988). However, this study may not adequately represent Swedish snus users, as it did not evaluate snus products, used nicotine as a biomarker rather than cotinine which is a better biomarker, and was conducted in cigarette smokers that had switched to STPs after an overnight abstinence from smoking. More research is necessary to have sufficient data and information to evaluate the use of cotinine as a biomarker for comparing exposure to nicotine among various tobacco-containing products.

In addition, the pH of STPs has been shown to be a significant factor in contributing to nicotine bioavailability (see Section 2.2.4). In a study with 10 male volunteers having used smokeless tobacco for a mean of 12.5 years, four brands of US-type moist snuff were tested that had comparable nicotine contents: *Copenhagen, Skoal Long Cut Cherry, Skoal Original Wintergreen* and *Skoal Bandits* (Fant et al. 1999). The maximum mean increase in plasma nicotine concentrations were: *Copenhagen* (19.5 ng/ml), *Skoal Long Cut Cherry* and *Skoal Original Wintergreen* (14.9 ng/ml), and *Skoal Bandits* (4.2 ng/ml). Plasma nicotine concentrations increased much more rapidly following administration of Copenhagen than for *Skoal Original Wintergreen* and *Skoal Long Cut Cherry* (10 ng/ml was reached after 4, 10, and 15 minutes after administration and 15 ng/ml after 6, 20 and 25 minutes, respectively). These differences correlated with the pH values of the STP in suspension, namely 8.6, 7.6 and 7.5, respectively.

A study was conducted by Cobb and colleagues (2009) to evaluate clinical laboratory parameters of toxicant exposure (plasma nicotine levels, expired CO) and abstinence symptom suppressing effects of noncombustible PREPs in 17 male cigarette smokers. The authors hypothesized that, relative to the participants' own brand of cigarettes, noncombustible PREPs would expose users to lower levels of nicotine and CO and suppress tobacco abstinence symptoms less effectively than cigarettes. The products tested included *Ariva* (Star Scientific, compressed tobacco tablet), *Marlboro Snus* (Philip Morris USA), *Camel Snus* (RJ Reynolds), *Commit* nicotine lozenge (GlaxoSmithKline; 2 mg), own brand cigarettes, *Quest* cigarettes (Vector Tobacco; delivers very low levels of nicotine), and sham smoking (i.e., puffing on an unlit cigarette). However, Swedish snus was not investigated. In each of seven sessions, the product was administered twice (separated by 60 minutes), and plasma nicotine levels, expired air CO, and subjective effects were evaluated using the Tiffany-Drobes Questionnaire of Smoking Urges. For *Camel Snus* and *Marlboro Snus*, participants were asked to place the pouch between their lip (location unspecified) and gum for 15 minutes. Overnight cigarette abstinence occurred before each session. Relative to baseline, participants smoking their own brand was associated with significant increases in plasma nicotine level at nearly every time point, and the greatest mean increase was observed five minutes after the first (mean = 20.7 ng/ml) and second (mean = 20.6 ng/ml) product administration. Relative to baseline, mean *Camel Snus* plasma nicotine level was significantly greater 15 minutes after the second product administration (7.6 ng/ml) and was 2.9 ng/ml for *Marlboro Snus*. Relative to mean plasma nicotine levels in the smoking their own brand condition, levels observed for all other study conditions were significantly lower 5 and 15 minutes after the first and 5, 15, and 30 minutes after the second PREP product administration. According to the authors, the results indicated that the non-combustible products delivered less nicotine than the participants' own brand cigarettes, did not expose smokers to CO, but failed to suppress tobacco abstinence symptoms as effectively as combustible products. The authors concluded that clinical laboratory methods can be used to evaluate the short-term effects (toxicant levels, abstinence symptoms) of non-combustible PREPs for smokers (Cobb et al. 2009).

3.4 Biomarkers of Exposure Studies with Snus

The Swedish snus product, *General*, which is manufactured according to the GothiaTek® quality standards designed to minimize nitrosamine levels among others, has been reported to contain relatively low levels of TSNAs, compared to other STPs, such as traditional US-type products (see Section 2.2.7.1 and the respective Section in Appendix II). There are several available studies conducted to evaluate exposure biomarkers such as levels of NNAL or its glucuronides, and nicotine or cotinine in humans following the use of snus (Andersson et al. 1994; Andersson et al. 1995; Cobb et al. 2009; Gray et al. 2008; Hatsukami et al. 2004b; Holm et al. 1992; Lunell and Lunell 2005; Österdahl and Slorach 1988; Post et al. 2005). These studies are described below.

A study by Holm et al. (1992) was conducted to evaluate the rate of absorption of nicotine from a single 2 g pinch of Swedish moist snuff (*Ettan* brand) and the steady-state blood nicotine levels of 10 regular users during usual everyday use. Absorption of nicotine from a single 2 g pinch of Swedish moist snuff in 10 users resulted in average plasma nicotine concentrations of 9.9±6.5 ng/ml after 10 minutes and peaked at 14.5±4.6 ng/ml shortly after discarding at 30

minutes. Among groups of habitual Swedish oral snuff takers and cigarette smokers, peak blood nicotine levels measured directly after using a pinch of their usual snuff or smoking one cigarette (without prior abstinence) were similar, averaging 36.6±14.4 ng/ml and 36.7±16.1 ng/ml, respectively (Holm et al. 1992). Blood levels of nicotine fall more slowly after removing exposure to the STP compared to after smoking a cigarette, presumably due to absorption of nicotine that has been swallowed and the nicotine remaining in the buccal epithelium. Individuals who smoke cigarettes are primarily exposed to nicotine through inhalation, whereby the nicotine is directly absorbed into the bloodstream. Swallowing the juice from STPs is prevalent in users and nearly 80% of nicotine that is absorbed from the intestine is metabolized to cotinine in the first pass through the liver and never reaches the systemic circulation (LSRO 2007)

Nicotine plasma levels related to one day's use of four Swedish brands of snus were compared with those from Nicorette gum in a cross-over study (Lunell and Lunell 2005). The extractable nicotine was determined by the difference in nicotine content between used and unused snus. The mean extraction ranged between 1.55 and 2.74 mg per portion for the different brands (approximately 3 to 8 mg/g dry weight, see Section 2.2.4 for details), resulting in 22 to 44% extraction of the total nicotine content. The mean extracted amounts were 2.74, 1.55, 2.00, and 1.08 mg/pouch for *General* (1 g, pH 8.4), *Catch Licorice* (1 g, pH 8.5), *Catch Mini* (0.5 g, pH 8.4) and *Catch Dry Mini* (0.3 g, pH 7.3) snus, respectively. Nicotine plasma levels with *General* portion snus (C_{max} was 29.0 + 8.5 ng/ml) were sustained at higher levels than nicotine replacement products (C_{max} ranged from 10.85 to 23.79 ng/ml). Based on the mean nicotine plasma concentration measured in the snus users in this study, the bioavailable doses were calculated to be between 40 and 60%[36]. The authors concluded that consumption of the tested traditional snus brands once hourly produced similar blood nicotine levels as moderate to heavy cigarette smoking (e.g., use of *Catch* once hourly resulted in blood levels similar to smoking 15-20 cigarettes per day, whereas use of *General* once hourly was similar to blood nicotine resulting from smoking 25-40 cigarettes per day). Based on the results from Lunell and Lunell (2005) and Andersson and colleagues (1994), Fagerstrom (2005) determined that the nicotine intake from portion-packed snus varied between 1.2 and 2.2 mg/g snus, in the same range as the estimated amount of 1.5 mg nicotine absorbed from smoking of 1 g of tobacco (approximately 1 cigarette) (Fagerstrom 2005).

One study conducted by the Swedish National Food Administration investigated the extraction of TSNAs from Swedish moist snuff and measured TSNA levels in the saliva of 4 habitual male snuff dippers during and shortly after snuff use (Österdahl and Slorach 1988). Three of the investigated snuff dippers used snuff pouches of which the total TSNA content (NNK, NNN, and NAT) was determined to be 9.2 µg/g. The extracted amount of the total TSNAs in two samples measured between 0.3 and 0.9 µg/g, which was mainly due to decreases in NNK and NNN content. The TSNA content in one used sample was slightly increased by 0.3 µg/g, in spite of the fact that high TSNA concentrations were found in the saliva of the respective snuff dipper. The authors noted that this could be due to *in vivo* formation of TSNA in the saliva. The

[36] The mean areas under the curve (AUC) for the plasma concentration-time curves of snus users following hourly use of the different snus brands were compared with the AUC from 2-mg nicotine gum, which served as reference standard with assumed 55% bioavailability.

average total TSNA levels in the saliva of the 4 individuals during 30 minutes of snuff dipping ranged from 15 to 125 ng/g. The investigators calculated that with a saliva production of approximately 60 mL per hour, the snuff dippers were exposed to 0.9-7.5 µg TSNAs per hour snuff dipping. However, it should be noted that the TSNA content measured in the snus samples in this study was considerably higher than TSNA concentrations detected in snus in recent years (see Section 2.2.7.1)

Andersson and colleagues (1994) investigated the uptake and metabolism of nicotine and evaluated changes in the oral mucosa in users of portion-bag packed oral moist snus[37] compared with the changes in the mucosa of loose snus users. The subjects included 54 habitual users of smokeless tobacco: 22 loose snus users and 23 users of portion-bag packed snus and 9 users of chewing tobacco. The average duration of use was 14.5 years (loose snus), 7.4 years (portion-bag snus), and 9.5 years (chewing tobacco). The average consumption was higher for users of loose (20.8 g/day) than for users of portion-bag snus (14.4 g/day) and lower for users of chewing tobacco (7.2 g/day), which was statistically significantly less than the snus users. The tobacco was kept in the mouth for about the same number of hours a day by all three groups, with average values ranging from 12.3 to 13.1 hours. The average systemic dose of nicotine estimated as nicotine equivalents excreted during 24 hours was 35 mg/24 hr for the snus users and was 54 mg/24 hr for users of chewing tobacco. The average steady-state saliva cotinine concentration was 342 ng/ml for the portion bag snus users, 325 ng/ml for the loose snus users, and 471 ng/ml for the chewing tobacco subjects. Less pronounced clinical changes in the oral mucosa were recorded in snus pouch users compared with loose snus users. The snus pouch users showed predominantly Degree 1 and 2 lesions, while users of loose snus had more Degree 3 lesions. The clinical findings observed in the oral mucosa of users of chewing tobacco were leukoedema and slight clinical “snus changes”. The clinical severity of buccal mucosal changes did not correlate with the markers for exposure (i.e. nicotine and TSNAs extracted from the tobacco) nor with the biological markers for uptake of tobacco components (i.e., nicotine equivalents excreted during 24 hr and saliva cotinine concentrations). Twice as much nicotine was extracted from loose (94.7 mg/24 hrs) than from portion-bag snus (47.6 mg/24 hrs), however, there was no difference in saliva cotinine concentrations or the systemic dose of nicotine between the two groups of snus users. The authors stated that the discrepancy between the amount extracted and the actual uptake of nicotine may be due to the fact that users of loose snus have a higher salivary secretion rate and therefore spit or swallow much more saliva than users of portion-bag snus.

Andersson and colleagues (1995) conducted two studies to evaluate the short-term effects and long-term effects on consumption and nicotine intake resulting from switching to low-nicotine snus. In Study 1, consumption data, oral mucosal soft tissue changes, and nicotine intake were measured in a group of 24 habitual users of Swedish snus pouches[38], both during use of their ordinary snus (Brand A) for 2 weeks and during consumption of the low-nicotine product (Brand B) for 10 weeks. In Study 2, the same data were measured during 2 weeks in a reference group of 18 snus users who had been habitual users of the low-nicotine snus (Brand B) for at least one year. On Day 6 of Weeks 1, 2, 4, 8 and 12 in Study 1 and of Weeks 1 and 2 in Study

[37] No additional details about snus type provided
[38] No additional details about snus type provided

2, urine samples were collected for 24 h; saliva samples were collected on Day 7 of the same weeks. Urine was analyzed for nicotine and its metabolites, including cotinine; saliva was analyzed for cotinine concentration. In Study 1, although there was no increase in number of hours of daily consumption, the amount of snus consumed increased on average by 2 g a day (15%) when switching from Brand A to the low-nicotine Brand B. The Brand B reference group (Study 2) consumed about 3 g less snus a day during the same number of hours as the participants in Study 1 who had switched to Brand B. The authors concluded that these results indicate that snus users compensate to a small extent for the lower nicotine delivery by increasing their consumption on short-term switching, but the same does not apply to long-term users. In addition, there was a significant reduction in cotinine saliva and urine levels in the participants that switched from Brand A to Brand B in Study 1, to about the same level as participants in Study 2. Nicotine intake decreased by about 50% when participants switched from "normal" nicotine snus to a snus having half the concentration of nicotine. The study indicates that the biomarker of exposure used in these studies (cotinine) may be used to evaluate exposure to components in different STPs such as snus.

In a study conducted with 54 STP users and 51 cigarette smokers, participants were asked to switch from their usual US brand of smokeless tobacco to Swedish snus (*General*), from their usual brand of cigarettes to *Omni* cigarettes (the manufacturer, Vector Tobacco, claims that it has 53% less NNK than conventional cigarettes marketed in the US), or a nicotine patch (Hatsukami et al. 2004b). Switching to snus for 4 weeks resulted in a statistically significant decrease in levels of urinary total NNAL (approximately 50%) in the STP users, relative to levels obtained during 2 weeks of *ad libitum* use of widely used brands of STPs in the US. The authors concluded that this decrease in total NNAL levels was not a result of decreased STP use because study participants used similar amounts of snus and, by the end of treatment, had similar cotinine levels as they had during use of their usual STP. The authors concluded that tobacco products are available that can reduce exposure to carcinogens, and that users of snus had lower exposure to the carcinogen NNK than did users of STPs sold in the US (Hatsukami et al. 2004b).

A cross-sectional study was conducted by Post and colleagues (2005) in Stockholm, Sweden, to validate self reports of cigarette and snus use in 520 adolescents by evaluating saliva cotinine levels and answers to a questionnaire. Using a cut point of 5 ng cotinine per ml saliva to discriminate active tobacco use from non-use, there was a 98% concordance between self reported non-use in the past month and cotinine concentration. The sensitivity of the questionnaire compared to the saliva cotinine test, used as the gold standard, was 90% and the specificity 93%. One hundred and fifteen out of 520 participants (22%) reported monthly tobacco use and among these, 67% of the exclusive cigarette smokers, 82% of exclusive snus users, and 94% of dual users (cigarettes + snus) had cotinine concentrations above 5 ng/ml. Among participants reporting daily use, 96% had saliva cotinine concentrations above the cut point. The authors concluded that this study confirms the reliability of adolescents' self reported tobacco use and that a cut off for saliva cotinine of 5 ng/ml discriminated tobacco users from nonusers.

Gray and colleagues (2008) conducted a study to develop methods to evaluate withdrawal suppression and toxicant exposure associated with potential reduced exposure products

(PREPs) and STPs. Participants that had used STPs on a daily basis for at least 12 months and had smoked 5 or fewer tobacco products in the last 6 months were included in the first study. The subjects' "usual brands" of STPs included the US-type products *Skoal*, *Kodiak*, and *Copenhagen*. In the second study, participants that had used STPs at an average rate of 5.2 uses/day for 8.1 years were included in the study. The usual brands in this study included the US-type products *Skoal, Copenhagen, Kodiak*, and *Hawken*. The participants were administered one of the following products: usual brand, *Bacc-off* (non-tobacco, non-nicotine containing smokeless product); *Stonewall* (compressed tobacco tablet); or *General* snus for four 4-hour increments, each separated by 48 hours for 5 days. Each condition was separated by at least 72 hours. Urinary total NNAL (NNAL and NNAL-*O*-glucuronide), and cotinine levels were measured, along with expired CO in study 1 and study 2. Participants in study 2 were also evaluated for withdrawal symptoms. Compared with their own brands, *Stonewall* was associated with lower levels of cotinine and NNAL, while *General* snus was associated with similar levels of cotinine but lower levels of NNAL. According to the authors, abstinence symptoms generally did not differ across tobacco conditions, and they concluded that clinical laboratory methods can be used to evaluate the toxicant exposure and abstinence symptom suppression associated with PREPs for STP users (Gray et al. 2008).

3.5 Biomarkers of Effect Studies with Snus

A few studies have evaluated biomarkers of effect for cardiovascular effects in snus users, smokers, and/or never-smokers, including evaluating atherosclerotic changes (carotid bulb or carotid artery intima-media thickness (IMT) or femoral artery thickness) (Bolinder et al. 1997a; Wallenfeldt et al. 2001). Studies have also evaluated levels of markers of inflammation (fibrinogen, C-reactive protein) (Bolinder et al. 1997a; Eliasson et al. 1991; Eliasson et al. 1995; Wallenfeldt et al. 2001) and markers of lipid metabolism (total serum cholesterol, high-densitiy lipoprotein (HDL), low-density lipoprotein (LDL), cholesterol, or apolipoprotein A-1 (Bolinder et al. 1997a; Eliasson et al. 1991; Hirsch et al. 1992; Wallenfeldt et al. 2001) in snus users and non-tobacco users. There were no significant differences between the biomarkers in snus users and never users of tobacco; however, there were significant differences between snus users and smokers for atherosclerotic changes (with snus users having less severe changes than smokers). One study did report an association between the use of Swedish snus and elevated triglyceride levels (Wallenfeldt et al. 2001). All of these studies are described in greater detail in Chapter 5.

Studies may be conducted to determine if biomarkers of effect will be instrumental in comparing early health effects associated with various tobacco-containing products to snus. According to LSRO (2008), changes in lipids, biomarkers of inflammation, and measures of arthrosclerosis are weighted more heavily than are changes in systolic blood pressure and heart rate. Based on the available studies that evaluated these biomarkers of effect in smokers and snus users, LSRO (2008) concluded that snus users appear to have a lower degree of cardiovascular disease risk from use of these tobacco products compared with cigarette smokers.

3.6 Summary of Biomarkers

According to LSRO (2007), biomarkers of exposure are of greatest value in assessing exposure if they have the following attributes: 1) tobacco specificity or substantial difference between

smokers and nonsmokers; 2) intra-individual variation that mirrors variation in smoking (or STP use) behavior; 3) existing database on the biomarker's pharmacokinetics; 4) low analytical method variation; 5) sensitivity and chemical specificity; and, 6) existence of other biomarkers that can confirm the exposure. There are several limitations regarding the use of currently-available biomarkers of exposure or effect for substances in tobacco-containing products, including lack of specificity or sensitivity, difficulty in obtaining tissues, technical difficulty or expense in performing assay(s), confounding variables, wide individual variation, or relationship to disease is unknown. Biomarkers are more informative and better disease risk markers when measured in the target tissue through biopsies (e.g., oral mucosa, lung, bladder); however, target tissue may be difficult to obtain (IOM 2001). As a result of this limitation, biomarker assays have been developed for surrogate tissues and fluids (e.g., expired breath, saliva, blood, urine) in an attempt to estimate the dose to the target tissue.

Scientific knowledge regarding biomarkers of exposure and effect remains incomplete and the extent of reduction in biomarker levels that is required to reduce disease risk or the threshold of change that is required for reduced risk is not known for the available biomarkers of exposure or effect for tobacco-containing products (Hatsukami et al. 2007). There are currently no biomarkers of exposure or effect that have been validated as reliable, independent predictors of differences in tobacco-related disease risk among users of different tobacco products, including snus (Hatsukami et al. 2004a). However, biomarkers may be useful for comparing tissue dose levels and potential effects for various tobacco-containing products with products such as snus.

NNAL and NNAL-Glucs in urine, urinary cotinine levels, aromatic amine-Hb adducts, urinary mutagenicity, and SCEs are presently considered to be the most practical biomarkers of exposure or effect for use in studies evaluating new tobacco products. However, although the relationship of some biomarkers to cancer risk may be plausible, this has not been demonstrated. Furthermore, even though measures of exposure to individual carcinogens or the presence of DNA adducts are useful tools for research, a difference in a single measure has not been established as predictive of a difference in individual or population disease risk. To date, there is no comprehensive set of biomarkers of exposure or biological effects that serves as a predictive measure of the adverse health effects (e.g., cardiovascular, cancer) related to exposure to components in tobacco or tobacco smoke (Hatsukami et al. 2007).

There are a very limited number of studies available in the scientific literature that report measurement of exposures biomarkers in snus users. In these studies, researchers have measured cotinine or NNAL and its glucuronides in saliva, urine, or both. Most studies that have been conducted have not measured biomarkers in various exposure groups (e.g., snus, other STPs, cigarettes) within the same study, so it is not feasible to draw conclusions regarding levels of specific biomarkers among users of various STPs. More research is necessary to determine whether the use of biomarkers of exposure for components in tobacco and biomarkers of effect will be useful for comparing and predicting health risks of various STPs, including snus. There are also differences in how the products are administered (oral vs. inhalation) that affect the toxicokinetics of substances such as nicotine that need to be taken into consideration when evaluating results from exposure and/or effect biomarker studies for components in tobacco in different products.

4 Toxicological Studies with Snus Ingredients and Snus

4.1 Introduction

The toxicity of Swedish snuff or snus has been examined in a limited number of *in vitro* studies and *in vivo* carcinogenicity studies. Most of the *in vitro* studies were conducted with extracts of snus being administered to various to cell types, whereas only a few studies evaluated effects in tissues of snus users (e.g., urine mutagenic activity, protein levels in oral lesions or biopsies). *In vitro* toxicology assays have been extensively used over several decades to assess the biological effects of various chemical exposures and for cigarette smoke, however, the effects of smokeless tobacco products such as Swedish snus have been less thoroughly investigated. As stated by Johnson et al. (2009), "Almost all of the available *in vitro* toxicology methods: (a) were not developed for testing tobacco and tobacco smoke toxicity, (b) are not reliably quantitative to allow valid comparisons of substantially different tobacco products with differing yields of complex chemical mixtures, (c) provide data that cannot reliably be extrapolated to infer human cancer risk, and (d) were intended primarily as screening methods for chemicals to identify possible humans carcinogens." Existing *in vitro* methods need to be evaluated and validated, and new ones developed, to address these issues related to tobacco products.

Very few *in vivo* toxicological studies have been conducted to evaluate the effects of orally administered snus. Most of the studies used an experimental model in which Swedish snus (*Röda Lacket*, Svenska Tobaks AB, Sweden) was administered to rats through surgically created oral test canals. Because there are so few available studies with Swedish snus, toxicological studies that have been conducted with orally administered TSNAs are briefly reviewed, as these are the substances that are primarily associated with the carcinogenic effects of tobacco products. The *in vitro* and *in vivo* studies that have examined the mutagenic, genotoxic, immunotoxic, and carcinogenic potential of Swedish snus are summarized in Appendices I and R.

4.2 *In Vitro* Studies with Swedish Snus[39]

4.2.1 Mutagenicity and Genotoxicity of Snus

Curvall and colleagues (1987) examined the mutagenic activity of concentrates of 24-hour urine collections from 8 Swedish wet snuff users in comparison to urine from 6 nonusers of tobacco and 8 cigarette smokers, using a *Salmonella typhimurium* reverse mutation assay (i.e., Ames test). Eight male volunteers used 15-40 g Swedish wet snuff per day. There was also a smoking group consisting of 8 volunteers that smoked 15-38 filter cigarettes per day. Urine was collected for 24 hours. The mean mutagenic activity reported was 1.3×10^3 revertants per 24 hours in the snuff users, 0.9×10^3 revertants per 24 hours in the non-users and was 8.6×10^3 revertants per 24 hour in smokers. There were no significant differences in the urinary levels of nicotine and cotinine between snuff users and smokers or between snuff users that had abstained for one week and non-users. Data from this study indicated no significant difference in mutagenic activity between the 24-hour urine collections from Swedish wet snuff users and urine from nonusers of tobacco.

[39] Most of the *in vitro* studies do not provide details about the product evaluated in the study and in many cases only refer to it as Swedish (wet or moist) snuff and do not provide manufacturer or brand name. When more detailed information was provided by the investigators, it was included in this report.

Jansson and colleagues (1991) investigated the genotoxic potential of aqueous and methylene chloride extracts of Swedish snus. The test systems selected included assays for the induction of mutations in four strains of *Salmonella typhimurium* (i.e., Ames test) in the presence and absence of S9 metabolic activation, sister chromatid exchanges (SCEs) in human lymphocytes, chromosome aberrations and gene mutations in V79 Chinese hamster lung cells, and micronuclei in mouse bone marrow cells. Additionally, the methylene chloride extract was tested for the induction of sex-linked recessive lethal mutations in the fruit fly, *Drosophila melanogaster*. The aqueous extract induced SCE in human lymphocytes and chromosome aberrations in V79 cells in the presence and absence of S9 metabolic activation. The aqueous extract did not induce point mutations in either the Ames test or in the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus of V79 cells, and the micronucleus test in mice was negative. Results from the Ames test were positive in the presence of metabolic activation for the snus extract, indicating that metabolic activation is required. The methylene chloride extract also induced genotoxicity in the SCE test and the chromosome aberration test in the presence of S9, but did not cause gene mutations in the HPRT locus of V79 cells. The methylene chloride extract did not cause induction of micronuclei in mice or of sex-linked recessive lethal mutations in *Drosophila melanogaster*. In conclusion, positive results were obtained for the methylene chloride extract in the Ames test, in contrast, no induction of mutations in mammalian cells *in vitro* was observed. The authors concluded, "*Based on these results, the carcinogenic potential of Swedish 'Snus' should be considered to be low, a conclusion in agreement with the low incidence of oral cancer in Sweden compared to other countries*."

A study by Rickert and colleagues (2009) was conducted to characterize several types of STPs available on the Canadian market using the modifications of the Official Health Canada chemical and toxicological methods developed for cigarettes. The main objectives of this study was to determine the levels of analytes in STPs with mandated Canadian reporting requirements and to compare the cytotoxicity, genotoxicity, and mutagenicity results obtained on extracts of these products. The samples tested included seven types of STPs: 1) fine-cut moist snuff reportedly made by US Smokeless Tobacco Company (UST) and imported into Canada; 2) long-cut moist snuff made by UST and imported; 3) pouched moist snuff made by UST and imported; 4) low-moisture snuff reportedly manufactured by McChrystal's in the UK and imported into Canada; 5) loose-leaf and plug chewing tobacco reportedly made in US by Swedish Match North America and imported into Canada, 6) pouched snus, reportedly made in Sweden and imported into Canada by Imperial Tobacco Canada; and 7) a gutkha-type product imported from India. Several types of moist snuff samples tested had TSNA and B[a]P levels slightly above the GothiaTek® standard while samples of snus, low-moisture snuff (McChrystal's), and US-style chewing tobacco (made by Swedish Match) did not exceed the standard. Different doses of eleven sample brands with vehicle controls (saliva, dimethyl sulfoxide, or dichloromethane) were evaluated *in vitro* for mutagenicity, cytotoxicity, and clastogenicity. Extracts of various products were evaluated for mutagenicity in several assays using the Ames assay with *Salmonella* tester strains TA98, TA100, TA102, TA1535, and TA1537. Sample brands were also evaluated for cytotoxicity using the neutral red uptake (NRU) assay and clastogenicity using the micronucleus assay. The Manikchand Gutkha sample had the highest cytotoxicity based on the NRU assay and the lowest clastogenicity with the micronuclei test (there was no explanation for these observations). No other differences

were detected between the remaining samples tested, including the snus sample. Most of the cytotoxicity assays did not reach the 50% cytotoxicity target and none of the Ames assays reached the two-fold rule for a positive mutagenicity response. According to the investigators, "Use of *in vitro* assays to assess STP toxicity was of limited utility in distinguishing STP types." These assays for mutagenicity, cytotoxicity, and clastogenicity have been successfully used to characterize the toxicological properties of cigarette smoke; however, when Rickert and colleagues (2009) applied them to extracts of STPs, the results were only a small fraction (less than 10%) of those observed for extracts of mainstream cigarette smoke condensate. In the case of mutagenicity, low activities of STP extracts are consistent with the finding by Curvall and colleagues that the mutagen levels in snuff users' urines were no higher than those of in the urines of nonusers, and abstinent snuff users (Curvall et al. 1987).

Costea et al. (2009) conducted a study with aqueous extracts prepared from moist Toombak and Swedish snuff (*Ettan*, Gothia Tobak AB) that were added in serial dilutions *in vitro* to cultured cells. The cells included primary normal human oral keratinocyte (NOK) and fibroblast (NOF) cells isolated from superfluous tissues of clinically healthy buccal mucosa from adult volunteers undergoing surgical removal of wisdom teeth and commercially available dysplastic oral keratinocytes (DOK cells). Cell viability, morphology and growth, DNA double-strand breaks, apoptosis, and cell cycle were assessed after various exposure time periods. Significant decreases in cell number, DNA double-strain breaks, morphological and biochemical signs of apoptosis were detected in all oral cell types exposed to clinically relevant dilutions of Toombak extract, although to a lesser extent in normal oral fibroblasts and dysplastic keratinocytes. Cell cycle arrest was also detected in normal oral keratinocytes and fibroblasts exposed to clinically relevant dilutions of Toombak extract. Swedish snuff extract had less adverse effects on oral cells, mainly at non-clinically relevant (high dose) dilutions. DOK cells were less sensitive than their normal counterparts, suggesting that they might have acquired a partially resistant phenotype to Toombak-induced cytotoxic effects while still being prone to DNA damage that could lead to further malignant progression. There were significant differences in the levels of NNN, NNK, NAT, and NAB in the Toombak and Swedish moist snuff extracts, with the levels being approximately 100-fold lower in snus extracts. The concentration of nicotine in the Toombak extract was 3 mg/ml and was 1.7 mg/ml in the snus extract. The investigators concluded that this study indicates a greater potential for Toombak to induce adverse effects on normal oral mucosa than Swedish snuff.

4.2.2 Effects of Snus on Immunological Parameters

Hasseus and colleagues (1997) assessed the effects of Swedish moist snuff extract (*Röda Lacket*, Svenska Tobaksbolaget, Göteborg, Sweden), alkaloids, and TSNAs (NNK, NDMA) on T-cell mitogenic response to concanavalin A (con A) in order to explore the potential effects of Swedish moist snuff extract on the immune system. Con A is a mitogenic substance that is used to induce T-cell proliferation. The investigators used oral mucosal epithelial, T, and spleen cells from 8- to 10-week old rats in a series of *in vitro* assays. Results indicated that high concentrations of the water-soluble extract from Swedish moist snuff significantly inhibited con A-stimulated T-cell proliferation induced by accessory cells from rat oral epithelium, implying cytotoxicity and the potential for local immunosuppression. Alkaloids and TSNAs however, were found to have no significant effect on cell proliferation in spleen, epithelial, or T-cells.

Swedish snuff extract, alkaloids, and TSNAs showed no mitogenic properties. Based on the results of this study, the authors concluded that although the individual alkaloid and TSNA components of snuff demonstrated no significant cytotoxic effects, these ingredients may have a synergistic action together with other snuff components that could result in interference with regulation of cell growth.

4.2.3 Effects of Snus on Cellular Proliferation and Epithelial Changes

Three studies were identified that have attempted to understand the cellular mechanisms involved in snuff-induced epithelial changes using oral tissues or cells (Ibrahim et al. 1996; Merne et al. 2002; Wedenberg et al. 1996). These studies examined the effect of Swedish snuff on indicators of cellular proliferation (e.g., Ki-67) and on tumor suppressor and differentiation markers (e.g., p53 tumor suppressor gene). Two studies compared p53 and Ki-67 expression in oral lesions obtained from Swedish snuff users to expression in normal oral tissue from non-snuff users (Merne et al. 2002; Wedenberg et al. 1996). In addition, Andersson et al. (2006) conducted a study to examine the effect of snus on the growth of periodontal ligament fibroblast cells from healthy subjects.

A study by Ibrahim and colleagues (1996) evaluated p53 protein staining in snuff-induced premalignant oral lesions and oral squamous cell carcinomas (SCC) among 15 Swedish snuff and 22 Sudanese snuff users, and 115 non-snuff users. None of the suspected pre-malignant oral lesions from Sudanese snuff dippers or non-snuff-dippers expressed p53. Only 2 out of the 15 oral fibro-epithelial hyperplastic lesions from Swedish snuff-dippers expressed p53. Of the 14 SCCs from Sudanese snuff-dippers, 21% (3/14) expressed p53. Of the 14, 60, and 41 SCCs from non-snuff dippers from the Sudan, Sweden, and Norway, 64% (9/14), 65% (39/60) and 68% (28/41) expressed p53, respectively. A statistically significant difference in expression of p53 was found in SCCs from Sudanese snuff dippers compared to those from non-snuff-dippers from the 3 countries. There was a statistically significant lower relative frequency of p53 lesions in the Sudanese and Swedish snuff dippers than the non-snuff dippers, indicating that the pathogenesis of oral lesions from snuff dippers may involve a p53-independent pathway.

Wedenberg and colleagues (1996) observed increased expression of both p53 and Ki-67 proteins in upper lip biopsy samples among 15 snus users compared to biopsy samples from four control individuals (no history of snuff dipping or tobacco consumption) with normal oral mucosa. The investigators reported overexpression of Ki-67 protein and mutant tumor suppressor p53 protein in the biopsy samples from the snus users and no expression in the control biopsies. None of the snuff-induced lesions (SIL) reportedly showed any clinical or histopathological signs of epithelial dysplasia or squamous cell carcinoma.

Merne and colleagues (2002) evaluated the expression of proteins involved in cell proliferation (Ki-67, PCNA), cell cycle regulation (p53, p21), and keratins in biopsy specimens from 14 men with SIL to 12 biopsy specimens from normal buccal mucosa of men who had never used any type of tobacco products. All participants in the snuff group had used “loose non-fermented Scandinavian moist snuff” and three were also using portion-packed snuff. Merne and colleagues noted that SILs were characterized by a thickened epithelium, but found that this was not due to increased cellular proliferation; rather, it was due to an increased lifespan of the differentiating cells. The SILs were associated with a significant decrease in Ki-67 expression

and with no significant difference in the percent of cells staining for p53. None of the biopsy specimens showed epithelial dysplasia, supporting the view that premalignant or malignant changes are rare in SILs. The authors interpreted these findings to indicate that SILs are associated with a decrease in cell proliferation and speculated that this is why lesions induced by Swedish snuff are not likely to become dysplastic.

In an *in vitro* study, Merne and colleagues (2004) reported that a snus extract disturbed epithelial differentiation but did not stimulate cellular proliferation. A cell line of immortalized epithelial skin (HaCaT cells) was grown for 6 to 18 days in the presence of 1% commercial moist Swedish snus extract (*Ettan*®, Gothia Snus, Sweden) and then examined histochemically. Treatment of the cells for more than 12 days resulted in morphological changes (loss of basal cell layer, apoptotic cells, and impaired cellular adhesion) and disturbances in the differentiation process, but cellular proliferation was not increased.

Andersson and colleagues (2006) conducted a study to examine the effect of Swedish snus (*Ettan*, Gothia Tobak AB, Sweden) on the growth of periodontal ligament fibroblast cells from healthy subjects. The aim of the study was to evaluate the effect of Swedish moist snuff on fibroblast growth and hard tissue production, and compare results to moist snuff from the US. Cells from three healthy volunteers were isolated and grown in culture and snus extract was added in varying concentrations (0.3%, 1%, 3%). After 24 hours, the cells were evaluated for alkaline phosphatase levels (marker of cell differentiation) and changes in growth and morphology. Low concentrations of snus generally had little effect on cell numbers, whereas the highest concentration resulted in a reduction in the number of cells as well as the production of alkaline phosphatase. The authors concluded, "Further studies have to be made to understand the effect of smokeless tobacco on periodontal tissues. However, from this study can be concluded that smokeless tobacco has biological effects in terms of reduced PDL cell growth and production of alkaline phosphatase."

4.3 *In Vivo* Toxicological Studies

4.3.1 Studies with Tobacco-Specific Nitrosamines

Very few *in vivo* toxicological studies have been conducted to evaluate the effects of orally administered snus in experimental animals. However, several toxicological studies have been performed to evaluate the potential health effects of TSNAs, which are believed to be the most important carcinogens in tobacco products. Studies have reported that TSNAs are transformed into methylating agents through their metabolic activation and methylated TSNAs are able to form DNA adducts which may cause mutations in oncogenes for proteins involved in cellular proliferation, cell cycle arrest, and tumor development (Hecht et al. 1993; Hoffmann et al. 1994; Zhang et al. 2009). Importantly, DNA adducts are considered only one factor in the complex process of tumor development and the mere presence of DNA adducts is probably not sufficient for the carcinogenic process to proceed.

As previously discussed, TSNAs are formed during tobacco production and processing. Compared to other oral tobacco products, snus contains very low levels of TSNAs, as does the saliva of Swedish snus users (Hoffmann and Adams 1981; Österdahl and Slorach 1988). TSNAs are of particular public health concern because six different TSNAs (NAB, NNN, NNAL,

NAT, iso-NNAC, and NNK[40]) have been shown at high doses in animal models to cause benign and malignant tumors (Hecht 1998). However, tumor formation occurred at sites other than the oral cavity, and with the exception of one study (Hecht et al. 1986), TSNAs have not been reported to cause oral malignancies via the oral route of administration in animal studies.

Zhang et al. (2009) conducted a study to evaluate the potential for NNN treatment to result in the formation of pyridyloxobutyl (POB)-DNA adducts in the nasal olfactory, nasal respiratory, and oral mucosa of F344 rats treated with (R)-NNN or (S)-NNN in the drinking water (10 ppm, 1-20 weeks). Adduct levels in the nasal respiratory mucosa exceeded those in the nasal olfactory and oral mucosa. (R)-NNN treatment generated 2-4 times more adducts in the nasal olfactory and respiratory mucosa than did (S)-NNN at most time points. In the oral mucosa, the opposite stereoselectivity was observed, with (*S*)-NNN treatment producing 3-5 times more POB-DNA adducts than did (*R*)-NNN. Studies conducted previously by these investigators showed that adducts from (*S*)-NNN predominated in esophagus and liver, while adducts from (*R*)-NNN were greater in lung. This study showed that adducts from (*S*)-NNN predominated in oral mucosa, while those from (*R*)-NNN were greater in nasal olfactory and respiratory mucosa. This tissue-related stereoselectivity of POB-DNA adduct formation by NNN enantiomers probably results in part from the tissue distribution of NNN enantiomers after oral administration. The authors concluded that POB-DNA adduct formation in the nasal olfactory and nasal respiratory mucosa was similar to that previously observed in the lung, whereas in the oral mucosa, the trend resembled that in the esophagus. In addition, the types of adducts differed between the nasal olfactory and the oral mucosa. The authors further concluded that these results indicate that different mechanisms are involved in NNN metabolism and tumorigenesis in rat nasal and oral tissues: NNN enters the nasal mucosa through the circulation, and tissue-specific metabolism is important, while in the oral mucosa, direct exposure and local activation both play significant roles. The investigators recommended that carcinogenicity studies using (R)– and (S)–NNN would be useful to further assess the importance of POB-DNA adducts in the induction of tumors by NNN in these tissues.

One study reported increased rates of non-malignant oral papillomas in test rats induced by 131 weeks of oral swabbing of NNN and NNK (Hecht et al. 1986). However, the investigators only included one NNN/NNK dose group, which precludes an assessment of the dose-response relationship between TSNA exposure and tumor formation. The relevance of these data is somewhat confounded by the observation of fewer oral cavity tumors among rats after oral swabbing with a US snuff extract enriched with NNN and NNK (3 tumors/30 rats) compared to the number of oral cavity tumors observed among rats after oral swabbing with the same dose of NNN and NNK (8 tumors/30 rats).[41] Hecht and colleagues speculated that the snuff extract may have contained inhibitors of tumor formation. The relevance of this finding to snus is unknown because US moist snuff and Swedish snus are processed differently and are considered different types of STPs. Concern over the potential health effects of these two

[40] TSNAs acronyms are as follows:
NAB=N-Nitrosoanabasine, NNN=N'-Nitrosonornicotine, NNAL=4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, NAT=N-Nitrosoanatabine, iso-NNAC=4-(Methylnitrosamino)-4-(3-pyridyl)butyric acid, and NNK=4-(Nitrosomethylamino)-1-(3-pyridyl)-1-butanone.

[41] The tumor incidences were not statistically significantly different between the two groups. In addition, the snuff extract used by Hecht et al. was prepared from a leading commercial US moist snuff (brand not specified).

TSNAs is also tempered by the finding that the only oral tumors observed by Hecht and colleagues (1986) after oral swabbing with NNN and NNK were papillomas, which are benign epithelial tumors. Additionally, the daily dose of the two TSNAs (NNN and NNK) chronically administered to rats in the Hecht et al. (1986) study were significantly higher (400 µg/kg/day) than the estimated daily oral exposure of TSNAs from Swedish snus (1.6 µg/kg/day) (Österdahl and Slorach 1988). Furthermore, the levels of TSNAs in snus have decreased since 1988, so the estimated daily oral exposure levels are likely to be even lower than the doses chronically administered to rats in the Hecht et al. (1986) study.

Differences have been reported for how NNK is metabolized in different organs for the same species and between the same organ from different species, possibly due to differences in experimental methods used to evaluate NNK metabolism. Because of this uncertainty, Richter et al. (2009) evaluated the *in vitro* kinetics of NNK metabolism under identical experimental conditions using A/J mice, Fischer 344 rats, and human lung tissue explants in tissue culture and compared these results to previously published results for Syrian golden hamster using identical experimental conditions. The concentration-dependent percentage contribution of the three major pathways of NNK metabolism (carbonyl reduction, α-hydroxylation, and *N*-oxidation) showed large interspecies variation. The metabolism of NNK by α-hydroxylation is considerably lower in human lung as compared to that observed in rodent species, suggesting that extrapolation of *in vitro* rodent data to man may result in invalid conclusions about the capacity of the human lung to activate NNK under realistic conditions of NNK exposure expected to occur in man. The authors concluded, "In summary, the results of this study clearly demonstrate that human lung metabolism of NNK differs from that observed in rodent species across the entire NNK substrate concentration range." In addition, they stated, "The data further suggest that extrapolation of rodent data obtained from *in vitro* studies performed with high NNK substrate concentrations (which are never achieved in man under realistic conditions of NNK exposure) probably results in invalid conclusions since the capacity to metabolize NNK by α-hydroxylation in rodents is further accentuated on extrapolation to low substrate concentrations."

Nilsson (1998) extrapolated the cancer risk of TSNAs in snus using toxicological data, and compared these results to background cancer rates taken from the Swedish Cancer Committee. Using the results of two chronic rat studies that exposed rats to NNK or NNN, Nilsson calculated two cancer potency factors using the linearized, multistage model.[42] Nilsson assumed two different NNK and NNN levels in snus (at two different time points), a 70 kg body weight, 60% absorption of TSNAs from snus, 20 g snus consumed per day, and a population base of 200,000 snus users. The cancer potency factors were multiplied by these assumptions to estimate the number of extra cancer cases per year.[43] Nilsson then compared the incidence of extra cancers from TSNA exposure (i.e., 6 and 44 extra cancer cases at the two different time

[42] Cancer potency factors were not based on the Hecht et al. (1986) dataset because of a lack of a dose-response relationship (Hecht et al. used only single doses). In addition, the linearized, multistage model implemented by Nilsson required multiple dose groups, which precluded the use of the Hecht et al. dataset. As such, two separate chronic animal studies that investigated the carcinogenicity of NNK and NNN were used to derive cancer potency factors (Rivenson et al. 1988, Griciute et al. 1986).

[43] Nilson estimated that NNK and NNN absorbed doses were 10 µg/day and 46 µg/day (using 1981 TSNA levels in snus) and 24 µg/day and 460 µg/day (using 1981 TSNA levels in snus), assuming 10 grams of snus per day (adjusted dry weight), and 60% absorption.

points)[44] to the background incidence of cancer of the oral cavity, lips, and pharynx in the total Swedish nonsmoking population, based on data from the Swedish Cancer Committee. This background incidence was 4 cancer cases per 200,000 persons per year, which is much lower than the approximately 6 and 44 extra cancer cases predicted from the two animal studies, using 1983 and 1981 levels of TSNAs, respectively.

As is evident from the above calculations, extrapolation of rodent data appears to overestimate the risk of human oral cavity, lip, and pharynx cancers due to TSNA exposure for users of snus. Nilsson attributes this discordance to possible pharmacokinetic differences between rats and humans, as well as modulation of the carcinogenic effects of TSNAs by anticarcinogens in snus. This analysis suggests that toxicological studies with TSNAs cannot be relied upon as the sole determinant for evaluating the potential carcinogenicity of snus. Differences in how NNK is metabolized between different species should also be considered (Richter et al. 2009).

4.3.2 Toxicological Studies with Swedish Snus

Seven toxicological studies were identified that evaluated the potential carcinogenicity of Swedish snus in mice or rats (Hirsch et al. 1984; Hirsch et al. 1986; Hirsch and Johansson 1983; Hirsch and Thilander 1981; Larsson et al. 1989; Sand et al. 2002; Stenstrom et al. 2007). Six of these studies examined the potential for the development of oral tumors in rats, whereas one study was designed to evaluate gastric tumors in mice following treatment with Swedish snus. Details about the study designs and results are provided in Appendix III-I.

Six studies used an experimental model in which Swedish snuff (*Röda Lacket*, Svenska Tobaks AB, Sweden) was administered to rats through a highly invasive method involving surgically created oral test canals (Hirsch et al. 1984; Hirsch et al. 1986; Hirsch and Johansson 1983; Hirsch and Thilander 1981; Larsson et al. 1989; Sand et al. 2002). In each study, 200 mg of Swedish snuff was injected in the test canal twice a day, five days per week, for up to 30 months, and remained there until manually removed. This model was designed to mimic prolonged use of Swedish snuff in the oral cavity. Experimental observations from these six studies were unremarkable with the exception of increased tumor incidence (in both oral cavity and distant sites) in animals that were infected with herpes simplex virus-1 (HSV-1) and treated with Swedish snuff (Hirsch et al. 1984; Larsson et al. 1989).

Although there is a suggestion of synergism between HSV-1 and Swedish snuff, the two studies reporting this interaction did not yield consistent results (Hirsch et al. 1984; Larsson et al. 1989). One study (Hirsch et al. 1984) reported a statistically higher number of tumors in rats treated with snuff (18 tumors/10 rats) or snuff and HSV-1 (16/7 rats) compared to the number of tumors

[44] 1983 levels of TSNAs:

$$\frac{(0.0016 \text{ mg NNK/g snuff} \times 10 \text{ g snuff/day} \times 0.6)}{70 \text{ kg}} \times \frac{0.086 \text{ cancers}}{\text{mg/kg/day}} \times 200{,}000 \text{ persons} = 2.4 \text{ extra cancers}$$

$$\frac{(0.0076 \text{ mg NNN/g snuff} \times 10 \text{ g snuff/day} \times 0.6)}{70 \text{ kg}} \times \frac{0.029 \text{ cancers}}{\text{mg/kg/day}} \times 200{,}000 \text{ persons} = 3.8 \text{ extra cancers}$$

1981 levels of TSNAs:

$$\frac{(0.004 \text{ mg NNK/g snuff} \times 10 \text{ g snuff/day} \times 0.6)}{70 \text{ kg}} \times \frac{0.086 \text{ cancers}}{\text{mg/kg/day}} \times 200{,}000 \text{ persons} = 5.9 \text{ extra cancers}$$

$$\frac{(0.077 \text{ mg NNN/g snuff} \times 10 \text{ g snuff/day} \times 0.6)}{70 \text{ kg}} \times \frac{0.029 \text{ cancers}}{\text{mg/kg/day}} \times 200{,}000 \text{ persons} = 38.2 \text{ extra cancers}$$

in control rats (11/10 rats) or HSV-1 inoculated rats (10/7 rats). The tumors included both benign and malignant tumors (thyroid, forestomach, breast, liver, ovary, and skin) in the snuff only or in the snuff and HSV-1 infected rats. HSV-1-infected test animals administered snus in the Hirsch et al. (1984) study developed squamous cell carcinomas of the oral cavity at a greater incidence than control rats (2/7 vs. 0/10). In contrast, Larrson and colleagues (1989) did not identify an increase in oral squamous cell carcinomas in HSV-1-infected test animals administered Swedish snuff compared to other test groups.[45] However, these authors did observe a statistically significant increase in the total number of non-oral cavity malignancies (ear duct, nasal, forestomach tumors) in HSV-1-infected rats administered Swedish snuff compared to other test groups.

HSV-1 is a ubiquitous human oral pathogen, is the cause of recurrent herpes labialis (cold sores), and approximately 70% of the adult population in the US and western Europe is seropositive for HSV-1 (Larsson et al. 1989; Silverman, Jr. 1998). Sufficient experimental data are not available to explain the apparent synergism between HSV-1 and snus observed in the two animal studies (Hirsch et al. 1984; Larsson et al. 1989), although mechanisms have been postulated, such as the effect of HSV-1 on DNA repair mechanisms, proto-oncogene activation, and immunosuppression (Larsson et al. 1989). Hirsch and colleagues (1984) hypothesized that Swedish snuff may restrict HSV-1 cytolysis, enabling an infected cell to transform into a malignant phenotype instead of being destroyed. Because more than 70% of western Europeans are seropositive for HSV-1, it would be expected that many Swedish snuff users would develop malignancies at either oral or non-oral sites; however, a review of epidemiological data clearly establishes that this has not occurred.

One of the six studies that employed surgically created test canals in rats was designed to evaluate the effect of long-term administration of cancer-promoting substances on oral subepithelial mast cells (Sand et al. 2002). Mast cells are believed to be involved in the development of and defense against tumors, and some studies have shown that animals that are deficient in mast cells show increased tumor incidence after exposure to carcinogens. Rats received either 4-NQO (a known carcinogen for the oral cavity of experimental animals), HSV-1, oral Swedish snuff, HSV-1 plus oral snuff, or 4-NQO plus oral snuff in the surgically created canal. The number of mast cells was significantly decreased in rats who received 4-NQO compared to those who received a control treatment, but not in those who received snuff alone, HSV-1 alone, or snuff plus HSV-1. Thus, snus (either alone or with HSV-1) had only minimal effects on mast cells.

A study was conducted to evaluate whether the addition of the *General* snus administered in the diet (5-9% for 6 months) was associated with the development of gastric cancer in wild type mice (WT) and a strain of mice genetically predisposed to developing gastric cancer (INS-GAS) (Stenstrom et al. 2007). INS-GAS mice are hyper-secretors of gastrin and they inevitably develop gastric cancer. The study also evaluated whether concomitant infection with *Helicobacter pylori* (Hp) (which increases risk of gastric cancer) influenced the effect of the snus. The treatment groups included 11 WT controls, 8 WT + snus, 17 WT + snus + HP, 8 INS-

[45] Non-oral malignancies in the HSV-1-infected rats administered Swedish snus included squamous cell carcinomas of the ear duct, adenocarcinomas of the breast, and sarcomas of the stomach, salivary gland, and scrotum.

GAS, 8 INS-GAS + snus, and 12 INS-GAS + snus + HP treated mice. There were no gastric tumors and no significant gastric pathology in either untreated WT mice or WT mice that consumed snus (mild morphological changes in the stomachs of WT mice treated with snus alone). INS-GAS mice had a 25% rate of gastric cancer without snus consumption and a 50% rate with snus consumption. Hp infection markedly increased the rate of gastric cancer in both the WT + snus group (53%) and the INS-Gas + snus group (100%). The investigators concluded that snus potentially accelerates gastric cancer development when present in combination with hypergastrinemia and/or Hp infection in mice. However, this study has some methodological weaknesses, including small numbers of animals as well as the failure to include a control group of either WT or INS-GAS mice that received only Hp infection. Thus, it is not possible to draw informed conclusions about the interaction of snus and Hp infection. Furthermore, epidemiological studies show no relationship between the use of snus and an elevated risk of stomach cancer.

4.4 Summary of Toxicological Studies

Swedish snuff/snus has been investigated in *in vitro* assays for genotoxicity, cellular proliferation, and epithelial changes in human biopsy samples and cell cultures. The results from the Curvall et al. (1987) and the Jansson et al. (1991) studies support the conclusion that snus is not genotoxic in mammalian cells. The studies by Rickert and colleagues (2009) and and Costea et al. (2009) also reported that snus is not mutagenic, clastogenic, or cytotoxic. There is limited data from a single *in vitro* assay to suggest that snus may inhibit the ability of the oral mucosa to instigate a local immune response; however, the biological relevance of this *in vitro* finding is unclear. Three studies examined the effect of snus on markers of cellular proliferation and differentiation; the results are not entirely consistent (perhaps due to methodological differences), but suggest that p53 mutations are not frequent in snus-induced lesions. A single study suggests that snus has effects on the growth of periodontal ligament cells, but the significance of these findings is not clear. The value of these diverse and few *in vitro* studies for evaluating the health effects of snus in humans is limited. The differences between the results of *in vitro* studies with snus and/or other tobacco products may be attributed to the use of different tobacco brands, as well as different cell types and species. Different methods of extraction and quantification of the extract concentration are also likely to produce different results, and a correlation between the concentrations of snuff extract used in various studies is difficult to achieve, as various methods for obtaining the extracts are used. Thus, to accurately compare the *in vitro* biological effects of two different types of smokeless tobacco products, extracts should be obtained at the same time following the same protocol.

The extrapolation of *in vitro* toxicology data to human risk is complicated (Johnson et al. 2009). Some of the reasons for this include the use of cells and modes of action where: (a) the mode of action and/or metabolic conditions in the cell culture model may not exist in humans; (b) chemicals may exert their carcinogenic effects in humans via nongenotoxic mechanisms for which there are very limited toxicology assays; (c) many cell models have mutations and increased cell proliferation that are not present in normal human cells; (d) many cell models do not have cellular processes that are present in humans (e.g., DNA repair or detoxification pathways); and (e) the effects in cell cultures and humans occur at different levels of exposure (Johnson et al. 2009). Another important limitation is that toxicology assays assess short-term

exposures and immediate effects, whereas cancer develops in humans over a long exposure and latency period.

There are a limited number of oral carcinogenicity studies with Swedish snus using a surgically prepared oral canal rat model and one dietary study with snus to evaluate gastric cancer in mice. Although the oral canal models may not accurately represent the types of exposure that snus users would experience, Swedish snus was not found to be tumorigenic in the oral canal studies, where the animals were exposed to relatively high doses of snuff for a long period of time. Groups such as LSRO have recommended that future research develop new, less invasive validated animal models of exposure and diseases that are relevant to STP use in order to evaluate the safety of different tobacco products (LSRO 2008).

The Institute of Medicine (2001) has stated that *in vitro* and *in vivo* toxicological studies with tobacco containing products are very important for: 1) determining those products that are not likely to result in measurable harm reduction, 2) identifying unforeseen reactions (e.g., if a product reduces exposure but does not decrease tumors, then there might be some component or combination of component or combination of components that is either new or more important than those changed in the product), 3) providing supportive evidence for the use of a particular bioassay in humans (e.g., if a biomarker predicts cancer risk in experimental animals), and 4) assessing the dose-response and the shape of the regression of risk for the potentially reduced exposure product as exposure is reduced, although the data should be considered qualitative or semiquantitative and cannot be extrapolated directly to human smoking risk.

5 Human Health Effects of Snus

5.1 Introduction

During the past 60 years, the potential adverse effects of snus on human health have been examined in an increasing number of epidemiological studies. These studies have been performed to determine whether use of snus is associated with an increased risk of developing any of various conditions and diseases or an increased disease-specific mortality risk. These conditions include: dental effects, oral mucosal lesions, oral cavity, gastrointestinal, or other cancer, cardiovascular disease, diabetes, and adverse pregnancy outcomes.

This systematic review of the potential health effects of snus begins by delineating the epidemiological investigations conducted to evaluate potential associations between snus use and various health conditions. With only one or two exceptions, these are studies of Swedish snus that were published in the English-language literature. A comprehensive and systematic review of the validity of each of these studies is beyond the scope of this document. Commentaries on many of these studies have appeared in the peer reviewed literature and elsewhere and are cited when they directly relate to the purpose of this report.

The studies discussed here assessed differences in prevalence, incidence or mortality related to different levels of snus use (ranging from none to frequent or heavy use). Although no individual study can determine a causal relation, all of these studies contribute to our knowledge of the potential effects of snus use when considered in the broader context of other research (epidemiological as well as chemical and toxicological). Epidemiological studies of the highest quality contribute the most to a causality determination. The design and careful planning and conduct of the study are important in considering a study's contribution to the weight of evidence for the determination of a causal association between exposure and outcome in humans. Epidemiological study designs include intervention studies and several types of observational studies. The study participants' exposure status is under the control of the investigator in *intervention studies* such as clinical trials. There are no intervention studies of the long-term health effects of snus use in humans.

Evidence of the long-term health effects of snus comes from a variety of types of observational studies including: cohort, case-control, cross-sectional and ecologic studies. In *cohort* studies, people with the exposure of interest are followed over time and observed for the development of one or more health outcomes. The rates of these health outcomes are compared to persons without the exposure under study. *Case-control* studies are comparisons of cases (who have the outcome of interest) and suitable controls to determine if they have different odds of exposure. This type of study is used for rarer outcomes (such as specific types of cancer) where a low number of cases are expected in a population. *Cross-sectional* studies assess the exposure and health outcome of interest at a single point in time, and thus cannot necessarily establish the temporal sequence for dynamic exposures.

Unlike the study types above, where the units of analysis are individuals, *ecologic* studies compare the populations with different prevalences of the exposure (for example, cancer rates in Sweden, where snus is available, compared to other European countries where snus use is less common).

As syntheses of the accumulated evidence are more informative than any single study, systematic reviews and meta-analyses are listed in Appendix V and discussed where applicable. In the last several years, researchers investigating the health effects of snus have used meta-analysis to quantitatively combine the results of different epidemiological studies. Meta-analysis is the statistical combining of effect estimates from separate but similar epidemiological studies, leading to a single quantitative estimate of the pooled individual study results. To determine if studies are "similar" enough to combine, scientists develop criteria for including studies in the analysis that consist of similarity of exposures, referent populations and other study characteristics, such as consideration of other risk factors, including smoking and alcohol consumption. Whether studies are similar enough to be combined can also be measured statistically (called "heterogeneity"), and if heterogeneity exists, then the sources of heterogeneity should be investigated and reduced, if possible, by combining only the studies that are similar with respect to exposure and study characteristics.

For any study type, it is important to evaluate several methodological issues, including (but not limited to) the following: (1) exposure and outcome assessment; (2) consideration of other risk factors; and (3) appropriateness of the data analysis and other potential sources of error and uncertainty. Differences in these aspects of study methodology are important to consider as these may contribute to variation in the study results.

5.1.1 Exposure Assessment

Studies of the health effects of snus use typically rely only on self-reports of snus use. As with all studies of self-reported behavior, this may result in misclassification and affect the study results. Although this report focuses on Swedish snus, it is possible that some of the participants in the studies discussed below used other STPs (instead of or in addition to snus). The most simplistic exposure assessment differentiates people who did and did not ever use snus, yielding a lifetime prevalence estimate of snus use. Snus use is sometimes further delineated: current and former snus users are compared to those who never used snus or participants who use snus daily are compared to occasional users and never users.

Assessments of the duration of snus use, amount ("dose") of snus used and time since cessation (among former users) are less common. Understanding these snus use variables and the potential for bias is important for reviewing and evaluating the literature about trends in snus use and the health effects of snus. An example of the possible snus use variables is in Table 5-1. The most common method of snus use is to deposit 1 to 2 grams (g) of loose product or 1 pouch of snus in the vestibular area inside the upper lip (Andersson et al. 1995). Andersson and colleagues found that 73% of snus users used only loose snus, 13% used only snus pouches, and 14% used both loose snus and snus pouches (Andersson et al. 1994). A later survey of 2,914 snus users between the ages of 18 and 72 years in Sweden found that 38% used only loose snus, 59% used only snus pouches and 3.5% used both loose snus and snus pouches (Digard et al. 2009). Much of the difference is likely attributable to temporal changes but different eligibility criteria, gender (females are more likely to use snus pouches (Digard et al. 2009) and random error (less than 50 snus users participated in the study by Andersson et al. (1995)) may have also contributed to the difference.

The size of portions of loose snus and pouches, number of portions used per day and the amount of time that users keep snus in their mouth vary considerably. Several authors reported that the average duration of snus use ranges from 7 to 16 hours per day (Andersson et al. 1994; Axell et al. 1976; Digard et al. 2009; Mornstad et al. 1989). The mean daily consumption is approximately 19 g of loose snuff or 10 g portion-bag-packed snuff (Axell 1998; Nyren 2001). Grams of snus per day may be reported as either a continuous variable (e.g., Digard et al. 2009) or a categorical a variable (Hergens et al. 2007) and is likely to be imputed from responses to questions about the number of portions or packages (tins) used.

Information about snus use patterns is crucial for understanding the epidemiology but the lack of consistency in how snus use is defined makes it difficult to compare studies. It is unknown to what extent measurement error contributes to the results of the studies discussed here are there is no gold standard against which to validate self-reported snus use.

Table 5-1: Mean Daily Snus Use in Sweden (Standard Deviation)

Pouched Snus	**Male (n=1,380)**	**Female (n=333)**
Packages per day	0.54 (0.3)	0.49 (0.2)
Portions per day	12.0 (6.6)	10.4 (5.6)
Consumption per day (g) from packages[1]	12.4 (7.2)	9.3 (6.6)
Consumption per day (g) from portions[2]	11.8 (7.0)	8.5 (6.2)
Time per day (hrs)	13 (10.9)	7.7 (5.9)
Length of time in mouth (min)	69.7 (51.8)	47.3 (35.0)
Loose Snus	**Male (n=1,075)**	**Female (n=23)**
Packages per day	0.59 (0.3)	0.58 (0.3)
Portions per day	12.3 (6.6)	13.5 (7.0)
Consumption per day (g) from packages[1]	29.3 (16.5)	29.0 (14.2)
Consumption per day (g) from portions[2]	32.1 (22.7)	33.8 (21.8)
Time per day (hrs)	12.7 (7.3)	14.6 (11.0)
Length of time in mouth (min)	69.6 (41.6)	56.1 (27.1)

Source: Digard et al. 2009
1. Consumption calculated from the (self-reported) number of packages (tins) of snus used per day.
2. Consumption calculated from the (self-reported) number of portions of snus used per day.

Assessment of the outcome is crucial for studies of snus use. Disease-specific mortality is assessed in many of the studies of the health effects of snus, although some of the cohort studies measure incidence and cross-sectionals studies typically measure prevalence. Incidence is a good measure of mortality for diseases with a high case fatality rate (e.g., lung or pancreatic cancer) but not for diseases with a lower fatality rate (e.g., oral cancer).

5.1.2 Consideration of Other Risk Factors

Adequate consideration of other risk factors (quantitatively as well as qualitatively) is important for studies of the health effects of snus. Other risk factors (e.g., alcohol use and diet) must be considered separately for each outcome being studied and appropriate data analysis techniques such as stratification or multivariable regression must be applied. Smoking is an example of one such risk factor and deserves careful consideration as it is one of the major causes of many of the outcomes discussed below and STP users may be likely to smoke or to have previously smoked. Smoking is an established strong risk factor for some outcomes (such as lung and oral cancer) such that the best analytic strategy is to conduct separate analyses for smokers and non-smokers. Attempting to control for the effects of such strong risk factors by including smoking in a statistical multivariable model may not be adequate to investigate the independent effect of snus use on health outcomes. All else being equal, a study of oral cancer that "controls" for smoking by including a variable that merely differentiates current, former and never smoking is less informative for assessing the independent effects of snus use on oral cancer risk than a study that presents separate analyses for smokers and non-smokers. Controlling for smoking in a multivariable model will prevent the assessment of potential differences in the effect of snus use between smokers and non-smokers.

5.1.3 Appropriateness of the Data Analysis and Other Potential Sources of Error and Uncertainty

The most commonly measured source of error in epidemiological studies is random error (as assessed by p-values and confidence intervals). Adequate sample size is an important consideration when assessing the contribution of study results to an accumulation of evidence as it affects the power to detect a true association if it exists. The smallest stratum, which has the greatest effect on whether an effect estimate is statistically significant, in many of these studies is the number of exposed cases. Although sample size (and the consequent statistical significance) is important to consider, it is merely one element of a critical review of the epidemiological literature. Statistical significance is a reflection of random error and the other important potential sources of error in studies of the health effects of a behavior such as snus use are likely non-random (e.g., the aforementioned potential misclassification of snus use).

5.1.4 Determination of Etiology

Though epidemiological studies can be designed carefully to minimize the likelihood of bias, to account for alternative explanations from other risk factors, and to maximize the likelihood of getting a "true" result, no epidemiological study can ever be totally devoid of flaws or shortcomings. A single well-conducted study can raise the likelihood of detecting a causal relationship; however, the establishment of causality necessitates replication of study findings and is far more complex. Many associations represent a situation when exposures and health effects happen together, not a causal relationship. The exposure and health effect may be associated because they are both commonly associated with another risk factor or by coincidence. This why it is important to conduct robust studies that can be replicated and critically review all the available literature, including epidemiological studies, as well as toxicological and other studies.

Guidelines for reviewing the literature with the aim of assessing causation have been developed (e.g., Elwood 1998; Hill 1965) but there is no checklist that can be used to identify a causal relationship. Some of the elements of these guidelines are used as a framework for this report and include: strength of the association; dose response (increased likelihood of the health effect at greater levels of exposure); consistency in the literature; ruling out alternative explanations (as discussed above); and a reasonable biologic mechanism (discussed in the chemistry and toxicology sections).

Science is seldom clear cut, but the more rigorous the process, the more likely scientists will be able to determine if there is a causal relationship. Ultimately, however, concluding that an exposure causes a health effect requires judgment—and this judgment must be based on what is known to be important in the particular relationship of interest. Because judgment is required, not all scientists may arrive at the same conclusion about causality in the context of a particular exposure-health outcome scenario. Furthermore, judgments about causality may have to be revised as new information becomes available.

5.2 Non-Carcinogenic Oral Effects

5.2.1 Overview

This section presents a review of studies conducted to evaluate non-carcinogenic oral effects in individuals that use snus[46]. Studies that have been conducted to evaluate the risk of oral cancer associated with snus are not included in this discussion, as they are reviewed in the section on cancer. In evaluating the potential effects of snus on the oral cavity, it is useful to be familiar with the gross anatomy of the mouth. Briefly, the oral cavity is divided into the following seven anatomical sites (Dimitroulis and Avery 1998):

- Lips
- Buccal mucosa (i.e., the cheek membrane)
- Retromolar trigone (i.e., the back of the mouth)
- Anterior two-thirds of the tongue
- Floor of the mouth
- Gingivae (i.e., the gums)
- Hard palate (i.e., bony part of the roof of the mouth)

STPs such as snus possess physicochemical properties (e.g., pH, ingredient composition, particle size, humidity, and molality) that can affect the teeth and the oral mucosa (Andersson et al. 1995). Several studies have reported that non-carcinogenic oral conditions, such as dental effects, periodontal disease, and oral mucosal lesions, occur among STP (e.g., snus, snuff, chewing tobacco) users. However, these studies have many methodological weaknesses, including cross sectional design, small numbers of participants, lack of information about product identification and exposure levels, lack of data on individuals who do not use snus, and

[46] Most studies of the non-carcinogenic oral effects included individuals that have used “snuff” and they did not specify that the product was snus or what the brand name was.

failure to control for important confounders (e.g., dietary and oral hygiene habits), and thus no firm conclusions can be drawn from these studies. The use of snus does not appear to be associated with dysplastic oral lesions or pre-carcinogenic effects on the oral cavity. Additionally, there is no clinical evidence to suggest that when dysplastic lesions occur in snus users, they transform into malignancies. Details of the available studies conducted to evaluate non-carcinogenic oral effects in snus users are provided below.

A recent review by Kallischnigg and colleagues (2008) was conducted to evaluate the relationship between STP and non-neoplastic oral diseases in Europe and the US. The authors identified 50 principal studies, 20 of "snuff" conducted in Scandinavia (2 in Denmark, 1 in Finland, 17 in Sweden), 29 of chewing tobacco, snuff or other STP conducted in the US, and one of chewing tobacco conducted in England. Some of the problems encountered with these studies included small numbers of participants or exposed cases, nonrepresentativeness of the studied populations, inconsistently defined outcomes, and heterogeneous methods of exposure assessment. Exposure details such as specific brand or type of product, duration and frequency of use were often not reported. Several studies and/or case series presented data only on populations selected by smokeless tobacco or snuff use and/or presence of oral lesions, and therefore, did not allow estimation of prevalence and odds ratio. Other weakness included incomplete presentation of findings and failure to adjust for potential confounders, such as age, smoking, education, or frequency of dental visits. In addition, many of the studies did not present results separately for major subgroups, particularly regarding alternative tobacco consumption, either smoked or smokeless, making it difficult to draw conclusions about the reported findings to snus users.

Although the review provided results from the US and England studies, only the Scandinavian studies cited by Kallischnigg and colleagues (2008) are discussed for the purposes of this report; those included were studies of snuff users and did not include studies on chewing tobacco. Fifteen of the studies in Scandinavia included in the review by Kallischnigg and colleagues (2008) provided information relating snuff use to oral mucosal lesions; 11 of these studies used the endpoint "snuff-dipper's lesion" as defined by Axell (1976) (3 studies used an endpoint which appeared to be similar) and 1 study used "oral leukoplakia" as an endpoint. Fourteen of the fifteen Scandinavian studies were with moist snuff only and not other types of products (one study by Andersson and colleagues (1994) did include 9 users of chewing tobacco and 45 snuff users). Most of the 14 Scandinavian studies that evaluated oral lesions had participants that were selected on the basis of snuff induced lesions (SILs) being present. Severity of the SILs was associated with the length of time snuff was used and amount of snuff consumed per day, though the statistical significance of these relationships could not always be determined in the Scandinavian studies. The relationship of severity with duration of use was less marked and severity was also lower in users of portion-bag snuff than in users of loose snuff. The results from seven Swedish studies that evaluated periodontal and gingival diseases showed no significant relationship between snuff use and the presence of plaque or calculus, pocket depth, attachment loss, alveolar bone level, bone height, or periodontal disease. One study reported that snuff users had a significantly increased gingival index, but others did not report a relationship with gingivitis, gingival index, or gingival bleeding. Two Swedish studies showed no association between tooth loss and snuff use (Bergstrom et al. 2006; Rolandsson et

al. 2005), but one study (Hirsch et al. 1991) reported significant increases in the mean number of decayed missing and filled teeth in snuff users ages 14-19 years.

5.3 Dental Effects and Periodontal Disease

Ten studies identified in the literature address the effects of snus on the teeth and the periodontal tissues. Included in this report are nine cross sectional studies (summarized in Appendix A-1) and one case-control study (summarized in Appendix A-2). As discussed below, a review of these studies indicates that there is limited evidence to suggest that snus may be associated with some non-carcinogenic oral conditions, including dental effects (such as dental caries, tooth wear, and tooth loss) and periodontal disease.

5.3.1 Dental Effects

Five studies offer some data on the relationship between use of snus and the presence of various dental effects, including dental caries, tooth wear, and tooth loss (see Appendix A-1). Caution should be exercised in interpreting the data on dental effects as the studies were all descriptive in nature (most were cross-sectional), the exposures are poorly characterized, the product type is not described (snuff or snus, loose or pouches), and they suffer from important limitations, including failure to control for important confounders such as diet, dental habits, and socioeconomic status.

One study examined the effect of snus on dental caries. Hirsch and colleagues (1991) investigated tobacco use (including snus use) in a population of 2,145 Swedish teenagers (age 14-19 years), including 197 snuff dippers. This study found that snuff dippers had significantly higher numbers of decayed, missing, and filled teeth than did nonusers of tobacco. However, the authors acknowledge that a definitive conclusion cannot be made, given the lack of adjustment for dietary and oral hygiene habits.

A study by Ekfeldt and colleagues (1990) was designed to investigate factors associated with occlusal wear of the teeth in a population of 585 dentate Swedish adults ages 20-80. Snuff use was characterized simply with a “yes” or “no” response. The authors found that the following factors were significantly correlated with increased incisal and occlusal wear: number of existing teeth, age, sex, bruxism, use of snuff and saliva buffer capacity (pH). Use of snuff and saliva pH was a minor factor, accounting for less than 2% of the variance.

A study by Johansson and colleagues (1994) compared dietary intakes and various lifestyle factors among edentulous middle-aged people from Sweden and those who still had natural teeth, using data from the World Health Organization’s (WHO) Multinational Monitoring of Trends and Determinants in Cardiovascular Disease project. The emphasis of the project was on diet and other cardiovascular risk factors, but data were also collected on use of snuff. Analysis of information from 1,287 men and 1,330 women showed that edentulous and dentate individuals did not differ with respect to the regular use of snuff.

Hellqvist and colleagues (2009) conducted a study to evaluate the use of tobacco (cigarette smoking and Swedish snuff) and changes in its use over time among individuals aged 15-70 in Sweden, and to analyze tobacco habits in relation to socioeconomic conditions, personality aspects and dental care habits. The study comprised three cross-sectional studies conducted

in 1983, 1993, and 2003 in which 704, 686, and 625 male and female participants, respectively, were administered a questionnaire about demographics (e.g., income, marital status), medical and oral health history, dental habits, tobacco habits, and oral hygiene habits. The results revealed a statistically significant reduction of tobacco product use from 34% in 1983 to 27% in 1993 and 28% in 2003 (the main decrease was seen among smokers). At the same time, the number of users of snuff increased in all the age groups between 20 and 60 years of age. The overall prevalence of tobacco use was therefore largely unchanged between 1993 and 2003. In 2003, there was a statistically significant difference between users and nonusers of tobacco when it came to the frequency of dental visits; more tobacco users than nonusers did not visit a dentist at all or did not do so regularly. In 1993, nonusers brushed their teeth more frequently than tobacco users and this difference was statistically significant. The authors concluded that during the 20-year study, there was a reduction in the number of smokers and an increase in the number of snuff users. There was a difference between tobacco users and nonusers of tobacco based on the frequency of dental visits and oral hygiene habits, however, the results were not separated for snuff users vs. smokers. Based on the results from this study, it is possible that the reduced frequency of dental visits and oral hygiene habits in tobacco users may impact the potential for periodontal disease or other oral effects that have been evaluated in other studies on snuff or snus users.

A study was conducted by Julihn and colleagues (2008) to evaluate risk factors for incipient alveolar bone loss and subgingival calculus in 696 Swedish 19-year-olds (358 males, 328 females). The participants were from seven public dental clinics in suburban Stockholm that answered a questionnaire on general health, tobacco habits, oral hygiene habits, and their parents' socioeconomic background. The clinical and radiographic examination included registration of plaque, bleeding on probing, supra- and subgingival calculus, caries, and restorations. Incipient alveolar bone loss was recorded when the distance from the cemento-enamel junction to the alveolar crest was >2.0 mm. There were 80 participants that reported that they were daily snuff users and 26 of participants were evaluated for incipient alveolar bone loss. The adjusted odds ratio (OR) for incipient alveolar bone loss for snuff users was not statistically significant (OR=1.15, 95% confidence interval (CI) 0.7 – 1.89). The only risk factors that were statistically significantly correlated with incipient bone loss were subgingival calculus and proximal restoration $\geq$1.

5.3.2 Periodontal Disease

Periodontal disease, commonly known as gum disease, is an infection of the tissues surrounding and supporting the teeth. The most common symptom is bleeding gums, but loosening of the teeth, receding gums, abscesses in pockets between gums and the teeth, and necrotizing ulcerative gingivitis may be present as the disease progresses. The early stage of periodontal disease is characterized by gingivitis, an inflammatory condition in which the gums become swollen and bleed easily. At this stage, the disease is still reversible and can usually be eliminated by daily brushing and flossing. Later stages of periodontal disease (known as periodontitis) are irreversible, and are marked by receding gums, loosened teeth, and bone loss.

Five descriptive studies (Andersson and Axell 1989; Bergstrom et al. 2006; Modeer et al. 1980; Monten et al. 2006; Wickholm et al. 2004) and one case-control study (Kallestal and Uhlin 1992) examined the relationship between the use of Swedish snuff and periodontal disease (Appendix

A-1). A few of these studies (Bergstrom et al. 2006; Monten et al. 2006; Wickholm et al. 2004) found a modest association between snuff use and periodontal disease.

Bergström and colleagues (2006) examined the relationship between use of Swedish moist snuff and periodontal bone loss (as assessed by bone height) among healthy men who were current, former, or never-users of snuff. Following responses to the questionnaire, participants were classified as current (n=25), former (n=21), and never-users (n=38) of moist snuff. After controlling for age, there were no significant relationships, even among those with heavy snuff use (who used for 15 years or more). The user groups also did not differ with respect to other clinical characteristics (periodontal pocket depth or percentage of sites exhibiting gingival bleeding on probing).

Wickholm and colleagues (2004) compared the prevalence of periodontal disease in 4 groups of Swedish male and female adults (n=1,654), based on mutually exclusive lifetime tobacco use, nonusers of tobacco (n=549); exclusive cigarette smokers (972), exclusive snus users (54), and mixed users (99). All groups of tobacco users had a higher prevalence of periodontal disease than never-users of tobacco. There was a significant association between smoking and periodontal disease (compared to never-smoking), but there was no significant association between current snuff use and periodontal disease (compared to neverusers). The OR associated with former snuff use (n=31) was elevated, although was not statistically significant (OR=2.25, 95% CI 0.71, 5.95). In addition, the proportion of participants with unhealthy periodontal conditions did not correlate with increasing can-years of snuff use.

A study by Monten and colleagues (2006) reported that the use of snus is associated with gingival recessions, but not a number of other periodontal conditions among adolescent 19 year old Swedish boys (33 snuff users, 70 controls). The study outcomes were plaque score, gingivitis, probing pocket depth, clinical attachment loss, alveolar bone level, and gingival recessions. There were no significant differences between boys who used snus but did not smoke and boys who had never used tobacco with any of the first 5 outcomes. However, participants with gingival recessions had significantly increased odds of using snus (OR=3.7; 95% CI: 1.40-9.87), after adjusting for plaque, gingivitis, and tooth-brushing. The authors concluded that, in this population of Swedish adolescents, use of snus was not associated with the prevalence of periodontal disease except for a significantly higher prevalence of gingival recessions.

Two older studies suggest that the use of snus (loose or portion-bag snuff) may result in gingivitis in some participants (Andersson and Axell 1989; Modeer et al. 1980). These studies do not provide strong enough evidence to associate the use of snus with periodontal disease. Andersson and Axell (1989) reported that STP associated gingival recessions were found in 42/184 (23.5%) of the participants that used loose snuff compared to 2/68 (2.9%) of the participants that used portion-bag snuff. Modeer and colleagues (1980) reported that of 21.5% of 232 children ages 13-14 smoked (boys and girls) and 11% used snuff regularly (boys). Snuff usage was significantly correlated with gingival index after controlling for plague. However, the evidence to support an association of snuff with gingivitis is limited by the inability to control for confounding variables in these studies. Finally, one case-control study of factors associated with buccal attachment was identified in which data on snuff users were collected (Kallestal and

Uhlin 1992) (see Appendix A-2). The authors did not present any quantitative data on the relationship between STP use and loss of buccal attachment, but they stated that cases and controls did not differ in the use of STP.

While the evidence is conflicting, some researchers have ascribed a reduction in blood flow caused by nicotine-induced vasoconstriction as linking tobacco use to periodontitis and gingivitis. In experimental studies performed by Mavropoulos and colleagues (2001; 2002), healthy study participants experienced increased (as opposed to decreased) gingival blood flow in and around the site of snus exposure. Blood flow was also found to increase in the contralateral gingiva and forehead skin. This vasodilation is likely due to both autonomic and antidromic reflexes. Mavropoulos and colleagues (2002) noted that the tissues of chronic tobacco users may be impaired in their ability to respond to injury and noxious stimuli, which could increase susceptibility to infections and diseases like periodontitis.

5.3.3 Oral Mucosal Lesions

A specific, well-recognized mucosal reaction is associated with use of Swedish snuff (Axell et al. 1976). It is characterized by thickening or discoloration of the oral mucosa, and occurs almost exclusively in men, the predominant users of Swedish snuff (Axell 1987). Histologic changes observed in SILs include hyperplasia of the epithelium with large, vacuolated cells, and a chevron type of keratinization. The degree of damage is related to increased pH, increased nicotine content, and period of exposure (Mornstad et al. 1989).

The published literature examining the relationship between the use of snus and oral mucosal lesions consists of approximately 20 cross-sectional studies.[47] These studies do not provide quantitative estimates of the risk of oral mucosal lesions associated with use of snus. Furthermore, many of the available studies draw from the same population of snuff users, which narrows the scope of available data. Eight studies described characteristics of oral mucosal lesions in the same population of snuff-using Swedish workers initially described by Andersson and Axell (1989). Seven studies examined the prevalence of snuff use and the characteristics of oral mucosal lesions in a large population of Swedish adults initially described by Axell (1976). These cross-sectional studies are detailed in Appendix B and most of the studies are discussed below.

5.3.4 Severity of Oral Mucosal Lesions

As previously stated, oral mucosal lesions commonly occur in users of snus with varying degrees of severity. Most of the studies summarized in Appendix B graded clinical changes associated with oral mucosal lesions on a four-degree scale that was proposed by Axell and colleagues (1976) and is still in use today (e.g., Roosaar et al. 2006):

Degree 1: A superficial lesion with a color similar to the surrounding mucosa, and with slight wrinkling. No obvious mucosal thickening.

[47] A publication by Warnajulasuriya and Ralhan (2007) reviews the literature on the clinical, pathological, cellular and molecular lesions associated with oral smokeless tobacco. However, the review is not comprehensive and fails to specify exactly what type of smokeless tobacco is studied. Other sections co-mingle information on malignant and benign lesions and fail to stress the non-malignant nature of snuff dippers' lesions.

Degree 2: A superficial, whitish, or yellowish lesion with wrinkling. No obvious mucosal thickening.

Degree 3: A whitish-yellowish to brown, wrinkled lesion with intervening furrows of normal mucosal color. Obvious thickening of the mucosa.

Degree 4: A marked, white-yellowish to brown and heavily wrinkled lesion with intervening, deep, and reddened furrows and/or a heavy thickening of the mucosa.

In studies that reported the prevalence of oral mucosal lesions in snus users, it appears that most of the participants had degree 2 and 3 lesions. The severity of oral mucosal lesions appears to be related to the duration, amount, as well as the form of snuff used daily (i.e., loose snuff vs. portion-bag snuff). For example, Hirsch and colleagues (1982) found that patients with degree 3 and 4 lesions used snuff approximately twice as long per day as patients with degree 1 and 2 lesions. With regard to the form of snuff used, Andersson and colleagues (1989; 1994) concluded that use of snuff pouches is associated with less pronounced changes to the oral mucosa than loose snuff.

Andersson and colleagues (1994) conducted a study to find out if the less pronounced clinical changes in the oral mucosa in users of oral moist snuff pouches(snus) compared with the changes in the mucosa of moist loose snus users are correlated to exposure and uptake of tobacco components such as nicotine. The study included 54 habitual users of STPs: 22 loose snus users, 23 users of snus pouches, and 9 users of chewing tobacco. The average duration of use was 14.5 years (loose snus), 7.4 years (portion-bag snus), and 9.5 years (chewing tobacco). Less pronounced clinical changes in the oral mucosa were recorded in snus pouch users compared with loose snus users. The snus pouch users showed predominantly Degree 1 and 2 lesions, while users of loose snus had more Degree 3 lesions. The clinical findings observed in the oral mucosa of users of chewing tobacco were leukoedema and slight clinical "snus changes". The average systemic dose of nicotine estimated as nicotine equivalents excreted during 24 hours was 35 mg/24 hr for the snus users and was 54 mg/24 hr for users of chewing tobacco. The average steady-state saliva cotinine concentration was approximately 300 ng/ml for both categories of snus users and was 471 ng/ml for the chewing tobacco subjects. The clinical severity of buccal mucosal changes did not correlate with the markers for exposure (i.e. nicotine and TSNAs extracted from the tobacco) nor with the biological markers for uptake of tobacco components (i.e., nicotine equivalents excreted during 24 hr and saliva cotinine concentrations).

Rolandsson et al. (2006) examined 80 adolescent males between 16-25 years of age, including 40 snuff users and 40 nonusers. Out of 40 snuff users, 35 showed SILs. Data were collected using a questionnaire on general and oral health, daily oral hygiene and tobacco habits and a clinical examination was carried out by two dental hygienists. The clinical diagnosis of snuff users' mucosa showed snuff lesions of different severity clinically classified as degree 1, 2 and 3. Hours of daily snuff use and product type (portion-bag snuff vs. loose snuff) had a statistically significant effect on the development of snuff lesions of degree 2 and 3. There were no statistical differences between snuff users and nonusers regarding restored tooth surfaces, presence of plaque, gingival inflammation and probing pocket depth. There were no statistical

differences in prevalence in plaque and gingivitis between snuff users and nonusers. However, 17% of the cases with SILs showed loss of periodontal attachment as gingival recessions.

A prospective study by Roosaar and colleagues (2006) documented the natural course of snus-induced lesions (SILs) among 1,115 men over several decades. The total number of individuals initially examined was 16,144 (7,890 men and 8,254 women), and of those, 1,115 of the male participants had SIL; 183 were re-examined in 1993 (the investigators stated that because of limited resources, not all members of the original cohort could be included in the follow-up study). Among this subgroup, there was a strong and significant relationship between the current level of snus use (both number of hours used and number of g consumed per day) and the severity of the lesions. Of 176 users with grade 1-4 lesions in 1973-1974 who were reexamined in 1993-1995, the lesion had disappeared in 62/66 (94%) of those who stopped, and remained in 108/110 (98%) of those that continued to use snuff. The lesions reversed if snus use was discontinued, and they also tended to regress among long-time users who did not change their snus habits. During follow-up, 3 cases of oral cancer occurred (standardized incidence ratio=2.3, 95% CI: 0.5-6.7). None of the oral cancers occurred at the site of the original SIL and two occurred in individuals who were also daily smokers. The authors concluded that snus-induced lesions are probably no more than markers of current or recent snus consumption, and that oral cancers rarely occur at the site of such lesions.

5.3.5 Histologic Changes Accompanying Oral Mucosal Lesions

As opposed to describing oral mucosal lesions on a clinical scale (i.e., visible to the naked eye), oral mucosal lesions can also be described on a histologic, or microscopic, scale. Several of the studies summarized in Appendix B identified the following types of histologic changes among users of snus:

- Increased variable degrees of non-specific inflammation;
- Increased thickness of the epithelial surface layer (epithelial hyperplasia) displaying large numbers of vacuolated cells;
- Increased mitotic rates; and
- Rarely dysplasia.

5.3.6 Leukoplakia

Leukoplakia is defined as a white patch or plaque of the oral mucosa that cannot be removed by scraping and that cannot be classified clinically or pathologically as any other definable lesion (Pindborg et al. 1997). The lesion can occur in all areas of the oral cavity, but is most common on the buccal mucosa. Leukoplakia represents 80% of potentially malignant oral lesions (Bouquot et al. 2006). The term “leukoplakia” describes a clinical condition; it has no specific histopathologic meaning and does not describe a microscopic finding. Furthermore, leukoplakia is a diagnosis of exclusion, used only when another condition cannot be diagnosed. The term is somewhat controversial and continues to undergo refinement (Neville and Day 2002). Lesions occurring in snuff users are believed to represent a clinical entity that is distinct from leukoplakia.

In general, leukoplakia is believed to present a demonstrable, though extremely variable, risk of malignant transformation. Some clinical forms of leukoplakia are considered entirely benign, without malignant potential. Such benign lesions include frictional keratosis, chronic cheek-biting, and irritation due to dental restorations. Hairy leukoplakia, a clinical entity associated with HIV, also does not appear to predispose to malignancy (Silverman, Jr. 1998). The malignant transformation rate for leukoplakia ranges from 1 to 28%, with an average of about 4% (Bouquot et al. 2006); leukoplakia also has the potential for spontaneous reversibility (Pindborg et al. 1997).

Confusion exists surrounding the use of the term leukoplakia, especially as related to the use of oral snuff. This is reflected in the various terms used to describe the condition in snuff users such as snuff dipper's lesion, oral leukoplakia, smokeless tobacco lesions, smokeless tobacco keratosis (Bouquot 1994; Greer 2006) and tobacco pouch keratosis (Neville and Day 2002). These differences in terminology, combined with the multiple number of classification systems used to grade the severity of these lesions, make direct comparison of studies difficult.

Bouquot (1994) made a distinction between leukoplakia and smokeless tobacco keratosis, defining the latter as a chronic white or gray translucent mucosal macule in an area of smokeless tobacco contact that cannot be scraped off. In contrast to leukoplakia, however, these lesions disappear with cessation of the STP use, as discussed below. In fact, Neville and Day (2002) argued against including the term “tobacco pouch keratosis” under the broad umbrella of leukoplakia, because tobacco pouch keratosis has a specific known cause and prognosis. Microscopically, these lesions show hyperkeratosis (thickening) of the mucosal epithelium. True dysplasia is uncommon, and if present, generally mild. Most tobacco pouch keratoses will reverse within a matter of weeks if the individual ceases using snuff. However, the potential for malignant transformation of smokeless tobacco keratosis is not known (Bouquot et al. 2006). Investigations using large numbers of tobacco chewers have found few, if any, keratotic lesions with serious dysplasias, although older and smaller investigations reported that as many as 16% of biopsied cases show at least mildly dysplastic cells (Stotts et al. 1992 and Bouquot et al. 1991 as cited by Bouquot et al. 2006).

Examination of patients with leukoplakia has provided some information into the likelihood of transformation and predictors of malignant transformation. Einhorn and Wersall (1967) evaluated 782 Swedish patients with a clinical diagnosis of leukoplakia; the participants included both tobacco users (smokers, snuff dippers) and nonusers of tobacco. Oral carcinoma developed in 2.4% of patients after 10 years, and in 4% of patients after 20 years. It was primarily the small group of cases of leukoplakia in persons not using tobacco that were responsible for the excess morbidity from oral carcinoma; among tobacco users with leukoplakia the figure was considerably lower. Another study of patients with dysplastic leukoplakia suggested that aneuploid status (having a chromosome number that is not an exact multiple of the normal number) was the most significant determinant of transformation to cancer, while tobacco use was a poor predictor of cancer (Greenspan and Jordan 2004; Sudbo et al. 2004).

The incidence of malignant transformation of leukoplakia is also reported to be related to any of the following factors: location on the floor of the mouth; non-homogeneous visible appearance, in particular an erythematous or verrucous component; dysplastic microscopic features;

overgrowth with the fungus *Candida albicans*; alcohol abuse, particularly when co-incident with the use of cigarettes; and nutritional deficiencies of iron, folate or vitamin B_{12} (Dimitroulis and Avery 1998; Macigo et al. 1996; Silverman, Jr. 1998).

5.3.7 Dysplasia

The effect of snus on the occurrence of pre-carcinogenic conditions such as dysplasia has been investigated in a limited number of epidemiological studies. For a lesion to be a valid indicator of carcinogenic activity, the lesion must be shown to be composed of an abnormal population of cells that are precursors of neoplasms (Williams 1999). Relatively few oral cancers in western populations are preceded by a recognizable premalignant lesion (Dimitroulis and Avery 1998). Squamous epithelial dysplasia is considered a precancerous lesion of stratified squamous epithelium characterized by cellular atypia and loss of normal maturation and stratification short of carcinoma *in situ* (Pindborg et al. 1997). The general disturbance of the epithelium is designated dysplasia and the potential for developing invasive carcinoma increases with its severity (Pindborg et al. 1997).

Historically, the available literature has provided limited insight into the relationship between snuff use and dysplasia. Among 21 male users of Swedish snuff, 5 cases of mild epithelial dysplasia were observed (Frithiof et al. 1983). The authors noted that the premalignant significance of the dysplasia was questionable, and that the dysplasia may have been a reactive change due to inflammatory infiltration. Follow-up was not performed on these 5 cases of dysplasia, so it cannot be determined whether any of the dysplastic lesions became malignant (Frithiof 2000). Hirsch and colleagues (1982) observed slight dysplasia in 9 of 50 (18%) patients. In this study, patients with dysplasia used snuff for more years compared to patients with no dysplasia (23.9 years vs. 19.5 years).

5.3.8 Reversibility of Oral Mucosal Lesions

There is evidence that snuff-induced oral mucosal lesions are reversible. In 20 of 29 snuff users (69%) followed by Larsson and colleagues (1991), histological data indicated that oral lesions were reversible in participants who had quit the use of snus. Frithiof and colleagues (1983) reported that snuff-induced mucosal lesions were almost entirely reversed 14 days after quitting the use of snus, even in patients who had used snus for decades. Andersson and Warfvinge (2003) showed that clinical and histological changes became significantly less pronounced when heavy snuff users switched to snuff with lower pH and lower nicotine content.

In the long-term follow-up study conducted by Roosaar and colleagues (2006), SILs initially seen in 1973-1974 reversed if snus use was discontinued, and they also tended to regress among long-time users who did not change their snus habits. The authors speculated that the regression of SILs over time among men who had not decreased their snus use could reflect changes in commercially available snus over the years (e.g., the introduction of portion bags). These findings are important because they indicate that oral mucosal lesions are generally not dysplastic (i.e., characterized by irreversibility). According to Crissman and colleagues (1993), the presence of dysplasia is the single most important factor predicting risk for the subsequent development of invasive neoplasia.

5.3.9 Miscellaneous Oral Changes

One published investigation was identified that examined the use of snus and the induction of miscellaneous oral changes (also summarized in Appendix B). Axell and Hedin (1982) examined whether the use of tobacco products, including snus, increased oral melanin pigmentation. According to Axell and Hedin (1982), oral melanin pigmentation is sometimes observed with rare pathological conditions such as Addison's disease or Peutz Jeghers' syndrome. Among 1,541 individuals examined, 42 were snus users. Prevalence of pigmentation in snuff dippers (4.7%) was not significantly higher than that among nonusers of tobacco (3.0%). In contrast, the prevalence of pigmentation in cigarette smokers (21.9%) and pipe smokers (16.8%) was significantly greater than in nonusers of tobacco. Axell and Hedin (1982) concluded that the use of snus did not significantly elevate the prevalence of oral melanin pigmentation.

5.4 Summary of Non-Carcinogenic and Pre-Carcinogenic Oral Conditions

Based on descriptive epidemiologic data, the following conclusions can be made about the use of snus and its effect on non-carcinogenic and pre-carcinogenic oral conditions:

- Some cross-sectional epidemiology studies have suggested that use of snus might be associated with dental conditions (caries, tooth wear, and tooth loss) and periodontal conditions (gingivitis and gingival recession). However, these studies have many methodological weaknesses (including small numbers of participants, cross-sectional study design, lack of data on individuals who do not use snus, insufficient product identification, and failure to control for important confounders, such as dietary and oral hygiene habits, SES, alcohol use, etc.), and no firm conclusions can be drawn from these studies.
- It is generally accepted that use of snus is associated with a characteristic type of oral mucosal lesion. The lesions are localized to the area where snuff is placed and have been found to be reversible following cessation of snus use.
- While snus does exert an effect on the oral mucosa, the available epidemiologic data fails to support that snus is associated with dysplastic lesions or with pre-carcinogenic effects on the oral cavity. Furthermore, there is no clinical evidence to suggest that when dysplastic lesions occur in snus users, they transform into malignancies.
- There are no hypothesis-testing or controlled studies presenting quantitative risk estimates of the role of snus in non-carcinogenic or pre-carcinogenic oral lesions. For this reason, the risk for these conditions, if any, associated with the use of snus, is unsubstantiated.
- Caution should be exercised in interpreting the available data on oral conditions related to the use of snus, as the studies are largely descriptive in nature (e.g., cross-sectional), are not controlled, and suffer from several important limitations including small sample sizes, and failure to control for important confounders.

5.5 Cancer

As previously discussed, snus contains low levels of several proven animal carcinogens, including TSNAs. Over the past 60 years, investigators have examined human and animal populations exposed to snus in search of an association between snus use and cancer. This

section discusses human and animal data that have been published on the relationship between snus and various types of cancer.[48]

5.5.1 Head and Neck Cancer

The term "head and neck" cancer includes a broad category of cancers that occur throughout the oral cavity, pharynx, larynx, esophagus, and nasal cavity. These cancers involve a variety of organs with distinct histological characteristics, each of which has different susceptibilities to carcinogens. Approximately 2% of cancers in the body are located in the oral cavity (EU Working Group on Tobacco and Oral Health 1998)[49]. The oral cavity contains several types of tissue, and each of these tissues contains several types of cells. Different cancers can develop from each type of cell. For example, squamous cells are flat, scale-like cells that form the lining of the oral cavity and oropharynx. Malignant squamous cells can develop into squamous cell carcinomas or verrucous carcinomas. The majority of oral cancers (approximately 90%) are squamous carcinomas that arise from the mucosal surface, which is lined with a stratified squamous epithelium. The remainder of oral cancers are adenocarcinomas (e.g., salivary gland tumors) or sarcomas (e.g., bone tumors) (Dimitroulis and Avery 1998; EU Working Group on Tobacco and Oral Health 1998).

In evaluating the epidemiological studies of snus use and the potential association with oral cancer, both the types and location of oral tumors (both malignant and benign), particularly those that develop in the squamous epithelium at or adjacent to the location of snus use (e.g., upper vestibular area of oral cavity), are important considerations. Appendices C-1, C-2, and C-3 describe epidemiologic studies that evaluate the effect of snus use on oral cancer. Details are provided on study design and findings, and include, when known, information on tumor types and location. Data regarding oral cancer rates in Sweden are considered to be very reliable because of the method of reporting cancer cases. The Swedish National Board of Health and Welfare administers the Swedish Cancer Registry. Since 1958, the Board has received compulsory reports of cancer diagnoses from all physicians in Sweden, as well as independent compulsory reports of cancer biopsy diagnoses made by pathologists, cytologists, and forensic pathologists (Anneroth et al. 1983). According to Ostman and colleagues (1995), reporting to the Registry is close to 100% and approximately 94% of reported cases are morphologically verified. During the time period 1960-1989, 1.8% of all newly diagnosed cancers in Sweden were malignant oral tumors (Ostman et al. 1995).

Ten studies have addressed the effects of snus on head and neck cancers. Included are two descriptive studies (summarized in Appendix C-1), four case-control studies (summarized in Appendix C-2), and four cohort studies (Appendix C-3). Data are discussed below first for oral and pharyngeal cancer and then for cancers at other sites in the head and neck.

[48] A number of review papers on smokeless tobacco and cancer are interesting, but are ultimately not useful in assessing the health profile of Swedish snus, as they mingle studies of U.S. and Swedish products and draw conclusions about smokeless tobacco in general (Cnattingius et al. 2005; Goldenberg et al. 2004; Kuper et al. 2002; Rodu and Cole 2002; Warnakulasuriya et al. 2005). All of the relevant studies on Swedish snus cited in these papers are discussed individually in the following sections of this report.

[49] It is not known whether this percentage is specific to European populations.

5.5.1.1 Oral and Pharyngeal Cancer

Two dated descriptive studies (Ahlbom 1937; Axell et al. 1978) report the prevalence of snus use and other tobacco use among older male participants with oral cancer, and, by design, cannot estimate the risk of oral cancer associated with tobacco use. Ahlbom (1937) did not examine the effects of snuff independently, but examined the prevalence of "snuff and chewing tobacco in the mouth" among patients with various types of oral cancers. He drew no specific conclusions about the use of snuff, but noted the relationship between site of usual placement of tobacco or snuff in the mouth and location of carcinoma. The paper also acknowledged the many other risk factors, especially heavy tobacco consumption, that play a role in oral cancers. Axell and colleagues (1978) examined snuff habits among 49 snuff-users with oral cancer. These authors concluded that snuff use is a factor that contributes to the occurrence of cancer, but that the risk for the individual snuff taker of getting oral cancer as a consequence of his snuff usage is very slight. These authors state that use of Swedish snuff is a considerably less risky tobacco habit than smoking.

Three more recent population-based case-control studies carried out specifically to study the relationship between snus and oral cancer (Lewin et al. 1998; Rosenquist et al. 2005; Schildt et al. 1998b) have found no evidence that use of snus was associated with a statistically significant increased risk of oral cancer.

Lewin and colleagues (1998) examined risk of cancer of the oral cavity among men aged 40 to 79 who were either ever-, current, or ex-users of snuff, compared to never-users of snuff. After adjustment for potential confounders (including smoking and alcohol), no significantly elevated relative risk estimates were identified. The relative risk estimate for cancer of the oral cavity among ever-users of snus was 1.4 (95% CI:0.8-2.4) and for current users it was 1.0 (95% CI:0.5-2.2). The relative risk estimate for cancer of the pharynx among ever-users of snus was 0.7 (95% CI:0.4-1.3) and for current users it was 0.7 (95% CI:0.3-1.5).

Schildt and colleagues (1998b) examined 354 cases with oral cancer, including 117 women. Snuff use (whether active, former or ever-use) was not associated with significantly increased risk of oral cancer. Odds ratios were not adjusted for potential confounding factors (e.g. alcohol), other than the matching characteristics of gender, age and county. When analysis was restricted to a small group of never-smokers, active snuff use was not associated with increased risk of oral squamous cell carcinoma (OR=0.7; 95% CI:0.4-1.2).[50]

Schildt and colleagues (2003) analyzed tumor samples from the oral cancer participants in their case-control study to determine whether various exposures (including smoking, snus, alcohol, infections, etc.) were associated with biological markers for oral cancer. The tumors were evaluated by immunohistochemistry for alterations in various genes, antigens, and proteins (p53, PCNA, Ki-67, and bcl-2) that are involved in the development of oral squamous cell

[50] Note that two papers address the co-occurrence of snus use and infection with human papilloma virus (HPV) (Sand et al. 2000b; Sand et al. 2000a). This is of interest because of a potential relationship between HPV and oral cancer. Neither of these papers found any correlation between oral lesions, snus use, and HPV infection. It is notable that Schildt et al. (1998a) did not find HPV in any oral lesions in their study of oral cancer, nor did these study authors find a relationship between use of snus and oral cancer. Cancers in the Schildt et al. (1998b) study comprised oral squamous cell carcinomas.

cancers. Although the number of snus users was very few, there was no clear relationship between snus use and any of the biological markers studied. However, oral infection (especially herpes simplex virus (HSV) infection) was associated with increased risk for all tumors and for those that had p53 mutations. This finding suggests that it is important to control for HSV infection in studies of the etiology of oral cancer.

Rosenquist and colleagues (2005) investigated the relationship between smoking, alcohol consumption, and snuff use and oral and oropharyngeal squamous cell carcinoma (OOSCC) in a case-control study. Regardless of the way snuff use was assessed (ever, current, ex; duration of <30 or ≥30 years; exposure in hours per day; or consumption in g per day), there were no significant associations between snuff use and increased risk of OOSSC. Odds ratios were adjusted for alcohol consumption and tobacco smoking, as well as the matching characteristics of age, sex, and county; however, the number of participants who had used snuff was quite low. All current snuff users in this study had clinical lesions; thus, this study provides additional evidence that, although oral mucosal lesions are common among snuff users, they are not likely to transform to cancer.

Two of three cohort studies that looked at the development of cancers in general have also failed to find a significant association between the use of snus and increased risk of oral and/or pharyngeal cancer. Details of these studies are presented in Appendix C-3. The most recent of the two (Luo et al. 2007) involved an analysis of the Swedish construction worker cohort. This cohort has much strength, including its large size, high prevalence of snus use, and its long and almost complete follow-up. There was no association between the use of snus and increased risk of oral cancer among the 125,576 never-smokers in this cohort after 20 years of follow-up. Though this study has much strength, this finding was based on only 10 exposed cases of oral cancer. Additionally, snuff habits were assessed only at study entry with follow up data collected for only a small portion of the cohort. Interestingly, ever-use of snus was associated with a statistically significant *decrease* in risk of oral cancer when all members of the cohort (regardless of smoking or snus status) were considered (risk ratio (RR)=0.7; 95% CI:0.5-0.9), compared to never-users of tobacco. The authors suggest that the reduced risk of oral cancer among snus users could have been due to residual negative confounding. Rodu (2007) presented data from Luo and colleagues (2007) that show that the rate of death from oral cancer among current snus users was less than half that of smokers, and was nearly the same as never-tobacco users in this cohort.

Boffetta and colleagues (2005) studied more than 10,000 Norwegian men who had been enrolled in a cohort study since 1966 to understand the relationship between snus use and subsequent development of a number of forms of cancer. Approximately 31% of these men were regular users of snus (either current or former). The authors found what they called a "modest, non-significant" increase in risk (adjusted for smoking) of oral/pharyngeal cancers (RR=1.10; 95% CI:0.50-2.41) among ever-users of snus compared to never-users. The risk was not significantly elevated among current and former users, was based on 9 exposed cases and the authors concluded that it is unlikely that the use of STPs in Europe and the US entails a substantial increase in the risk of these cancers.

Roosaar and colleagues (2008) examined roughly 10,000 Swedish men who had been enrolled in a cohort study in 1973 and followed up until 2002 in order to evaluate the effects of tobacco smoking and snus use on the risk of subsequent development of oral and pharyngeal cancer and cancer in general. Only 9% of this population were ever daily snus users (never smokers), while 7% of this population were both ever daily smokers and snus users. The authors conclude that their results are inconsistent with claims that the use of snus is without demonstrable risk of oral and pharyngeal cancer based on an observed hazard ratio (HR) of 3.1 (95% CI:1.5-6.6) among ever daily snus users. Though this finding was adjusted for smoking, it is possible that some residual confounding may remain. The risk estimate for ever daily snus users among never smokers was not statistically significantly elevated (HR=2.3; 95% CI:0.7-8.3). Both analyses are based on a small number of snus users; 11 and 5 exposed cases respectively). Overall, the authors conclude that the relative risks for oral cancer associated with snus are consistently lower than those associated with smoking.

Thus, a growing body of evidence finds that there is no consistent significant association between the use of snus and oral cancer. In 2004, Rodu and Jansson (2004) concluded in a review of smokeless tobacco and oral cancer that "the use of Swedish moist snuff is associated with no demonstrable risk." The IOM's 2001 report "Clearing the smoke: Assessing the science base for tobacco harm reduction," states that, based on recent epidemiologic studies, "Swedish snus does not increase the risk of oral cancer" (Stratton et al. 2001). Weitkunat and colleagues (2007) and Boffetta and colleagues (2008) conducted meta-analyses that examined the risk of oral cancer from the use of a range of smokeless tobacco and snuff products (both snus and traditional US STPs) and these researchers concluded that no increased risk from use of snus was observed. A third meta-analysis, conducted by Lee and Hamling (2009b), also did not show an elevated risk of oropharyngeal cancer among smokeless tobacco users generally, or specifically among snuff users in Scandinavia. The SCENIHR Working Group (2008), charged with assessing the health risks of smokeless tobacco use, also concluded that the available literature indicates that "an increased risk of oral cancer has not been proven in snus users."

5.5.1.2 Cancer at Other Sites in the Head and Neck

Four analytic studies have examined the association between snus use and cancers at other sites in the head and neck; all concluded that snus does not pose significant risks.

Lewin and colleagues (1998) examined many variables related to snus use (age at start, duration of usage, total consumption, and intensity of usage) and estimated relative risk estimates associated with overall cancer of the head and neck. After adjustment for potential confounders (including smoking and alcohol), no significantly elevated relative risk estimates were identified (see Appendix C-2). In an analysis with never-users of tobacco as the reference category, significantly elevated risks of head and neck cancer were seen for ever-users and ex-users of snuff (it is unclear whether these risk estimates were adjusted for any potential confounders). However, the authors note that precision was very low in these analyses because the numbers of participants was very small (9 cases and 10 controls).

Four studies present data on the relationship between use of snus and risk of esophageal cancer. A case-control study by Lagergren and colleagues (2000) (summarized in Appendix C-2) investigated the role of smoking, alcohol intake, and the use of oral snus in the etiology of

head and neck cancer. The authors concluded that there was no statistically significant association between the use of snus and the risk of developing either of the tumor types studied (esophageal adenocarcinoma and esophageal squamous cell carcinoma). Lewin and colleagues (1998) also presented data on risk of esophageal cancer associated with use of snus. After adjustment for potential confounders (including smoking and alcohol), the relative risk estimate for cancer of the esophagus among ever-users of snus was 1.2 (95% CI:0.7-2.2); for current users it was 1.1 (95% CI:0.5-2.4). The cohort study by Boffetta and colleagues (2005) (described above and summarized in Appendix C-3) reported only a "modest, non-significant" increase in risk of esophageal cancer (RR=1.40; 95% CI:0.61-3.24) among ever-users of snus compared to never-users. The risk was not significantly elevated among current (RR=1.06; 95% CI:0.35-3.23) or former (RR=1.90; 95% CI:0.69-5.27) snus users. More recently Zendehdel and colleagues (2008) conducted a study of the Bygghälsan cohort and reported significantly elevated risks of esophageal squamous cell carcinoma (RR=3.5; 95% 1.6-7.6) but not for adenocarcinoma of the esophagus among never-smoking, "snus" users (RR=0.2; 95% CI:0.0-1.9). These relative risks were adjusted for attained age and BMI, but the lack of lifestyle and alcohol information presents a severe limitation in this study, as the authors note that alcohol is a candidate confounding factor for associations of tobacco use and esophageal squamous cell carcinoma. Interestingly, no significant elevations of esophageal squamous cell carcinoma were observed among the group of "snus" users that also included smokers and were unadjusted for smoking, a well-established risk factor for the disease. Overall, 58% of the workers were current or former smokers at time of entry. The prevalence of "snus" use was 28% overall while 12% of the participants were never-smoking snus users. Relative risks were based on small numbers of cases (10 exposed cases of esophageal squamous cell carcinoma and one exposed case of adenocarcinoma), limiting precision and suggestive of potential chance variation or misclassification (see Appendix C-3).

Finally, Lewin and colleagues (1998) also presented data on risk of laryngeal cancer associated with use of snus. After adjustment for potential confounders (including smoking and alcohol), no significantly elevated relative risk estimates were identified. The relative risk estimate for cancer of the larynx among ever-users of snus was 0.9 (95% CI:0.5-1.5) and for current users it was 1.0 (95% CI:0.5-1.9).

The meta-analysis conducted by Boffetta and colleagues (2008), described earlier, found that the summary relative risk of esophageal cancer from use of snuff was significantly elevated, but only when the relative risk was based on five studies, one of which included US smokeless tobacco users, while the other four included Scandinavian populations (snus users). Of the four, only one study that was previously mentioned above, Zendehdel and colleagues (2008), reported a significantly elevated relative risk, though the summary risk for esophageal cancer limited to snuff users in Scandinavia was not significantly elevated. Of note, the appropriate relative risk from the Zendehdel and colleagues (2008) study that should be used in a meta-analysis for esophageal cancer is the subject of debate (Lee and Hamling 2009a). In a more recent meta-analysis conducted by Lee and Hamling (2009b), the summary relative risk of esophageal cancer from use of smokeless tobacco was not statistically significant, primarily due to the selection of different relative risks from the Zendehdel et al. (2008) study.

5.5.1.3 Population Attributable Risk of Oral and Esophageal Cancer Due to Use of Snus

The population attributable risk (PAR) represents the proportion of the cancer incidences or deaths in a population that could theoretically be prevented if a particular risk factor (such as use of snus) were totally eliminated. In calculating an attributable risk, the underlying assumption is that a causal relationship between an exposure and outcome exists, and often this has not been established. In addition, other risks for the disease are often not examined in the same study, and thus the purported risk factor may be taken out of context of other, more important, risk factors. Critchley and Unal (2003) calculated the PAR fraction for oral cancer among men in Sweden (based on data from the Lewin et al. (1998) and Schildt et al. (1998b) studies described above), and estimated that between 0 and 60 oral cancer deaths each year may be due to snus use. Boffetta and colleagues (2008) also calculated the PAR for esophageal cancer in three Scandinavian countries (based on data of total number of cancers from Ferlay et al. (2004)) and estimated the proportion of esophageal cancer cases among men attributable to smokeless tobacco use in 2002 to be 2.1% in Denmark (5 cases), 2.5% in Norway (5 cases) and 10.7% in Sweden (31 cases). However, such calculations are inappropriate until a causal relationship has been established (Hennekens and Buring 1987), and as the above sections of the report demonstrate, use of snus has not been causally linked to an increased risk of oral or esophageal cancer. These results are best interpreted among the population attributable risks of other causes of these diseases, including smoking and alcohol consumption, which were not presented in these analyses.

5.5.2 Pancreatic Cancer

Two recent cohort studies have examined the relationship between the use of snus and the development of pancreatic cancer (Boffetta et al. 2005; Luo et al. 2007). Both studies have shown that use of smokeless tobacco (the specific types are discussed below) is associated with an increased risk of pancreatic cancer in some subgroups of the populations studied; however, there are inconsistencies between the two studies with respect to the specific subgroups at risk. Details of the two studies are provided in Appendix D.

The cohort study described previously by Boffetta and colleagues (2005) is an update of an earlier study carried out by (Heuch et al. 1983) which provided the first suggestion that the use of snus (though the study was not specific on the type of STP used) might increase the risk of pancreatic cancer. In this recently updated cohort of more than 10,000 Norwegian men, the use of snus was associated with significant increases in risk of pancreatic cancer after adjustment for smoking: RR=1.67 (95% CI:1.12-2.50) for ever use; RR=1.80 (95% CI:1.04-3.09) for former use. There was a borderline non-significant increase in risk of pancreatic cancer for current snus use: RR=1.60 (95% CI:1.00-2.55). However, when risk was assessed by smoking status, a significant increase in risk was only seen among ever-users of snus who currently smoked (RR=1.86; 95% CI:1.13-3.05). The authors concluded that this study provides evidence that STPs may cause pancreatic cancer.

Luo and colleagues (2007) investigated the relationship between the use of snus and several types of cancer among 279,897 male construction workers followed for 20 years. Among all cohort members (regardless of smoking or snus status), use of snus was not associated with

increased risk of pancreatic cancer (RR=0.9; 95% CI:0.7-1.2), when compared to never-users of tobacco. However, when analyses were restricted to the 125,576 men who had never smoked, both ever-use of snus (RR=2.0; 96=5% CI:1.2-3.3) and current use of snus (RR=2.1; 95% CI:1.2-3.6) were associated with significantly increased risk of pancreatic cancer, after adjustment for age and BMI.

The authors suggest that there is a biologically plausible mechanism by which snus could increase the risk of pancreatic cancer, noting that rats treated with TSNAs in drinking water have been reported to develop pancreatic tumors. They concluded that the use of snus should be added to the list of tentative risk factors for pancreatic cancer. Because little is known about the etiology of pancreatic cancer, it's possible that unknown confounding may explain these observations of increased risk. As noted previously, the Swedish construction worker cohort has many strengths (large size, long and almost complete follow-up), but this analysis also suffers from some weaknesses. The authors did not adjust the risk estimates for pancreatitis, a recognized risk factor for pancreatic cancer. It is also possible that exposure misclassification may contribute to uncertainty in the risk estimates; Luo and colleagues reported that a sensitivity analysis that accounted for possible changes in cigarette use affected the risk estimates "no more than trivially." Importantly, though, the authors did observe a difference in misclassification of smoking among participants who were nontobacco users at the initial visit compared to snus users when a sample of these participants was observed at follow-up visits. The authors reported that 12% of never-smoking snus users who did not report current or former smoking during their first visit, were later recorded during the second visit as having smoked while only 7% of those who reported never using tobacco during the first visit and later reported smoking.

Thus, to date there are two studies that suggest that use of snus could be associated with increased risk of pancreatic cancer among some groups of the population. However, there are inconsistencies between the two studies with respect to the specific tobacco user subgroups at risk. Boffetta and colleagues (2005) found that the increased risk of pancreatic cancer was limited to snus users who were *also current smokers*. In contrast, Luo and colleagues (2007) found that snus use was significantly increased only among a subgroup of men who had *never smoked tobacco*. This finding is inconsistent with what is known about the association between smoking and risk of pancreatic cancer, as smoking is strongly associated with pancreatic cancer. In fact, the Surgeon General (2004) report on the health consequences of smoking, concludes that "the evidence is sufficient to infer a causal relationship between smoking and pancreatic cancer." It is not known why the two studies would have found that the increased risk was limited to two distinctly different subgroups. Further research is needed to clarify these questions.

5.5.2.1 Debate in the Scientific Community

This section provides additional information relating to the continuing debate in the scientific community regarding the association between snus use and pancreatic cancer (e.g., Boffetta et al. 2006; Colilla 2010; Lee and Hamling 2009a; Nilsson 2006; Ramström 2006; Rodu 2007; Rodu and Cole 2005; 2006). The Boffetta et al. (2005) study in particular has been the subject of much of this debate. Several methodological weaknesses of this study have been cited including:

- Failure to control for the confounding effect of alcohol;
- Failure to reassess tobacco habits after study enrollment (especially given that the follow-up was more than 30 years and tobacco habits may have changed);
- Evaluation of a different type of smokeless tobacco than snus (called “skra”) that was commonly used in Norway until the early 1980s; thus, the results are not relevant for the product that is now most widely used in northern Europe;
- Limitations in the statistical methods used to adjust for smoking;
- Likely selection bias (in that the cohort had a much higher prevalence of smokeless use than the general population);
- Inability to assess dose-response; and
- Unconventional exposure groups (specifically, creating a reference group that combined never and occasional users).

In rebuttal, Boffetta and colleagues (2006) have stated that their data show that alcohol is not a confounder of the association between snus use and pancreatic cancer in this cohort. They believe that snus and skra contain comparable amounts of carcinogenic components, and thus can be appropriately considered together. They do, however, agree that the small number of cases of pancreatic cancer among snus users who did not smoke is an important limitation of this study. After consideration of all submitted comments, they stand by their original conclusions.

Rodu (2007) conducted an analysis using data from Luo and colleagues (2007) to contrast the potential risk from snus to that of smoking, if the association between snus and pancreatic cancer was found to be causal. Dr. Rodu reported that the rate of death from pancreatic cancer among current snus users in the Luo et al. (2007) study was approximately 50% lower than that of smokers in this cohort, however the rate of death among snus users was approximately twice that of never-tobacco users.

The Boffetta and colleagues (2008) meta-analysis, mentioned previously, combined the pancreatic risk estimates from use of a range of smokeless tobacco and snuff products using data from four US studies and the Luo et al. (2007) and Boffetta et al. (2005) studies of snus users. Boffetta and colleagues (2008) report a significant elevated summary risk for pancreatic cancer, and concluded that these studies suggest an increased risk of pancreatic cancer among snus users. The SCENIHR Working Group (2008) also reports that these two Scandinavian cohort studies identify the pancreas as a main target organ among smokeless tobacco users.

An additional meta-analysis conducted by Sponsiello-Wang and colleagues (2008) also examined the risk of pancreatic cancer from the use of smokeless tobacco in Europe and North America. These researchers conclude that although some subgroup analyses suggest a possible association, the risk estimates are heavily dependent on the contribution from one specific study (Luo et al. 2007) with known weaknesses described previously. Thus, these authors state that before a potential causal link can be established, further research needs to be conducted.

More recently, Lee and Hamling (2009b) also conducted a meta-analysis that examined the risk of pancreatic cancer among North American and European smokeless tobacco users. No significantly elevated summary risk of pancreatic cancer was observed among smokeless tobacco users, which included the Luo et al. (2007) and Boffetta et al. (2005) studies that included the Swedish and Norwegian cohorts respectively. This combined summary risk estimate for pancreatic cancer among snuff users as used in Scandinavia was also not significantly elevated. As mentioned previously, selection of the appropriate relative risk to be used in a meta-analysis is the subject of debate (Lee and Hamling 2009a). These authors selected the smoking-adjusted relative risk on the basis that it provides greater power, as opposed to the selection by Boffetta and colleagues (2008) of the relative risk for never smokers from the Luo et al. (2007) study (Lee and Hamling 2009a). Lee and Hamling (2009a) noted an inconsistency in the stated approach by Boffetta and colleagues (2008) of selecting relative risks from never smokers when they selected the smoking-adjusted relative risk from the Boffetta et al. (2005) study, the higher of the two effect estimates.

5.5.3 Stomach Cancer

A review of the published literature identified five studies addressing the relationship between snus use and stomach cancer. This endpoint has been studied because saliva produced during the use of snus is often swallowed instead of expectorated. The term stomach cancer, also called gastric cancer, generally refers to adenocarcinoma (ACS 2000). Adenocarcinomas of the stomach are malignant neoplasms of the glandular epithelium, and are labeled cardia (closer) and noncardia (more distant) in relation to proximity to the esophageal junction. Less common types of gastric cancers are lymphomas, leiomyosarcomas, adenoacanthomas, squamous cell carcinomas, and carcinoids (ACS 2000).

There are three case-control studies (Appendix E-1) and two cohort studies (Appendix E-2) that examined the relationship between the use of snus and stomach cancer. Only one of these studies (Zendehdel et al. 2008) found (for one sub-analysis) that “snus” is associated with a significantly increased risk of stomach cancer.

Three population-based case-control studies looked at the effects of oral snuff use, tobacco smoking, and alcohol consumption on the risk of gastric cancers. No study found a statistically significant association between snuff use and gastric cancer, even after adjustment for several relevant potential confounders. In particular, Ye and colleagues (1999) examined the relationship between snus use among males and gastric cancer of various sub-sites and histologic types after adjustment for age, residence area, BMI, SES, and smoking. They found no significant association between snus use and cancer of the gastric cardia or cancer of the distal stomach (of either the intestinal or diffuse types). One concern regarding the negative findings for snuff dipping and alcohol use mentioned by the authors was the potential for differential recall among cases and controls. Hansson and colleagues (1994) found no elevated risk of gastric cancer associated with snuff dipping, although they focused on the role of cigarette and pipe smoking. The number of snuff users is not clearly stated, nor are details provided on the quantity and frequency of snuff use in these participants. Lagergren and colleagues (2000) did not find that risk of adenocarcinoma of the gastric cardia (the uppermost part of the stomach) was significantly elevated among snus users, even those who had used for more than 25 years or who used more than 35 quids per week.

Two cohort studies looked at the effects of snus use on the risk of gastric cancers. Boffetta and colleagues (2005) studied the relationship between snus use and development of stomach cancer among more than 10,000 Norwegian men who had been enrolled in a cohort study since 1966. Approximately 31% of these men were regular users of snus (either current or former). The authors found what they called a 'modest, non-significant increase' in risk of stomach cancer among ever-users of snus compared to never-users (RR=1.11; 95% CI:0.83-1.48). There was no increased risk among current snus users (RR=1.00; 95% CI:0.71-1.42). There are several weaknesses present in this study, that include the assessment of tobacco habits only at enrollment, lack of information about amount or duration of snus use, and failure to adjust for alcohol consumption.

Zendehdel and colleagues (2008) studied the relationship between smoking and "snus" use and the development of stomach cancer among 336,381 Swedish male construction workers who provided information on "snus" habits between 1971 and 1993 and were followed-up through 2004. After adjusting for attained age and BMI, a significantly elevated risk was found for noncardia gastric cancer among never-smoking "snus" users (RR=1.4; 95% CI:1.1-1.9). When analyzed by age group, this excess risk was limited to men aged 70 years and older (RR=1.7; 95% CI:1.2-2.5). No association was observed for "snus" users among ever-smokers unadjusted for smoking. It is surprising that an association was observed only among *never-smoking* "snus" users, considering significantly elevated risks of noncardia gastric cancer were consistently observed for almost all sub-analyses of former and current smokers. Additionally, information concerning lifestyle and dietary factors is lacking, which remain viable confounding factors.

The recent meta-analysis carried out by Lee and Hamling (2009b) did not observe a significantly elevated summary risk of stomach cancer among smokeless tobacco users that combined five Scandinavian studies among snus users with seven US studies among chew or other STP users. When limited only to studies of snus users in Scandinavia, no increased risk for stomach cancer was observed.

5.5.4 Kidney and Bladder Cancer

The cohort study by Boffetta and colleagues (2005) described previously also presents data on the relationship between snus use and development of kidney and bladder cancers (see Appendix F). The authors concluded that the use of snus (either current or former) was not associated with any increase in the risk of kidney or bladder cancer. In fact, current snus users had a significantly lower risk of kidney cancer than did never-users (RR=0.47; 95% CI:0.23-0.94).

The recent meta-analysis carried out by Lee and Hamling (2009b) did not observe a significantly elevated summary risk of bladder or kidney cancer among smokeless tobacco users that included studies of a variety of STPs including snus. A significantly elevated summary risk for kidney or bladder cancer among snuff users as used in Scandinavia was also not observed.

5.5.5 Lung Cancer

Three large cohort studies have collected data on the relationship between use of snus and lung cancer. These studies, which are summarized in Appendix G, found no evidence that use of snus increases the risk of lung cancer.

Two studies evaluated this relationship using data from the Swedish construction worker cohort. Bolinder and colleagues (1994) failed to find a significant association between "smokeless tobacco" use and increased risk of death due to lung cancer in their study population of 84,781 Swedish construction workers, regardless of age (either 35 to 45 years or 55 to 65 years). Precision was very low, however, since there were only 3 lung cancer deaths. Luo and colleagues (2007) also found no association between use of snus and increased risk of lung cancer among 125,576 never-smoking men in this cohort after 20 years of follow-up. Interestingly, ever-use of snus was associated with a statistically significant *lower* risk of lung cancer when all men in the cohort (regardless of smoking or snus status) were considered (RR= 0.7; 95% CI:0.6-0.7). The authors suggest that the reduced risk of lung cancer among snus users could have been due to residual negative confounding. Rodu (2007) presented data from Luo and colleagues (2007) that show the rate of death from lung cancer among current snus users was more than 13 times lower than that of smokers, and was actually lower than never-tobacco users in this cohort.

The cohort study by Boffetta and colleagues (2005) described previously also presents data on the relationship between use of smokeless tobacco and development of lung cancer among more than 10,000 Norwegian men who were followed for more than 30 years. The authors reported that use of smokeless tobacco was not associated with a statistically significant increase in the relative risk of lung cancer (all histological types and adenocarcinoma). However, the authors note that the analysis of lung adenocarcinoma was limited by the small number of cases.

Boffetta and colleagues (2008), as mentioned previously, conducted a meta-analysis that examined the risk of lung cancer from use of a range of smokeless tobacco and snuff products. The authors conclude that northern European studies of snus users suggest no excess risk of lung cancer and that any potential excess risk of lung cancer among snus users is especially lower than that of smokers.

The recent meta-analysis carried out by Lee and Hamling (2009b) also did not observe a significantly elevated summary risk of lung cancer among smokeless tobacco users that included studies of a variety of STPs, including snus. A significantly elevated summary risk for lung cancer among snuff users as used in Scandinavia was also not observed.

Rodu and Cole (2009) estimated how smoking-attributable lung cancer mortality would decline in other EU countries if they had the smoking prevalence of Sweden. The authors found that cigarette consumption among men in Sweden was inversely correlated with snus use, resulting in the lowest lung cancer mortality rate (LCMR) in Europe. They state that if all EU countries had the LCMR of men in Sweden, there would have been 92,000 fewer lung cancer deaths in 2002. Additionally, if all EU countries had the smoking rate of Swedish men, 274,000 smoking attributable deaths would have been avoided in 2002. They note that these large differences

occur only in men, and state that since it is unlikely that anti-smoking campaigns were differentially highly effective for Swedish men but not for women, evidence that suggests that the higher prevalence of snus use among men has played the primary role in the low LCMR among Swedish men.

5.5.6 Other Cancers

Six studies have examined the effect of snus use on risks of other types of cancer; these studies are summarized in Appendix H. All but one of these studies (Roosaar et al. 2008) evaluated participants drawn from a single population of Swedish construction workers.

The cohort study by Bolinder and colleagues (1994) described above also presents data on death due to any type of cancer among 84,781 male construction workers. There was no excess risk of cancer mortality among the 6,297 "smokeless tobacco (snuff)" users in this cohort. The study did not examine specific types of cancer, except for lung cancer, probably due to relatively small numbers of cancers (there were only 96 malignancies).

Also described previously, the cohort study by Roosaar and colleagues (2008) presents data on the risk of any type of cancer and also smoke-related cancers[51] among approximately 10,000 Swedish men. With respect to smoke-related cancers, a significantly elevated risk was observed among never-smoking ever-daily snus users (HR=1.6; 95% CI:1.1-2.5). Contrary to what would be expected, a significantly elevated risk was not observed among snus users that included smokers, as smoking was significantly associated with both the development of any cancer and smoke-related cancers in the analysis. For any cancer, no excess risk was observed among ever-daily snus users among never-smokers and snus users that included some smokers. Residual confounding is an important concern, and the authors conclude that relative risks are consistently lower among snus users than those associated with smoking.

Odenbro and colleagues (2005; 2007) examined the relationship between use of snus and several forms of skin cancer in two analyses of the construction worker cohort. An initial analysis (Odenbro et al. 2005) examined the effect of tobacco use on the risk of cutaneous squamous cell carcinoma (CSCC) among 337,311 male construction workers who were followed for 30 years. The authors found that snuff use was not associated with any increased risk; in fact, it was associated with a significantly decreased risk of CSCC (RR=0.64; 95% CI:0.44-0.95).

In their second analysis, Odenbro and colleagues (2007) examined data from 339,802 male construction workers to determine whether tobacco use was associated with any of three types of melanoma, including cutaneous malignant melanoma (CMM), melanoma *in situ* (MIS), and intraocular malignant melanoma (IMM). Snuff-only users had a significantly reduced risk of CMM (RR=0.63; 95% CI:0.48-0.81), a nonsignificantly reduced risk of MIS (RR=0.64; 95% CI:0.36-1.14), and there was no effect on IMM (RR=1.14; 95% CI:0.43-3.07). Risk of CMM decreased with increasing duration of snuff use. The authors note that the biological

[51] Smoke-related cancers include: oral & pharyngeal (ICD7: 140-148), esophageal & gastric (ICD7: 150-151), pancreatic (ICD7: 157), laryngeal and pulmonary (ICD7: 161-162), kidney, bladder & other urinary organs (ICD7: 180-181)

mechanisms behind these findings are unclear, and that this cohort is relatively young, with some workers not reaching the mean age for melanoma diagnosis.

Two analyses by Fernberg and colleagues (2006; 2007) investigated the role of tobacco use and BMI in the development of various hematopoietic malignancies. An initial study (Fernberg et al. 2006) evaluated the effect of these factors on the incidence of malignant lymphomas, specifically non-Hodgkin's lymphoma (NHL) or Hodgkin's disease (HD), among 335,612 male and female Swedish construction workers. There was no link between snuff use and risk of NHL, even among men who had used snuff for more than 30 years (incidence rate ratio (IRR)=0.69; 95% CI:0.41-1.15). With respect to HD, the overall analysis did not show snuff use to be associated with significant increased risk. However, men who had used snuff for more than 30 years had a significantly increased risk of HD (IRR=3.78; 95% CI:1.23-11.15). This is a novel finding that must be verified by additional studies, and it was based on only four cases, which limits the statistical power of the finding. Women who had ever used snuff were not at significantly increased risk of either NHL or HD.

In their second analysis, Fernberg and colleagues (2007) investigated the role of tobacco smoking, oral moist snuff use, and BMI on the incidence of leukemia and multiple myeloma (MM) among 336,381 Swedish male construction workers. The authors reported that exclusive use of snuff was not associated with increased risk of either acute lymphocytic leukemia (IRR=1.24; 95% CI:0.39-4.01), acute myelogenous leukemia (IRR=0.81; 95% CI:0.41-1.60), chronic myelogenous leukemia (IRR=1.17; 95% CI:0.60-2.28), or multiple myeloma (IRR=0.92; 95% CI:0.61-1.40), after adjustment for age and BMI.

The recent meta-analysis conducted by Lee and Hamling (2009b) did not observe a significantly elevated summary risk of overall cancer among smokeless tobacco users that included studies of a variety of STPs, including snus. A significantly elevated summary risk for overall cancer among snuff users as used in Scandinavia was also not observed.

5.5.7 Summary of Carcinogenicity Studies

The following conclusions can be drawn about the effect of snus on oral and other cancers:

- Two dated descriptive studies describe the prevalence of snus use among older men with oral cancer; however, such studies cannot, by design, estimate the risk of oral cancer associated with tobacco use.
- Three high-quality case-control epidemiology studies done specifically to study the relationship between snus and oral cancer (Lewin et al. 1998; Rosenquist et al. 2005; Schildt et al. 1998b) found no evidence that use of snus was associated with a statistically significant increased cancer risk. Two additional cohort studies that examined the development of cancers in general also found no relationship between snus use and oral cancer. A third cohort study found a statistically significant increased cancer risk among snus users that included smokers, but did not find a significant increased risk among never-smoking snus users.
- Three analytic epidemiology studies also found no significant association between the use of snus and cancer of the esophagus or larynx. A fourth study reported significantly

elevated risks of esophageal squamous cell carcinoma; however, due to study limitations any true relationship may have been overestimated.

- Two cohort studies suggest that use of Scandinavian smokeless tobacco could be associated with increased risk of pancreatic cancer among some subgroups of the population. However, there are troubling inconsistencies between the two studies with respect to the specific subgroups at risk (only individuals who were also current smokers in one study vs. only never-smokers of tobacco in the second study). It is not clear why the two studies would have found that the increased risk was limited to two distinctly different subgroups. Further research is needed to resolve these questions.
- Three case-control and one cohort study found no significant association between use of snus and stomach cancer. One additional study (Zendehdel et al. 2008) found (for one sub-analysis) that "snus" is associated with a significantly increased risk of stomach cancer, however, study limitations described previously raise questions that need to be addressed in further research.
- Several other cancer endpoints have been evaluated in a limited number of studies (kidney and bladder cancer, lung cancer, hematopoietic cancers, skin cancers, all cancers combined). The only statistically significant increase in risk associated with the use of snus and a specific cancer was for Hodgkin's lymphoma among men who had used snuff for more than 30 years. The finding was based on a very small number of cases, and is a novel finding that must be verified by additional studies. One other study found that the risk of smoke-related cancers among never-smoking ever-daily snus users was significantly elevated. A significant risk of any cancer was not observed among this group. Residual confounding is an important concern, and the authors conclude that relative risks are consistently lower for snus users than those associated with smoking.

5.6 Cardiovascular Effects

5.6.1 Overview of Cardiovascular Effects

The use of snus and its association with cardiovascular conditions, including acute cardiovascular effects, atherosclerosis, hypertension, and myocardial infarction (MI), has been investigated in a number of studies. Due to the presence of nicotine, which is known to have effects on vasoregulation, cardiac control, and autonomic homeostasis (Bolinder 1997), scientists have suspected that snus could affect the cardiovascular system. Several researchers have reviewed the available studies of potential cardiovascular effects of snus, and have concluded that snus is associated with acute increases in heart rate and blood pressure that disappear with abstinence, and that these effects are due to the nicotine (Asplund 2003; Boffetta and Straif 2009; Critchley and Unal 2004; Gupta et al. 2004). Many of these same reviewers found that with respect to numerous other cardiovascular parameters, snus users appear to be more similar to nonsmokers than to smokers. For example, snus users do not exhibit all the changes in biochemical risk factors for CVD typically seen in smokers, nor do snus users show evidence of the atherosclerotic processes generally seen in smokers (Bolinder 1997).

The body of published literature examining the relationship between use of snus and various measures of CVD includes eleven descriptive studies, four case-control studies, seven

prospective cohort studies, and one experimental study. The outcomes studied include long-term risk factors for CVD (e.g., fibrinolytic activity, hypertension, BMI), acute cardiovascular effects (e.g., elevated blood pressure and heart rate), and chronic CVDs (e.g., MI, coronary heart disease (CHD), sudden cardiac death (SCD), and total cardiovascular death).

Of the descriptive analyses, four utilize the same population of male Swedish firefighters (Bolinder et al. 1997b; Bolinder 1997; Bolinder et al. 1997a; Bolinder and de Faire 1998). Three case-control studies (Bolinder 1997; Huhtasaari et al. 1992; Huhtasaari et al. 1999; Wennberg et al. 2007) and two of the descriptive studies (Angman and Eliasson 2008; Eliasson et al. 1995) are derived from data from the MONICA Study (Monitoring Trends and Determinants in Cardiovascular Disease). The same population of male Swedish construction workers was utilized for several descriptive and cohort studies (Bolinder et al. 1994; Bolinder et al. 1992; Hergens et al. 2007; Hergens et al. 2008b; Hergens et al. 2008a). In addition, cardiovascular effects in the Swedish Twin cohort were reported by Hansson et al. (2009). Most of these studies include male participants only, so very little is known about the potential cardiovascular effects of snus in females. Furthermore, data derived from descriptive studies need to be considered cautiously, as these suffer from various limitations, including incomplete or nonexistent control for confounding factors and variations in the definitions of events included in the studies, and the cross-sectional nature of the studies.

A summary of the statistically significant findings for each parameter can be found in Table 5-2 below. While relative risk estimates are the more desired summary statistic to provide information on the potential association between snus use and the outcome, most studies did not provide this information. For the subset of studies that did provide effect estimates, these are summarized in detail in Appendix III-J (J-1 presents descriptive studies, J-2 presents case-control studies, J-3 presents cohort studies, and J-4 presents experimental studies) and specifically listed in Appendix IV.

Table 5-2: Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users

Cardiovascular Outcome	Statistically Significant* Association with Snus Use Found	No Statistically Significant Association with Snus Use Found
Acute Cardiovascular Events		
Acute effects on heart rate	**3 descriptive studies** • Bolinder and de Faire 1998 • Bolinder et al. 1997b • Hirsch et al. 1992	**2 descriptive study** • Eliasson et al. 1991 • Bolinder et al. 1997a
Acute effects on blood pressure	**3 descriptive studies** • Bolinder and de Faire 1998 • Bolinder et al. 1997b • Hirsch et al. 1992	**3 descriptive studies** • Eliasson et al. 1991 • Wennmalm et al. 1991 • Bolinder et al. 1997a
Indicators of or Risk Factors for Cardiovascular Disease		
Hypertension, blood pressure	**1 descriptive study** • Bolinder et al. 1992 **1 case-control study** • Hergens et al. 2005 **1 cohort study** • Hergens et al. 2008	**3 cohort studies** • Janzon and Hedblad 2009 • Ängman and Eliasson 2008 • Norberg et al. 2006

Table 5-2: Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users

Cardiovascular Outcome	Statistically Significant* Association with Snus Use Found	No Statistically Significant Association with Snus Use Found
Atherosclerosis or atherosclerotic index		**2 descriptive studies** • Bolinder et al. 1997a • Wallenfeldt et al. 2001
Cholesterol/hyperlipidemia/high density lipoprotein (HDL) or low density lipoprotein (LDL)		**4 descriptive studies** • Bolinder et al. 1997a • Wallenfeldt et al. 2001 • Eliasson et al. 1991 • Eliasson et al. 1995 **1 case-control study** • Hergens et al. 2005 **1 cohort study** • Norberg et al. 2006
Triglycerides	**1 descriptive study** • Wallenfeldt et al. 2001 **1 cohort study** • Norberg et al. 2006	**3 descriptive study** • Bolinder et al. 1997a • Eliasson et al. 1991 • Eliasson et al. 1995
Fibrinolytic activity		**3 descriptive studies** • Bolinder et al. 1997a • Eliasson et al. 1995 • Eliasson et al. 1991
Glucose levels		**6 descriptive studies** • Bolinder 1997a • Eliasson et al. 1995 • Eliasson et al. 1991 • Wallenfeldt et al. 2001 • Persson et al. 2000 • Eliasson et al. 2004 **1 cohort study** • Norberg et al. 2006
Insulin resistance or insulin response	**2 descriptive studies** • Eliasson et al. 1991 • Persson et al. 2000	**5 descriptive studies** • Bolinder 1997a • Eliasson et al. 1995 • Wallenfeldt et al. 2001 • Eliasson et al. 2004 • Persson et al. 2000
C-reactive protein		**1 descriptive study** • Wallenfeldt et al. 2001
Metabolic syndrome	**1 cohort study** • Norberg et al. 2006	**1 descriptive study** • Wandell et al. 2008
Diabetes	**1 descriptive study** • Persson et al. 2000	**1 case-control study** • Hergens et al. 2005 **2 descriptive studies** • Wandell et al. 2008 • Eliasson et al. 2004
Thromboxane A_2 production (possibly reflecting platelet activation)		**1 descriptive study** • Wennmalm et al. 1991
Oxygen uptake/work capacity	**1 descriptive study** • Bolinder and de Faire 1998	**2 descriptive studies** • Bolinder et al. 1997b • Wennmalm et al. 1991
Impaired endothelial function	**1 experimental study** • Rohani and Agewall 2004	

Table 5-2: Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users

Cardiovascular Outcome	Statistically Significant* Association with Snus Use Found	No Statistically Significant Association with Snus Use Found
BMI; change in body weight	**1 case-control study** • Hergens et al. 2005 **2 cohort study** • Norberg et al. 2006 • Nafziger et al. 2007 **2 descriptive studies** • Saarni et al. 2004 • Bolinder et al. 1992	**7 descriptive studies** • Sundbeck et al. 2009 • Bolinder et al. 1997a, b • Eliasson et al. 1995 • Eliasson et al. 1991 • Wallenfeldt et al. 2001 • Bolinder and de Faire 1998 **1 cohort study** • Rodu et al. 2004
Waist-to-Hip Ratio		**6 descriptive studies** • Bolinder et al. 1997a,b • Wallenfeldt et al. 2001 • Eliasson et al. 1995 • Sundbeck et al. 2009 • Bolinder and de Faire 1998
Cardiovascular Events		
Incidence of myocardial infarction (fatal or nonfatal)		**4 case-control studies** • Hergens et al. 2005 • Huhtasaari et al. 1992 • Huhtasaari et al. 1999 • Wennberg et al. 2007 **2 cohort studies** • Hergens et al. 2007 • Janzon and Hedblad 2009
Fatal MI; Sudden cardiac death	**1 cohort study** • Hergens et al. 2007	**1 cohort study** • Huhtasaari et al. 1999 **1 case-control study** • Wennberg et al. 2007
Incidence of coronary heart disease		**2 cohort studies** • Johansson et al. 2005; updated by Haglund et al. 2007 • Hansson et al. 2009
Mortality from all cardiovascular disease	**1 cohort study** • Bolinder et al. 1994	**2 cohort studies** • Hansson et al. 2009 • Roosaar et al. 2008

*Where available, effect estimates or p-values for current snus users were selected to determine significance, howeve if any particular subanalysis revealed a significant association for the specified outcome the corresponding study was placed in the statistically significant column.

5.6.2 Acute Cardiovascular Effects

Acute effects are those that can be linked temporally to a single exposure or brief series of exposures. Based on the nicotine content of snuff, which is known to affect blood vessel tone, it is expected that snuff use would produce an increase in heart rate and blood pressure in users. No studies have assessed the acute effects of snus use on blood pressure and heart rate in naive participants (those who have never used snus), but five reports have investigated these parameters among regular snus users. For example, Bolinder and de Faire (1998) used ambulatory blood pressure monitoring to compare blood pressure obtained during the daytime (presumably while using “smokeless tobacco”) with those obtained at night (presumably during abstinence). This study showed that diastolic blood pressure was significantly higher in snus users compared to nonusers of tobacco during daytime hours (6 am to 12 am) and that no

significant difference was observed at night (12 am to 6 am). Higher systolic blood pressure was also frequently observed in snus users compared to nonusers of tobacco. According to the authors, adjustments for confounders (i.e., age, BMI, waist-hip ratio, physical fitness, and alcohol consumption) had no significant effect on these findings. Further, a significant correlation was shown between blood pressures of the smokeless tobacco users and blood cotinine levels (the main nicotine metabolite), implying that the level of use was associated with these effects.

A second study by Bolinder and colleagues (1997b) found that after adjusting for confounders, heart rates of snus users were, on average, 6 beats per minute faster, systolic blood pressures tended to be 10-15 mmHg higher, and diastolic pressures tended to be 6 mmHg higher in "smokeless tobacco" users who had recently (< 2 hours previously) used "smokeless tobacco" than in those who had last used "smokeless tobacco" more than 2 hours before measurement. These differences, though acknowledged by the authors to lack statistical significance at many points during the investigation, were suggested to be consistent with temporal differences in acute nicotine exposure. These temporal relationships between use and effects on blood pressure and heart rate support an acute cardiovascular effect of "smokeless tobacco".

A third study failed to identify group differences in pulse rate or systolic or diastolic blood pressure between healthy young men who were either snuff users or nonusers of tobacco (Eliasson et al. 1991). The authors concluded that use of oral moist snuff does not appear to have a significant impact on these cardiovascular risk factors.

Hirsch and colleagues (1992) examined the hemodynamic effects of snus during rest and exercise in a placebo-controlled study of nine healthy, young people who used snus regularly. They reported that snuff intake induced significant increases in heart rate and blood pressure during rest but not during exercise.

Finally, Wennmalm and colleagues (1991) examined the effect of tobacco on several cardiovascular variables in a group of 577 young men, 127 of whom used only snuff. They found no differences between snuff users and nonusers of tobacco with respect to resting systolic and diastolic blood pressure. Snuff users who did not smoke were also similar to nonusers of tobacco with respect to various other thrombogenic factors (including thromboxane A_2).

5.6.3 Hypertension

Although snus use may be associated with acute changes in blood pressure among its users, considerable uncertainty exists as to whether snus use is associated with, and can cause, hypertension. A cross-sectional study of a large population of Swedish construction workers showed a significantly higher risk of mild hypertension (diastolic BP>90 mmHg or systolic BP>160 mmHg) among middle-aged smokeless tobacco users than among middle-aged nonusers of tobacco (Bolinder et al. 1992). Further, this study showed that among those who had received early disability pensions, there was a significantly higher risk of disability attributed to hypertension among middle-aged "smokeless tobacco" users compared to middle-aged nonusers of tobacco.

Hergens and colleagues (2008a) extended the follow-up of this cohort through 2003, and examined prevalent hypertension and the incident hypertension among those free of elevated blood pressure at baseline. These outcomes were identified from inpatient registers or separately, from repeated measurements made at health visits. Information on "snuff" use was obtained from follow-up visits starting in 1978 as "snuff" use data before that date was deemed incomplete. Among current "snuff" users, the overall prevalence of high blood pressure was significantly increased compared to never tobacco users (OR=1.25, 95% CI: 1.16-1.35). This was observed for all age groups except those who were older at baseline (60 years old or more), and was increased significantly among current snuff users using more than 12.5 g per day, but not among those using less than 12.5 g per day. An increased risk of incident high blood pressure (RR=1.34, 95% CI: 1.03-1.74) or hypertension (RR=1.43, 95% CI: 1.12-1.83) was also observed among current snus users who had been free of these conditions at baseline. This study has been criticized by Rodu and Heavner (2009) as containing errors and omissions that may have affected the study findings. Hergens and colleagues responded with some corrections, but stand by their findings of increased risk of blood pressure effects among "snuff" users (Hergens et al. 2009).

Janzon and Hedblad (2009) conducted a population-based cohort study that included male and female residents as part of the Malmö Diet and Cancer study. Residents ages 45-73 were invited to participate from 1991-1996 and followed for first incident MI through December 2004 using hospital discharge records. Participants completed a self-administered questionnaire on tobacco use and other lifestyle factors. The authors report that even after adjusting for age and BMI, mean blood pressure showed no statistically significant difference between male and female snuff users and nonusers. It is not clear if this estimate includes smokers.

The available studies of Swedish snuff use do not indicate an association between snus use and atherosclerosis. For example, a cross-sectional study of clinically healthy men by Wallenfeldt and colleagues (2001) found no statistically significant association between use of oral moist snuff and any ultrasound-assessed measures of subclinical atherosclerosis (intima-media thickness in the carotid bulb, carotid artery, or femoral artery, or carotid or femoral plaques). Similarly, two analyses of a population of healthy male construction workers showed no significant difference between smokeless tobacco users and nonusers of tobacco with respect to measurements of carotid wall thickness, lumen diameter, or the presence of carotid plaques (Bolinder et al. 1997a) or an "atherogenic index" (Bolinder 1997).

Furthermore, the literature does not present evidence of an association between use of snus and a wide range of risk factors for atherosclerosis. In the Wallenfeldt et al. (2001) study cited above, there were no associations between snuff use and numerous biochemical risk factors for CVD (cholesterol, apolipoprotein A1 or B, fasting blood glucose, plasma insulin, or C-reactive protein). The only significant finding in this study was that never-snuff users had lower serum triglyceride levels than previous or current snuff-takers. Other studies of risk factors for atherosclerosis (serum lipids, fibrinogen levels, fibrinolytic activity, insulin resistance, thromboxane A_2 production) have generally shown no significant difference in levels of these risk factors between smokeless tobacco users and nonusers of tobacco products (Bolinder 1997; Bolinder et al. 1997a; Eliasson et al. 1995; Wennmalm et al. 1991).

5.6.4 Other Indicators of Cardiovascular Disease

Numerous studies have examined the use of snus on indicators of cardiovascular health, sometimes as part of studies of other related outcomes, such as diabetes (see Table 5-2 and Section 6.9). A Swedish study of cardiovascular work capacity among healthy participants showed no significant differences between "smokeless tobacco" users and nonusers of tobacco with respect to maximal oxygen uptake or maximal work capacity (Bolinder et al. 1997b). Participants in this study used "smokeless tobacco" on average for 24-25 years, suggesting no effect of long-term snuff use on cardiovascular health. However, a large cross-sectional study of Swedish construction workers found a significantly higher risk of reporting cardiovascular/circulatory symptoms (i.e., breathlessness on slight effort, chest pain walking up hill, pain in the leg while walking, white finger symptoms) among "smokeless tobacco" users compared to nonusers of tobacco (Bolinder et al. 1992). Further, this study showed that among those who had received disability pensions, there was a significantly higher risk of attributing the disability to CVD among users of smokeless tobacco than among nonusers of tobacco.

An experimental study of 20 healthy, middle-aged men and women suggests that acute use of Swedish snuff may be associated with endothelial dysfunction. This is of interest because endothelial dysfunction is a predictor of cardiovascular morbidity (Rohani and Agewall 2004). However, the study suffered from a number of flaws, and thus the significance of this finding is unclear.

5.6.5 Chronic Cardiovascular Disease

Eleven epidemiology studies have evaluated the relationship between use of snus and various chronic CVDs. With the exception of one cohort, in which an update observed an increased risk only in a subanalysis of fatal MI, these studies of men failed to observe an increased risk of specific CVDs (e.g., MI, SCD) among snus users when compared to nonusers of tobacco.

Two studies by Huhtasaari and colleagues revealed a lack of significant risk (Huhtasaari et al. 1992; Huhtasaari et al. 1999). Huhtasaari and colleagues (1999) further noted that, from a cardiovascular perspective, cigarette smoking had greater deleterious effects than snuff. Huhtasaari and colleagues (1992) also included a comparison of cigarette smoking and snuff use, and found that cigarette smokers aged 35-54 had a significantly higher risk of MI compared to snuff users of the same age. This same effect was seen when participants of all ages were pooled, but not in the subgroup of men aged 55-64.

The study reported by Wennberg and colleagues (Wennberg et al. 2007), a prospective incident case-referent study, reported that snuff users are not at increased risk of MI or SCD. These investigators evaluated tobacco habits among 525 men who experienced a first MI (including 93 who died suddenly) and 1,798 matched controls. Snuff users who had never smoked did not have increased risk of either MI (OR=0.82; 95% CI:0.46-1.43) or SCD (with survival <24 hours; OR=1.18; 95% CI:0.38-3/70) compared to nonusers of tobacco. Snuff users who had smoked previously were also not at significantly increased risk, although the authors note that the odds ratio for MI was slightly increased (OR=1.25; 95% CI:0.80-1.96). In contrast, men who were current smokers and who did not use snuff were at significantly increased risk of both MI and SCD.

Hergens and colleagues (2005) conducted a population-based case-control study in two Swedish counties. Only men were included in the study due to a low prevalence of "snuff" use among women. In this study, the relative risk estimate for first acute MI among current "snuff" users who had never smoked was 0.73 (95% CI:0.35-1.5). When nonfatal and fatal cases were examined, the relative risk estimate for fatal MI among current "snuff" users who had never smoked was nonsignificantly elevated (OR=95.7; 95% CI:0.48-5.5).

In addition to the case-control studies, a cohort study by Johansson and colleagues (2005) found that incidence of CHD was no higher among men who used snus (but did not smoke) than among nonsmokers. Johansson and colleagues evaluated the association between smoking and snuffing habits and incidence of CHD among 3,120 healthy men aged 30 to 74 who were followed for an average of 11.2 years. Participants were divided into six mutually exclusive categories based on their smoking and snuff use habits. Men who used snuff daily but had never smoked were not at significantly increased risk of CHD (HR=1.41; 95% CI:0.61-3.28), after adjustment for age, physical activity, BMI, diabetes, and hypertension. In contrast, men who were daily smokers, former smokers, or who combined smoking and snuffing all had significantly higher hazard ratios than never-smokers. The greatest weakness of this study is that tobacco habits were assessed only at baseline and not during the follow-up period.

Haglund and colleagues (2007) examined the association between snus use and risk of fatal or nonfatal ischemic heart disease (IHD) following the methodology of the prior study (Johansson et al. 2005), but used an expanded cohort, an additional three years of follow-up, and was able to look at stroke outcomes in addition to other cardiovascular outcomes. In this study, no statistically significant excess IHD risk for snus users was observed. The authors noted, however, that the risk of mortality from IHD was slightly increased (RR=1.15, 95% CI: 0.54-2.41). The authors also noted that the risks for both incident IHD and IHD mortality, though not statistically significant, were elevated for dual users, that is, study participants who smoked and used snus had a significantly increased risk of fatal or non-fatal IHD. The number of fatal events was small, however (less than 10).

In contrast, a cohort study by Bolinder and colleagues (1994) reported a statistically significant association between "smokeless tobacco" use and increased risk of death from all CVDs in their study population of Swedish construction workers. Risks appeared to vary by age, however. Increased risks of all CVDs and IHD were seen among smokeless tobacco users aged 35-45 years, but not among participants aged 55-65 years. Although the exposure data on smokeless tobacco use was properly limited to include only "present smokeless tobacco use and no former or present smoking," tobacco habits were assessed only once at entry into the cohort. Therefore, this study did not account for any changes in tobacco habits or changes in other confounding factors that occurred during the ten years of follow-up. The authors presented unadjusted risk estimates, although they stated that adjustments for age, area of domicile, BMI, blood pressure, diabetes, history of heart symptoms, and use of blood pressure medication did not affect risk estimates, but did not adjust for other important confounding factors, such as cholesterol, family history of CVD, alcohol consumption, or SES. Some epidemiologists call into question the use of a single cause of death for statistical tabulations, as this does not provide a complete representation of comorbid events. In addition, Rodu and Cole (1995) criticized Bolinder et al.'s findings, and noted that an apparent excess of cardiovascular deaths observed

in “smokeless tobacco” users could be attributable to the inappropriate selection of the control group in the study, as nonusers of tobacco were exceptionally healthy.

Hergens and colleagues (2007) extended the follow-up of this cohort through 2003, and examined MI incidence and mortality. Information on “snuff” use was obtained from follow-up visits starting in 1978 as snuff use data before that date was deemed incomplete. Overall risk of total and nonfatal MI were not increased among current “snuff” users compared to never tobacco users, even when examined by daily snuff use. The relative risks for fatal MI, however, was significantly elevated overall (1.32, 95% CI: 1.08-1.61), and the highest risk for fatal MI was observed among heavy “snuff” users. The relative risk for fatal MI for those who reported using 50 or more g per day was 1.96 (95% CI:1.08-3.58).

Hansson and colleagues (2009) followed participants in the Swedish Twin Registry, born between 1926-1958, for stroke incidence or mortality. Participants had been asked about snus use through a telephone survey conducted from 1998-2002. Participants were followed for hospitalization or death due to MI or coronary revascularization (considered together as IHD). No statistically significant increase in IHD risk (or any CVD risk, including stroke) was observed among current or former snus users. Furthermore, there was no increased risk of IHD observed for heavy users (4 or more cans of snus per week) nor for those who had used snus for 20 or more years.

Janzon and Hedblad (2009) conducted a population-based cohort study that included male and female residents as part of the Malmö Diet and Cancer study. Residents ages 45-73 were invited to participate from 1991-1996 and followed for first incident MI through December 2004 using hospital discharge records. Participants completed a self-administered questionnaire on tobacco use and other lifestyle factors. Among males snuff users who were never smokers (9% of the male snuff users), the relative risk of first ever MI was not increased (RR=0.75; 95% CI: 0.3-1.8). No MI cases were observed among the 75 female snuff users. The authors concluded that snuff use is not associated with stroke risk in males.

5.6.6 Reviews, Meta-Analyses and Population Attributable Risk of Death from Cardiovascular Disease Due to Use of Snus

Several reviews of the potential cardiovascular effects among snus users have been conducted; many did not differentiate between snus and other types of smokeless tobacco in reaching conclusions (Colilla 2010; Critchley and Unal 2004; Gupta et al. 2004; SCENIHR 2007). Boffetta and Straif (2009) conducted a meta-analysis that examined risk of MI among ever users of STPs compared to never smokers. These authors included six studies of incident or fatal MI. When limited only to studies in Sweden, the summary risk of any MI among snus users was not elevated (RR: 0.87; 95% CI: 0.75-1.02), and was similar when the analysis was limited only to cohort studies in Sweden. For fatal MI, the summary risk was significantly increased (RR: 1.27; 95% CI: 1.07-1.52), and again, was similar when limited only to cohort studies in Sweden. These authors estimated that the fraction of all fatal MI in Sweden attributable to ever snus use is 5.6%, or a total of 346 deaths per year.

Lee (2007) conducted a meta-analysis that examined risk of IHD or acute MI using seven studies of snus users and these outcomes, and also examined briefly the evidence for an association between use of smokeless tobacco and risk factors for heart disease such as diabetes, hypertension, cholesterol, fibrinogen, platelet function and measures of atherosclerosis. The summary risk estimates included only the risk estimates among current snus users compared to never smokers (where these data were available). Using different models[52] produced slightly different results when the Swedish studies were combined; the summary risk estimate using the fixed effects model was not increased for risk of IHD or MI (RR:1.17, 95% CI: 0.83-1.37), nor using the random effects model summary risk estimate (RR: 1.06, 95% CI: 0.83-1.37). When the Swedish data were combined with the US studies, which have large population sizes, Lee (2007) found a clearly increased risk using the fixed effects model (RR: 1.15, 95% CI: 1.08-1.22), and the risk was of borderline significance with the random effects model (RR: 1.12, 95% CI: 0.99-1.27). Lee commented that combining the US and Swedish studies may be appropriate, as both contain nicotine exposures comparable to that of smoking, and because nicotine has been implicated in several processes related to risk of CVD. Bolinder (1997) commented, however, that nicotine is not thought to be linked to the atherosclerotic process in the same way as smoking.

Since these reviews have been conducted, additional studies of potential cardiovascular effects among snus users have been reported (Hansson et al. 2009, Janzon and Hedblad 2009), and for the Lee (2007) meta-analysis, the study by Haglund et al. (2007). These more recent studies did not observe a significant association between snus use and risk of MI.

As noted previously, the PAR represents the proportion of the deaths in a population that could theoretically be prevented if a particular risk factor (such as use of snus) were totally eliminated. Critchley and Unal (2003) calculated the PAR fraction for ischaemic heart disease in Sweden (based on data from the Bolinder et al. 1994; Huhtasaari et al. 1992; and Huhtasaari et al. 1999 studies described above), and estimate that between 0 and approximately 3,000 heart disease deaths each year may be due to snus use. However, such calculations are inappropriate until a causal relationship has been established (Hennekens and Buring 1987), and as the above sections of the report demonstrate, use of snus has not been causally linked to increased risk of death due to IHD.

5.6.7 Summary of Cardiovascular Effects

The following conclusions can be made about the use of snus and its effect on the cardiovascular system and risk factors for CVD:

- Several studies suggest that snus use is associated with acute cardiovascular effects, including increases in blood pressure and heart rate. Researchers appear to agree that these effects are most likely due to nicotine.
- It remains unclear whether snus use is associated with hypertension.

[52] Fixed-effects models are appropriate when heterogeneity between studies is low, and random-effects models are appropriate when heterogeneity between studies is observed.

- Snus does not appear to be associated with atherosclerosis or risk factors for atherosclerosis (serum lipids, fibrinogen levels, fibrinolytic activity, insulin resistance). This is significant because hypertension and atherosclerosis are more potent predictors of long-term IHD than are acute changes in heart rate and blood pressure.
- Numerous recent studies have not revealed an increased risk of MI or an overall increased risk of CVD. One study found an increased risk of fatal MI.
- Meta-analyses that combine studies with those from the US have reported significantly increased risk of MI. Combining studies of snus users with users of traditional US STPs may be appropriate if nicotine exposures are similar, and nicotine is the putative exposure for risk of MI. This remains to be determined.

5.7 Stroke

5.7.1 Overview

A stroke is a sudden interruption in the blood supply of the brain. Most strokes are caused by a blockage in the arteries leading to the brain; these are referred to as ischemic strokes. Another type of stroke (called a hemorrhagic stroke) occurs when there is bleeding into the brain when a blood vessel bursts. Seven analytic studies have explored the relationship of snus use and risk of stroke; none found that use of snus was associated with significantly increased risk of stroke overall. The studies are summarized in Appendix K-1 (case-control studies) and Appendix K-2 (cohort studies).

Asplund and colleagues (2003) conducted a nested case-control study in Northern Sweden, using data recorded prospectively in two cohort studies. The study involved 276 men (age 25 to 74) who had a first-ever fatal or nonfatal stroke (either ischemic or hemorrhagic), and 551 matched controls with no history of CVD. The risk of stroke in exclusive snuff users who had never smoked was similar to that of men who had never used tobacco (unadjusted OR=1.05, 95% CI:0.37-2.94). The odds ratio did not change appreciably after adjustment for multiple cardiovascular risk factors (OR=0.87, 95% CI:0.41-1.83). In contrast, the risk of stroke among regular cigarette smokers was higher (OR=1.74, 95% CI:0.85-3.54). The authors concluded that use of snus involves a much lower risk for adverse cardiovascular effects than smoking, and speculated that the important factor in increasing risk is chemicals produced by burning tobacco. A strength of this nested case-control design is that information on risk factors was collected before the strokes occurred, eliminating the possibility of recall bias.

A study by Koskinen and Blomstedt (2006) examined the relationship between snus use and subarachnoid hemorrhage (SAH) among 120 consecutive patients with spontaneous SAH and a reference population that was selected to match the distribution of smokers in 2001 and snuff users from 1996 to 1997. Snus use was not associated with increased risk of SAH among either men (RR=0.48; 95% CI:0.17-1.30) or women (RR=1.30; 95% CI:0.33-5.18). In contrast, smoking was associated with significantly increased risk of SAH among both men (RR=2.63; 95% CI:1.20-5.72) and women (RR=2.26; 95% CI:1.69-3.01). Consequently, the investigators suggest that it is unlikely that nicotine is solely responsible for the increase in risk of SAH. It

does not appear that potential confounders were considered in the statistical analysis of this study; this and other details are not presented by the authors.

In the cohort study of Swedish construction workers described earlier, Bolinder and colleagues (1994) examined the relationship between "smokeless tobacco" use and risk of death from a number of CVDs, including stroke, among men aged 35-45 years and among men aged 55-65 years through 1985 (see Appendix J-2). "Smokeless tobacco" users were those who were current users of smokeless tobacco and who had never smoked. "Smokeless tobacco" use was not associated with significantly increased risk of stroke death among either age group: the RR among younger men was 1.9 (95% CI: 0.6-5.7) compared to nonusers of smokeless, and it was 1.2 (95% CI:0.7-1.8) among older men. Adjusted risk estimates were not presented, although the authors stated that adjustments for age, area of domicile, BMI, blood pressure, diabetes, history of heart symptoms, and use of blood pressure medication did not affect risk estimates. Hergens and colleagues (2008a) extended the follow-up of this cohort through 2003, and examined both stroke incidence and mortality. Information on "snuff" use was obtained from follow-up visits starting in 1978 as "snuff" use data before that date was deemed incomplete. Overall stroke risk was not increased among current "snuff" users compared to never tobacco users, and no increased risk of hemorrhagic stroke was observed. Relative risks for ischemic stroke (1.72, 95% CI: 1.06-2.78) and for unspecified stroke (1.35, 95% CI: 1.02-1.80) were statistically significantly increased. Among current "snuff" users, however, there was no clear evidence of a dose-response relationship; a statistically significant risk of ischemic stroke was observed among those using less than 12.5 g per day of "snuff", and not among those using more than 12.5 g per day.

Haglund and colleagues (2007) examined the association between snus use and risk of stroke following the methodology of a prior study (Johansson et al. 2005), but used an expanded cohort, an additional three years of follow-up, and were able to look at stroke outcomes in addition to other cardiovascular outcomes. In this study, no excess stroke risk for snus users was observed. The authors noted, however, that the highest risks for stroke were observed among dual users, that is, study participants who smoked and used snus had a significantly increased risk of stroke mortality, and an elevated risk of stroke incidence. The authors commented that risks for active smoking are believed to remain elevated for five years following smoking cessation.

Hansson and colleagues (2009) followed participants in the Swedish Twin Registry, born between 1926-1958, for stroke incidence or mortality. Participants had been asked about snus use through a telephone survey conducted from 1998-2002. No statistically significant increase in stroke risk was observed among current or former snus users. The authors noted an indication of increased risk of stroke for users of 4 or more cans of snus per week, though this finding was not statistically significant, but no increased risk among those with more moderate snus use (< 4 cans/week). No increased risk was observed among those who had used snus for 20 or more years.

Janzon and Hedblad (2009) conducted a population-based cohort study that included male and female residents as part of the Malmö Diet and Cancer study. Residents ages 45-73 were invited to participate from 1991-1996 and followed for first incident stroke (or MI) through

December 2004 using hospital discharge records. Participants completed a self-administered questionnaire on tobacco use and other lifestyle factors. Among males snuff users who were never smokers (9% of the male snuff users), the relative risk of stroke was not increased (RR=0.59, 95% CI: 0.2-1.5). One stroke was observed among the 75 female snuff users, but no relative risk was calculated from this small number and the smoking status for this stroke case was not presented. The authors concluded that snuff use is not associated with stroke risk in males.

5.7.2 Literature Reviews and Meta-analyses of Effects on Stroke

Several major reviews of the epidemiological literature have been published (Asplund 2003; Boffetta and Straif 2009; Colilla 2010; Critchley and Unal 2004; Gupta et al. 2004; Lee 2007; SCENIHR 2008). Because four of the seven available studies were reported in 2007 or later, reviews conducted before 2008 had relatively few studies to consider. The SCENIHR (2008) report considered only three of the studies (Asplund et al. 2003; Bolinder et al. 1994; Haglund et al. 2007), and did not reach a conclusion regarding stroke risk among snus users.

More recent reviews were conducted by Colilla (2010) and Boffetta and Straif (2009) that included the Hergens et al. (2008a) update to the Construction Workers cohort first reported by Bolinder et al. (1994), in addition to the studies by Asplund et al. (2003) and Haglund et al. (2007). Colilla (2010) did not differentiate between exposures to snus and to US STPs, and based on the combined results of studies from these two exposures, concluded that increased ischemic stroke mortality, but not stroke incidence (new cases), may be associated with use of smokeless tobacco. In their meta-analysis, Boffetta and Straif (2009) also combined results of studies of snus and US smokeless tobacco users. In the analyses that combined studies only from Sweden, relevant to this review, no overall increased risk of stroke (RR=1.02; 95% CI: 0.93-1.13) or of stroke mortality (RR=1.25; 95% CI: 0.91-1.70) was reported.

5.7.3 Summary of Effects on Stroke

Seven analytic studies (two case-control and five cohort) were identified that examined the relationship between snus and risk of stroke. Males only were studied in all but two studies (Janzon and Hedblad 2009; Koskinen and Blomstedt 2006), though the study by Janzon and Hedblad had too few female snus users to report risk estimates. Thus the findings from the studies are applicable generally only to males.

The findings from the studies of stroke are summarized in Table 5-3. None found an increased risk of all stroke types combined among current or former snus users. No association between hemorrhagic stroke and snus use was observed in the two studies that examined this stroke type. In one study that examined ischemic stroke, an increased risk of ischemic stroke was observed among snus users, however, in this study, no dose-response relationship with ischemic stroke was observed. In the study by Hansson et al., the dose-response analysis was suggestive of a higher overall stroke risk for snuff users using four or more cans per week, but this finding was not statistically significant. The two recent reviews of stroke studies published through 2008, both reported no increased risk of stroke incidence. One of the recent reviews suggested an increased risk from fatal stroke based on one study in which a significant

increased risk of fatal ischemic stroke was observed, but when results of studies of fatal stroke were combined by Boffetta and Straif, the risk of fatal stroke was not significantly elevated.

Table 5-3: Studies of Stroke Among Swedish Snus Users

Stroke Type	Statistically Significant Association with Snus Observed	No Statistically Significant Association with Snus Observed
All		Bolinder et al. 1994 and Hergens et al. 2008* Asplund et al. 2003 Haglund et al. 2007 Hansson et al. 2009 Janzon and Hedblad 2009
All Fatal		Hergens et al. 2008 Haglund et al. 2007
All Nonfatal		Hergens et al. 2008
Ischemic		Hergens et al. 2008
Ischemic Nonfatal		Hergens et al. 2008
Ischemic Fatal	Hergens et al. 2008	
Hemorrhagic		Hergens et al. 2008
Hemorrhagic Nonfatal		Hergens et al. 2008
Hemorrhagic Fatal		Hergens et al. 2008
Subarachnoid Hemorrhagic		Koskinen and Blomstedt. 2006
Dose Response		
All: <12.5 g/day		Hergens et al. 2008
12.5-24.9 g/day		Hergens et al. 2008
25-49.9 g/day		Hergens et al. 2008
>50 g/day		Hergens et al. 2008
All: ≤ 4 cans/week		Hansson et al. 2009
All: ≥ 4 cans/week		Hansson et al. 2009
Ischemic: <12.5 g/day	Hergens et al. 2008	
12.5-24.9 g/day		Hergens et al. 2008
25-49.9 g/day		Hergens et al. 2008
>50 g/day		Hergens et al. 2008
Hemorrhagic: <12.5 g/day		Hergens et al. 2008
12.5-24.9 g/day		Hergens et al. 2008
25-49.9 g/day		Hergens et al. 2008
>50 g/day		Hergens et al. 2008

*Both studies report on the Swedish Construction Workers Cohort

5.8 Gastrointestinal Effects

Because saliva produced during the use of snus is often swallowed instead of expectorated, studies of the relationship between snus use and gastrointestinal effects should be considered in an evaluation of the potential health effects of snus. Two relevant studies were identified. Bolinder and colleagues (1992) evaluated the link between tobacco consumption and general health, including heartburn and peptic ulcer. Persson and colleagues (1993) examined whether the use of snus was associated with an increased risk of two different gastrointestinal diseases, Crohn's disease (CD) and ulcerative colitis (UC). The findings of these studies are summarized in Appendix L-1 and L-2, respectively, and are discussed below.

5.8.1 Heartburn and Peptic Ulcer

In a descriptive, cross-sectional study of approximately 40,000 subjects, Bolinder and colleagues (1992) found that Swedish users of "smokeless tobacco" (described as 'mainly moist snuff') did not have an elevated risk of peptic ulcer and that they had a significantly decreased tendency to suffer from heartburn compared to nonusers. These findings were based on 5,014 Swedish smokeless tobacco users who had never been regular smokers and 23,885 Swedish participants who had never used any type of tobacco. The reason for the lower risk of heartburn in "smokeless tobacco" users was not clear, but the authors speculated that the high pH of moist snus (8.5) could be important when saliva is swallowed.

5.8.2 Crohn's Disease or Ulcerative Colitis

Persson and colleagues (1993) examined two types of inflammatory bowel disease (IBD), CD and UC, in a case-control study. CD is a type of chronic inflammatory disorder of unknown cause that involves the gastrointestinal tract, specifically the terminal ileum of the small intestine (Glickman 1998). The incidence of CD in Western Europe and the US is estimated to be approximately 2 cases per 100,000 annually, and the prevalence is between 20 and 40 per 100,000. The major clinical features of CD are fever, abdominal pain, diarrhea (often without blood), weight loss, and generalized fatigability.

UC shares many of the features of CD. It is another category of IBD of unknown cause characterized by ulceration of the colon and rectum (Glickman 1998). The incidence of UC in Western Europe and the US is estimated to be approximately 6 to 8 cases per 100,000 annually, and the prevalence is between 70 and 150 per 100,000. The major clinical symptoms of UC include rectal bleeding, mucosal crypt abscesses, inflammatory pseudopolyps, abdominal pain, and diarrhea (Glickman 1998).

Persson and colleagues (1993) evaluated the relationship between the two types of IBD (CD and UC) and snus in a case-control study that also examined the role of cigarette smoking as a confounding or synergistic factor in the development of IBD. In this study, use of snus among never-smokers was not associated with any increase in risk of IBD. Among all participants (including those who were former or current smokers), ever-use of snus was associated with a two-fold increase in relative risk of both CD (RR = 2.1, 95% CI: 1.0-4.6) and UC (RR = 2.2, 95% CI: 1.1-4.4) after adjustment for age and cigarette smoking, but not for other potentially important factors that could be related to UC. However, only the finding for UC was marginally statistically significant. The authors found a synergistic interaction between cigarette smoking

and snus use, although it is not clear whether the interaction was tested statistically in a logistic regression model.

5.8.3 Summary of Gastrointestinal Effects

A descriptive study of the relationship between snus and heartburn and peptic ulcer showed that users of snus did not have any excess risk of peptic ulcer and that they had a significantly lower risk of heartburn. A single case-control study was identified that examined the relationship of IBD with oral moist snuff and cigarette smoking in Sweden. This study found no increased risk of CD or UC associated with snuff use when the analysis was limited to never-smokers.

5.9 Insulin Resistance and Type 2 Diabetes

There are reports in the literature that smokers are at increased risk of developing type 2 diabetes, as well as developing the conditions underlying diabetes (i.e., insulin resistance and impaired glucose tolerance). This finding has stimulated research into the relationship between snus use and these outcomes. Some studies described previously in this report (see Cardiovascular Effects) have addressed the effect of Swedish snuff use on insulin resistance, which is also a risk factor for heart disease. More recently, studies have examined the specific relationship between snus use and type 2 diabetes.

Diabetes occurs when there is an imbalance in the levels of glucose and insulin in the body. Two precursor conditions underlie this disease and are frequently studied in conjunction with diabetes. *Impaired glucose tolerance* refers to a condition in which blood glucose levels are higher than normal, but not high enough to qualify the individual as diabetic. *Insulin resistance* is a condition in which target tissues in the body (cardiac, skeletal, and adipose tissue) gradually become insensitive to the natural actions of insulin. Type 2 diabetes is the most common form of diabetes, and occurs when an individual's tissues become resistant to insulin (National Institute of Health 2009).[53]

5.9.1 Studies of Insulin as a Risk Factor for Heart Disease

The relationship between snus use and insulin resistance has been examined in four descriptive studies of risk factors for CVD (previously described in the “Cardiovascular Effects” section of this report; (Bolinder 1997; Eliasson et al. 1991; Eliasson et al. 1995; Wallenfeldt et al. 2001)). Three of the studies found no statistically significant associations between snus and insulin reactivity or plasma insulin levels, while one (Eliasson et al. 1991) suggested that serum insulin levels may be somewhat higher in snus users compared to nonusers of tobacco. None of the four studies found any significant association between snus use and blood glucose levels. Little can be concluded about the relationship between snus use and insulin resistance, because these cross-sectional studies do not permit an assessment of whether the snus use preceded or followed the observed increase in insulin.

5.9.2 Studies on Diabetes

In addition to studies evaluating insulin resistance as a risk factor for heart disease, four studies of varying designs have evaluated the relationship between Swedish snuff use and insulin

[53] National Institute of Health. 2009. http://diabetes.niddk.nih.gov/dm/pubs/overview/index.htm; accessed November 2009.

resistance or impaired glucose tolerance as these conditions underlie type 2 diabetes and one descriptive study that does not evaluate the relationship between Swedish snuff use and insulin resistance or impaired glucose tolerance in conjunction with diabetes. These studies are summarized in Appendices M-1 (two descriptive studies), M-2 (a cohort study), M-3 (an experimental study) and M-4 (a case-control study).

The strongest of these five studies (Eliasson et al. 2004) examined the effect of snus use and smoking on risk of type 2 diabetes among 3,384 men in a population-based cross-sectional and prospective cohort study (the northern Sweden MONICA study) (summarized in Appendix M-2). At study entry, the prevalence of clinically diagnosed diabetes was significantly higher among ever- and ex-smokers compared to never-tobacco users, but the prevalence was not significantly elevated among any category of snus users (ever, current, or ex). The prevalence of pathological glucose tolerance (defined as impaired glucose tolerance or undiagnosed diabetes) was not significantly elevated among snus users or smokers at entry. The risk of developing diabetes during follow-up was significantly elevated among exclusive smokers and ex-smokers, but no cases of diabetes developed among exclusive snus users. The authors concluded that the risk of diabetes was not significantly increased among snus users. Smoking was associated with both prevalent and incident cases of diabetes.

Eliasson and colleagues (2004) appropriately note that a causal link between tobacco use and disease cannot be claimed on the basis of cross-sectional prevalence data. However, their study also provides strong data on incidence (i.e., development of disease over time among individuals who were not diseased at study entry); causal conclusions can be drawn from such data. Other strengths of this study include: a large number of participants; about half of the incident cases of diabetes were confirmed by oral glucose tolerance test; and tobacco use was validated biochemically in a subgroup of participants. The Eliasson et al. (2004) study is the first study to use prospective data to demonstrate that snus does not carry the same increased risk for diabetes as smoking.

In contrast to Eliasson and colleagues (2004), a descriptive study by Persson and colleagues (2000) suggests that an association exists between oral snus use and type 2 diabetes. This cross-sectional study (summarized in Appendix M-1) examined a group of 3,128 Swedish men, half of whom had a strong family history of diabetes. All participants were given an oral glucose tolerance test and classified as having normal or impaired glucose tolerance, or type 2 diabetes. The authors then examined the correlation between snus use and the outcomes of interest among exclusive users of snus (i.e., those without a history of cigarette smoking). Exclusive users of snus had approximately a 4-fold increased prevalence of type 2 diabetes compared to never-users of tobacco (OR=3.9; 95% CI:1.1-14.3), based on only four cases of diabetes among snus users. Additional results indicated that exclusive snus users did not experience impaired glucose tolerance and that snus users (a category that may have included both exclusive snus users and former smokers) did not experience increased insulin resistance—conditions which, as previously discussed, are recognized precursors to diabetes.

Hergens and colleagues (2005) examined the association between “snuff” use and having diabetes among controls in their population-based case-control study. The relative risk estimate

for having diabetes among current "snuff" users was 1.5 (95% CI: 0.76-2.9), based on five cases observed among the controls.

Another study, a population-based cross-sectional study conducted by Wandell and colleagues (2008), examined the effect of snus use and smoking on risk of diabetes among 1,859 men, aged 60 years. The prevalence of newly diagnosed diabetes was not significantly elevated among any category of snus use (ex-smokers and current snuffers, ex-snuffers, current snuffers, current smokers and snuffers, low consumption of snuff, high consumption of snuff), based on 78 participants diagnosed with diabetes. The only risk factors found to be associated with newly diagnosed diabetes were waist size and high alcohol consumption.

As with all cross-sectional studies, these studies examined prevalence of disease, not incidence; thus, they can help identify factors that are correlated with diabetes, but cannot elucidate factors that affect the development of this disease. An important limitation of cross-sectional studies is that they cannot address temporal sequence (i.e., whether the snus use preceded the diabetes or not). Analytic studies, such as the Eliasson et al. (2004) study, do not suffer from this limitation.

Data on snus use and diabetes also come from a human experimental study by Attvall and colleagues (1993) (summarized in Appendix M-3). This study examined the acute effect of snuffing on insulin sensitivity in a small group of healthy habitual smokers. These individuals abstained from smoking for two days, then used snus in a controlled manner, and finally were tested for insulin resistance. There was no difference in insulin action between snuffers and abstainers. Experimental studies in theory should generate results with less variability than epidemiology studies, because outside factors influencing exposure data can be controlled.

The SCENIHR Working Group (2008), charged with assessing the health risks of smokeless tobacco use, also concluded that use of snus was not causally linked with insulin sensitivity or diabetes.

5.9.3 Summary of Effects on Insulin Resistance and Diabetes

The following conclusions can be made about the use of snus and its association with diabetes and risk factors for diabetes:

- One well-conducted analytic study (a cohort study that generated both prevalence and incidence data) found that use of snus was not associated with increased risk of diabetes.
- One cross-sectional study suggested that snus use may be linked to an increased prevalence of type 2 diabetes, while one other cross-sectional study did not. However, cross-sectional studies have significant limitations, including the fact that they cannot address temporal sequence (i.e., whether the snus use preceded the diabetes or not).
- A single human experimental study found that acute use of snus had no significant effect on insulin action.
- A single case-control study found that use of snus was not associated with diabetes among controls only.

5.10 Metabolic Syndrome

Two recent epidemiology studies investigated the relationship between use of snus and risk of metabolic syndrome (MetSy) (see Appendix N-1 and N-2). Individuals who have MetSy (a cluster of risk factors, including obesity, impaired glucose regulation, hypertension, and dyslipidemia) are at increased risk of heart disease and diabetes. Norberg and colleagues (2006) analyzed data from a population-based longitudinal study to investigate the relationship between a number of lifestyle factors, including use of Swedish snus, and risk of MetSy. Several factors were associated with increased risk of having developed MetSy, including heavy consumption of snus (OR=1.6; 95% CI:1.26-2.15), low education, physical inactivity, and former smoking. Heavy use was defined as more than 4 cans per week; use of $\leq$4 cans was not associated with increased risk of developing MetSy. Use of snus was associated with significantly increased risk of some of the individual elements of MetSy (high triglycerides and obesity) but not others (impaired glucose regulation, low HDL cholesterol, and hypertension). The authors concluded that heavy use of snus is independently associated with MetSy, even after adjustment for smoking.

This study suffers from a number of weaknesses, however. It appears that people who had the disease of interest were not eliminated at baseline, as is necessary in a cohort study. Consequently, this study cannot demonstrate a temporal relationship. Furthermore, those who had MetSy at baseline may have been more likely to die and not return for follow-up; the authors do not address how this was handled. In addition, the authors only considered baseline tobacco use as a predictor of development of MetSy. Participants may have changed their tobacco habits during the long follow-up period; this is especially likely given the nature of the intervention program, in which participants were advised at study entry of their risk profile for CVD and how to improve it. Thus, this study raises an important health effect that could potentially be associated with heavy use of snus, but further research is needed to understand whether the association is real.

As mentioned previously, the population-based cross-sectional study conducted by Wandell and colleagues (2008) examined the effect of snus use and smoking on risk of MetSy (as well as diagnosed diabetes) among 1,859 men, aged 60 years. The only significant finding in this study related to tobacco use was that ex-smokers had a significantly elevated prevalence of MetSy; the prevalence was not significantly elevated among any category of snus users (ex-smokers and current snuffers, ex-snuffers, current snuffers, current smokers and snuffers, low consumption of snuff, high consumption of snuff). However, the power of this study was relatively low, and the authors concluded that the study results could not exclude the possibility that snuff use, especially three or more cans per week, could be associated with increased risk of type 2 diabetes.

5.10.1 Summary of Studies on Metabolic Syndrome

Two epidemiology studies investigated the relationship between use of snus and MetSy. One longitudinal study suggests that MetSy may be associated with heavy use of snus while a cross-sectional study found that risk of MetSy was significantly elevated only among former smokers. Further research is needed to understand whether an association with snus use is real.

5.11 Effects on Body Weight

5.11.1 Overview

Two studies suggest that use of snus may be associated with intentional weight loss or may play a role in limiting the weight gain that is often seen after quitting smoking. These studies are summarized in Appendix O-1 and O-2 and described below. Saarni and colleagues (2004) examined the association between episodes of intentional weight loss (defined as 5 kg or more) and tobacco use in a cross-sectional sample of 4,521 young adult twins in Finland. The authors found that snuff use was associated with an increased likelihood of reporting intentional weight loss episodes in men. Snuff use was uncommon among women and there was no association with intentional weight loss among women.

Rodu and colleagues (2004) investigated the relationship between tobacco use (both smoking and use of snus), cessation of these habits, and subsequent weight gain in a study of Swedish men that provided both cross-sectional and prospective data. At study entry, the prevalence of being overweight varied by group, ranging from 28.7% among smokers to 32.5% among snus users to 42.1% among ex-smokers. Smokers who quit all tobacco during follow-up gained significantly more weight (average annual gain of 0.96%) than those who switched to snus (0.51%). The authors concluded that smokers who switch to snus may avoid the weight gain that typically occurs after quitting smoking.

In contrast, seven studies found a different relationship between snus use and weight gain (Bolinder et al. 1997a; Bolinder and de Faire 1998; Eliasson et al. 1991; Eliasson et al. 1995; Hergens et al. 2005; Nafziger et al. 2007; Norberg et al. 2006; Sundbeck et al. 2009; Wallenfeldt et al. 2001). Nafziger and colleagues (2007) conducted a longitudinal study that followed 14,867 adults who were not obese at baseline for 10 years in an effort to characterize those participants who did not gain weight. The outcome was “weight non-gain,” which was defined as losing weight or maintaining body weight within 3% of baseline weight. Snuff use was characterized only as “yes” or “no.” The authors reported that lack of snuff increased the chances of not gaining weight. The study objective was not specific to snus and the authors did not devote any discussion to the significance of this reported finding, nor did they speculate on a mechanism to explain the reported association.

Eliasson and colleagues (1991) conducted a descriptive study among Swedish men and found that BMI did not differ significantly between non-tobacco users and snuff-users.

Eliasson and colleagues (1995) conducted a descriptive study among Swedish men and women and found that BMI did not differ significantly between groups of tobacco users, which included nonusers of tobacco, ex-smokers, smokers, snuff dippers, and snuff and cigarette users. The waist-hip ratio for snuff users was also not significantly greater than the waist-hip ratio among nonusers of tobacco. Only men who were current or previous smokers had a greater waist-hip ratio than nonusers and snuff users.

Bolinder and colleagues (1997a) conducted a descriptive study among Swedish men and concluded that the BMI or waist-hip ratio of the group of “smokeless tobacco” users did not differ significantly from the never-users of tobacco.

Bolinder and de Faire (1998) conducted a descriptive study among Swedish men and found that BMI or waist-hip ratio did not differ significantly between "smokeless tobacco" users and never users of tobacco. Only smokers had a significantly higher waist-hip ratio compared with never users of tobacco.

Wallenfeldt and colleagues (2001) also conducted a descriptive study among Swedish men and found that oral use of moist snuff (in a univariate analysis considering snuff-years) is significantly associated with waist-hip ratio, but not with BMI. However, no significant differences in BMI or waist-hip ratio were observed among never, ex- and current snuff users when compared for differences.

Hergens and colleagues (2005) conducted a case-control study among Swedish men to investigate the relationship between "snuff" use and MI; however, a number of potential risk factors for MI were also investigated among the controls only, including being overweight (BMI ≥30 kg/m^2). Risk of being overweight was significantly elevated among current snus users. The study objective was not specific to being overweight and the authors did not devote any discussion to the significance of this reported finding, nor did they speculate on a mechanism to explain the reported association.

Norberg and colleagues (2006), as mentioned previously, analyzed data from a population-based longitudinal study to investigate the relationship between a number of lifestyle factors, including use of snus, and risk of MetSy. The potential relationship between use of snus and individual components of MetSy including obesity was also investigated. Obesity was defined as having a BMI of ≥ 30. The authors found that heavy use of snus (more than 4 cans per week) was significantly associated with obesity, whereas use of ≤4 cans was not associated with an increased risk of obesity.

Sundbeck and colleagues (2009) conducted a cross-sectional study among Swedish men and reported a relationship between increasing snuff consumption and abdominal obesity. Obesity was measured using the BMI, waist circumference, and waist-to-hip ratios, which define abdominal obesity. Although the study showed that abdominal obesity increased with snuff consumption, this association was limited to former smokers. The authors concluded that "the weight increase commonly seen among former smokers should be considered a possible causal factor." On the other hand, no association existed between any category of snuff use and overall obesity compared to nonusers. Further research is needed to understand whether the association is real.

Bolinder and colleagues (1992) also conducted a cross-sectional study and investigated the relationship between "smokeless tobacco" use and prevalence of being underweight or overweight among Swedish construction workers. "Smokeless tobacco" users did not differ from non-users in the prevalence of underweight (BMI<22) though prevalence of overweight (BMI>26) was significantly elevated among some age groups (36-45, 46-55 and ≥56 years) but not among those 35 or younger. The prevalence of underweight among smokers was significantly higher whereas the prevalence of overweight did not differ from non-users of tobacco. These findings were based on 5,014 Swedish "smokeless tobacco" users who had never been regular smokers and 23,885 Swedish participants who had never used any type of

tobacco. The authors note that the reasons for lower BMI among smokers and higher obesity among "smokeless tobacco" users could be related to behavior.

5.11.2 Summary of Studies on Effects on Body Weight

The following conclusions can be made about use of snus and body weight:

- Two studies (one descriptive and one analytic) suggest that use of snus may be associated with weight loss or with limiting weight gain associated with smoking cessation.
- One analytic study reported that not using snus was associated with not gaining weight and a cross-sectional study suggested that snuff consumption was positively associated with abdominal obesity, which was limited to former smokers.
- One longitudinal study found that heavy use of snus was associated with an increased risk of obesity.
- One cross-sectional study found that abdominal obesity was associated with snus use although this association was limited only to former smokers.
- One case-control study found that being overweight was significantly elevated among current snus users.
- Four descriptive studies found that BMI or waist-hip ratio was not significantly elevated among snus users while a fifth descriptive study found that moist snuff (in a univariate analysis considering snuff-years) is significantly associated with waist-hip ratio, but not with BMI. However, no significant differences in BMI or waist-hip ratio were observed among never, ex- and current snuff users when compared for differences in the fifth study.
- One additional cross-sectional study also found that BMI was significantly elevated among snus users and did not differ significantly from non-users with respect to prevalence of being underweight.
- No firm conclusions can be drawn on the basis of these studies.

5.12 Pregnancy Outcomes and Reproductive Effects

5.12.1 Overview

A single cohort study suggests that women who use snus on a daily basis while pregnant may have increased risk of some adverse pregnancy outcomes. The study, conducted by England and colleagues (2003), used data from the Swedish Medical Birth Register to compare the birth outcomes of 789 women who used snuff daily (but did not smoke cigarettes), 11,240 women who smoked cigarettes daily (but did not use snuff), and 11,495 women who used no tobacco products. Four health endpoints were evaluated: birth weight; small-for-gestational-age birth; pre-term delivery; and preeclampsia. Findings of the study are summarized in Appendix P-1 and described below.

- Birth weight: Compared to nonusers of tobacco, the average birth weight of babies born to snuff-users was reduced by 39 g, whereas that of cigarette smokers was reduced by 190 g.

- Small-for-gestational-age weight: Being small for gestational age was defined as having a birth weight that was more than 2 standard deviations below the mean birth weight for gestational age, according to gender-specific Swedish fetal growth curves. The risk of having a small-for-gestational-age baby among snus users was similar to that of nonusers of tobacco (OR=1.25, 95% CI:0.72-2.17), but it was significantly increased among cigarette smokers (OR=2.99, 95% CI:2.48-3.61).
- Preterm delivery: The risk of preterm delivery (i.e., before 37 weeks of gestation) was significantly elevated in both snuff users (OR=1.98, 95% CI:1.46-2.68) and cigarette smokers (OR=1.57, 95% CI:1.38-1.80), compared to nonusers of tobacco.
- Preeclampsia: Daily users of snuff were at significantly increased risk of preeclampsia compared to nonusers of tobacco (OR=1.58; 95% CI:1.09-2.27). The authors found that cigarette smoking was associated with a significant reduced risk of preeclampsia and indicate that this protective effect is well documented although the mechanism is unknown.

5.12.2 Effects on Infants

A single study reports that exclusively breastfed infants whose mothers used snus are exposed to measurable levels of nicotine (Dahlstrom et al. 2004). The authors estimated the daily oral dose of nicotine for an infant of a smoking and snuff-taking mother in this study to be about 7 µg/kg; they note that the "safe" level of nicotine for an infant is unknown.

5.12.3 Effects on Male Fertility

A single cross-sectional study does not suggest that the use of snus is associated with reproductive parameters in adolescent males (Richthoff et al. 2008). Though the authors' primary focus was on smoking, snus' potential association with male reproductive factors was investigated because it might have an impact directly or as a confounder or an effect modifier. None of the reproductive parameters (semen parameters, seminal biochemical biomarkers, hormone levels) investigated were associated with snus use. The authors conclude that since tobacco smoking was associated with negative impacts on male reproductive parameters, it is unlikely that tobacco itself causes these impacts but rather the compounds that are released by smoking.

5.12.4 Summary of Pregnancy Outcomes and Reproductive Effects

Based on a single cohort analysis, the following conclusions can be made about the use of snus and its association with negative pregnancy outcomes:

- Daily use of snus during pregnancy is associated with a modest reduction in average birth weight.
- Daily use of snus during pregnancy is not associated with risk of small-for-gestational-age birth.
- Daily use of snus during pregnancy is associated with a significant increase in risk of preterm delivery and preeclampsia.

In addition, there is evidence that breastfed infants of mothers who use snus are exposed to nicotine in breast milk; the effects of this exposure are unknown. A single cross-sectional study also suggested that use of snus does not affect male reproductive factors.

5.13 Other Health Effects

Recently, several isolated publications have addressed other health effects potentially associated with snus, including incidence of amyotrophic lateral sclerosis (Fang et al. 2006), complications after hernia surgery (Lindstrom et al. 2007) and delayed bone healing (W-Dahl and Toksvig-Larsen 2007). These cohort studies are summarized in Appendix Q-1. Two cross-sectional studies explored the potential relationship between snus use and circulating selenium and pain intensity among participants experiencing chronic pain (Ellingsen et al. 2009; Jakobsson 2008). One case-control study examined the relationship between tobacco smoking and Swedish snuff use and the risk of developing multiple sclerosis (MS) (Hedstrom et al. 2009). In addition, a study of disability related to neck and back pain provides some interesting data on snus (Holmberg and Thelin 2006); that study is also discussed briefly in this section.

5.13.1 Amyotrophic Lateral Sclerosis (ALS)

Fang and colleagues (2006) used data from the Swedish construction workers cohort to evaluate the relationship between snuff use and cigarette smoking and the development of ALS. The analysis involved 280,558 men who were followed for an average of 19.6 years. At study initiation, 13.6% of the participants were pure snuff dippers, 37.7% were pure smokers, and 17.3% were mixed snuff dippers and smokers. There was no increased risk of ALS among any group of tobacco users, including pure snuff dippers (RR=0.6; 95% CI:0.3-1.5); cigarette smokers (RR=0.7; 95% CI:0.5-1.1); or mixed snuff dippers and smokers (RR=0.9; 95% CI:0.6-1.4), after adjusting for age and county of residence. The authors concluded that this study provides no evidence that tobacco use is associated with increased risk of ALS.

5.13.2 Complications after Hernia Surgery

Another analysis of the Swedish construction worker cohort sought to determine whether smoking, use of snus, or obesity affected the outcome of surgery (Lindstrom et al. 2007). The participants were 12,697 male construction workers who had undergone a first-time inguinal hernia repair. The overall complication rate following this surgery was low (2.9%). Snus use was not associated with significantly increased risk of postoperative complications, nor was it associated with any increase in the mean length of hospitalization. In contrast, current smokers had a 34% increased risk of postoperative complications compared to never-smokers, although their length of hospitalization was unaffected. The authors concluded that use of snus does not appear to affect the complication rate after hernia surgery at all.

5.13.3 Delayed Bone Healing

A third analysis of the Swedish construction worker cohort was carried out in order to assess the effect of snuff use and smoking on the time for bone healing (W-Dahl and Toksvig-Larsen 2007). The participants were 175 male patients who were subsequently operated on by tibial osteotomy using the hemicallotasis technique. The cohort comprised of 41 smokers, 21 oral snuff users, and 113 non-smokers/non-snuffers, with habits documented preoperatively. There were no cases of delayed bone healing among snuffers and the authors concluded that snuff

does not have the negative effects—such as delayed bone healing and increased risk of post-operative complications—that cigarette smoking has.

5.13.4 Circulating Selenium

A cross-sectional study carried out by Ellingsen and colleagues (2009) sought to examine the relationship between smoking and snuff use, and the status of biomarkers of selenium. The participants were 98 blue-collar, male workers from southern Norway who submitted biological samples for quantitative analysis. At study initiation, 49 of the participants were non-smokers/non-snuff users, while 38 and 11 were smokers and snuff users, respectively. Snuff users had about the same selenium in serum (S-Se) and selenium in whole blood (B-Se) as the non-smokers although they had about the same amount of nicotine metabolites in their blood as the smokers. The authors concluded that smoking, not snuff use, is associated with lower concentrations of B-Se and S-Se.

5.13.5 Chronic Pain Intensity

Jakobsson (2008), also using a cross-sectional study design, evaluated the relationship between tobacco use and pain intensity among 384 male and female participants from southern Sweden, who reported experiencing chronic pain for a duration of at least 3 months. At study initiation, 12.5% reported ever using snuff, while 52.1% reported ever smoking cigarettes. The author concluded that there was no significantly higher pain intensity among those who used moist snuff compared with those who did not. In contrast, smokers experienced higher pain intensity than nonsmokers. This relationship was also found among former smokers. The study results are limited in that data on tobacco habits and chronic pain were collected simultaneously. Because it is suggested that tobacco is often used for coping with stress, it is possible that occasional smokers resorted to using tobacco more frequently to cope with their chronic pain and ended up being grouped with daily smokers.

5.13.6 Multiple Sclerosis

A case-control study carried out by Hedstrom and colleagues (2009) sought to examine the influence of tobacco smoking and snuff use on the risk of developing MS among 902 incident cases of MS and 1,855 randomly selected controls. Participants were from Sweden and included males and females. Smoking was found to be significantly associated with an increased risk of developing MS, while snuff use was not associated with an increased risk of developing MS. There was clear evidence of a dose-response relationship between the cumulative smoking dose and the development of MS. Snuff users, on the other hand, experienced a significantly lower risk of developing MS among those who had used snuff and may have been ever smokers for 5 or more years (OR=0.3; 95% CI:0.1-0.9) or more than 15 years (OR=0.3; 95% CI:0.1-0.8). A significant trend of decreasing risk of MS was also observed among ever smoking snuff users. Odds ratios for snuff users were adjusted for age, sex, ancestry, residential area and smoking. Results among never-smoking snuff users were limited in that confidence intervals were wide and imprecise, indicative of a small number of participants in these subgroups. The authors point out that their findings suggest that the association between MS and smoking is not a result of the influence of nicotine. To explore a potential mechanism for the protective effect observed among snuff users, the authors point out

that previous research provides evidence that suggests nicotine may have the ability to act as a neuroprotective agent.

5.13.7 Disability Related to Neck and Back Pain

Holmberg and Thelin (2006)[54] examined long-term health outcomes associated with neck and back pain in a prospective cohort study of 1,347 Swedish farmers and rural non-farmers. They found that neck or low back pain at study entry was a significant predictor of consultation with a primary care doctor and sick leave during 12 years of follow-up. Snuff use was considered as a possible confounder; surprisingly, it was identified as a strong independent predictor of disability pension due to neck or low back pain (OR=3.46; 95% CI:1.35-8.84). There is little information on snuff use and musculoskeletal symptoms; the authors note that this finding must be interpreted cautiously and that further research is warranted.

5.13.8 Summary of Studies of Other Health Effects

There were seven single studies identified that evaluated the relationship between snus use and different potential health effects involving ALS, complications after hernia surgery, delayed bone healing, circulating selenium, chronic pain intensity, disability related to neck and back pain and MS. The authors of only one study found a significant positive association with snus use and subsequent neck and low back pain, but note that the finding must be interpreted cautiously and that further research is warranted.

[54] This study is not summarized in Appendix Q as the hypothesis did not include snuff use. Instead, snuff use was only considered as a confounder.

6 Conclusion

ENVIRON has conducted a comprehensive review of the relevant published chemistry, epidemiology, and toxicology studies available for Swedish snus, including literature identified through systematic ongoing literature searches of Medline and several additional databases in Dialog® through December 31, 2009. This review was conducted to characterize the types of potential health risks reported to be associated with the use of Swedish snus. The review includes an overview of several topics regarding Swedish snus, including chemical properties and chemical analysis of snus, the manufacturing process, biomarkers of exposure and effect, and toxicological studies and epidemiological studies of Swedish snus.

Swedish snus is a heat-treated oral moist snuff tobacco product originally developed in Sweden. Snus mainly consists of air-cured tobacco, water, and salt. Other ingredients added in small quantities serve to retain moisture, and for preservation and flavoring purposes. The moisture content of traditional Swedish snus is approximately 50% and the pH close to 8.5. The manufacturing process of snus in Sweden must satisfy the hygienic requirements of the Swedish Food Act and all ingredients must comply with the Swedish Food Regulation.

Concentrations of TSNAs, traditionally the most frequently analyzed and reported trace-level components in smokeless tobacco products (STPs) due to their carcinogenic potential demonstrated in experimental animals, have significantly decreased in Swedish snus between the early 1980s and 2000. This appears to be mainly due to improvements in the Swedish snus manufacturing process that were introduced in the early 1980s, including both technical changes in the production process and the institution of more rigorous quality checks of the raw ingredients. Published data for most other trace-level components in STPs, including Swedish snus, is limited, and only in recent years more analyses on a variety of components other than TSNAs have become available (e.g., polyaromatic hydrocarbons, aldehydes, and metals).

This limited published analytical data on the chemical composition of traditional Swedish snus does not allow distinction between different brands of snus. There are differences in portion sizes and nicotine content and delivery between snus brands. This information needs to be taken into account when conducting an exposure assessment for critical chemical substances in Swedish snus. Furthermore, for a comparison of the potential exposure to critical components in traditional Swedish snus with other oral moist snuff products, such as new products marketed as snus and traditional US-type moist snuff, other factors, such as differences in moisture content, pH and resulting nicotine delivery need to be considered, along with use patterns.

Biomarkers of exposure and biomarkers of effect are being utilized in some studies of individuals that use various STPs. Measuring a chemical or metabolite in biological fluids or tissues (“biomarkers of exposure”) allows for the scientific estimation of external exposure levels that are necessary for characterizing health risks from STPs such as Swedish snus. Biomarkers of exposure include specific chemical components in tobacco or their metabolites. Biomarkers of effect may be used to evaluate the potential for the development of adverse health effects associated with exposure to tobacco or its chemical components. These biomarkers may be the products of different cellular responses following exposure, leading to a variety of biological responses. To date, there is no comprehensive set of biomarkers of

exposure or biological effects available for use to predict adverse health effects (e.g., cardiovascular, cancer) related to exposure to components in tobacco or tobacco smoke.

There have been a limited number of studies conducted to evaluate exposure biomarkers such as levels of NNAL or its glucuronides or cotinine in humans following the use of Swedish snus. Most studies that have been conducted have not measured biomarkers in different exposure groups (e.g., snus, other STPs, cigarettes) within the same study, so it is not feasible to draw conclusions regarding levels of specific biomarkers among users of different products. A few studies have evaluated biomarkers of effect in snus users; however, future studies may be needed to determine if biomarkers of effect will be instrumental in comparing early health effects associated with different tobacco-containing products to snus.

Well controlled epidemiological evidence indicates that Swedish snus is not associated with oral cancer. Though the studies are mostly consistent showing no association between Swedish snus use and esophageal or stomach cancer, a single recent study did observe increased risks for these cancer sites. Additional research will help resolve this uncertainty. A limited number of epidemiology studies have failed to demonstrate that Swedish snus is a significant risk factor for the following cancers: kidney, bladder, lung, skin cancer, hematopoietic cancers, and all cancers combined. Two studies suggest that Scandinavian smokeless tobacco may be associated with increased risk of pancreatic cancer among specific subgroups of the population. There are inconsistencies between the two studies and the interpretation of the studies has been the topic of much scientific debate. Further research is needed to resolve the relationship between use of Swedish snus and cancer at this site.

Snus extract has not shown to induce tumors in rat studies and it is not mutagenic or genotoxic in mammalian cells or clastogenic in *in vitro* assays. Snus contains low levels of TSNAs and analytical data clearly demonstrate that levels of TSNAs in snus have steadily decreased over the past 25 years. Extrapolation from animal studies that investigated the health effects of TSNAs appears to overestimate oral cavity, lip, and pharynx cancer risk for users of snus. The calculated margin of safety demonstrates that humans are exposed to levels far below those associated with significant health risks in animal models.

Studies have reported that the use of Swedish snus is associated with a characteristic type of oral mucosal lesion which is localized to the area where the snus is placed; however, the lesions are reversible following cessation of snus use and there is no clinical evidence to suggest that they transform into malignancies. Limited evidence from uncontrolled descriptive studies suggests that Swedish snus use may also be associated with acute cardiovascular effects such as increased blood pressure and elevated heart rate almost certainly due to nicotine. A single epidemiological study observed an increased risk of death from one specific stroke type among Swedish snus users; this finding has not been replicated in other epidemiological studies.

The literature indicates that use of Swedish snus is not associated with harmful gastrointestinal effects, including peptic ulcer, heartburn, Crohn's disease or ulcerative colitis. One well-conducted analytic epidemiology study found that use of Swedish snus was not associated with increased risk of diabetes. This is in contrast to a single descriptive epidemiologic study of

insulin resistance among Swedish snus users that concluded that only heavy users of moist snuff have an increased risk of type 2 diabetes. However, this descriptive study, by design, cannot determine true risk, and a single experimental study found no difference in insulin action between snuffers and abstainers. Though a single study has suggested that heavy use of Swedish snus could be associated with increased risk of MetSy, other studies have not observed this outcome, or associations with clinical markers of MetSy, such as insulin reactivity, so further research is needed to understand whether the association is real.

Multiple studies have examined weight (BMI), weight gain, and waist-to-hip ratios, and the results are mixed, making it difficult to draw firm conclusions. Prospective cohort studies are necessary to examine this potential association with Swedish snus use.

A single epidemiological study suggests that daily use of Swedish snus during pregnancy is associated with some adverse consequences (a modest reduction in average birth weight and a significant increase in risk of preterm delivery and preeclampsia) but not others effects (no increase in risk of small-for-gestational-age birth). One study reported that breastfed infants of Swedish snus-using mothers are exposed to nicotine, but the health effects of this exposure are not known.

This comprehensive review of the published scientific literature confirms the lack of serious adverse health effects associated with Swedish snus. The use of Swedish snus is not associated with oral cancer or cancer of any part of the respiratory tract. At this time, the most likely health risks associated with chronic use of Swedish snus appear to be acute, reversible cardiovascular effects probably due to nicotine. Overall, there is very little evidence that current use levels of snus in Sweden are associated with any significant long-term health effects, and ongoing research is hoped to provide additional information to resolve remaining areas of uncertainty. The areas where firm conclusions cannot be drawn include the relationship between Swedish snus use and pancreatic cancer, potential cardiovascular risks, and possible metabolic syndrome or weight gain issues.

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Appendix I

Description of Literature Search

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Identification of relevant literature on the composition, use, and potential health effects of snus has been ongoing for several years. The basic search strategy consists of the following terms, though variations on this set of terms may have changed over time:

"tobacco, smokeless" [MeSH Terms] OR chew tobacco* OR oral tobacco* OR snuff OR plug tobacco* OR spit* tobacco* OR smokeless tobacco* OR loose leaf tobacco* OR dip tobacco* OR dipping tobacco* OR snus OR Swedish snuff OR Swedish tobacco

Literature searching is conducted primarily using the National Library of Medicine's PubMed database, and ENVIRON continually monitors the literature using the PubMed alert system, which notifies subscribers when a publication that meets the search criteria is entered into the system.

In the development of this report, targeted outcome terms were used in addition to the basic exposure terms listed above, for example, cancer or neoplasms, oral lesions, cardiovascular, stroke, etc.

In addition to using PubMed, periodic literature searches using similar key words have been performed in Dialog® (a commercial compilation of more than 650 databases), as well as in other databases such as Toxnet, an online toxicology database, and the World Wide Web, to identify any published reports that may have been missed. The detailed literature search was conducted at least ten times: October 2001; September 2002; January 2004; June 2005; August 2006; August 2007; March, June, September, and December 2008. Each literature search was designed to identify relevant literature published since the previous search.

Following the identification of articles and abstracts (as available), they are reviewed for potential relevance. Those studies that appear relevant are retrieved and evaluated for inclusion in the systematic review of snus. Once actual articles are obtained, the reference lists of these publications are "tree-searched" to identify other relevant studies or publications that may have been missed in the data base searches.

ENVIRON maintains a Reference Manager database that contains 1,562 citations and of those citations, maintains a library of 1,278 smokeless tobacco-related electronic copies of the publications.

Appendix II

Appendix to Chapter 2 Chemical Properties of Snus

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This Appendix intends to supplement the information presented in Chapter 2 on the chemical properties of snus with detailed analytical data as provided in the more recently published literature (presented in tables) and to compare it with information available in the same studies on new products marketed as snus and US-type moist snuff. Therefore, the outline of this appendix closely follows that of Chapter 2.

Because the epidemiological research conducted in Scandinavia is based on use of traditional products, i.e., Swedish snus, Chapter 2 focuses only on traditional Swedish snus. However, much of the published literature that reports analyses of the chemical composition of Swedish snus also includes data on US-type oral moist snuff. Furthermore, more recent studies (published 2004 to present) have also investigated newer products that are marketed as snus. While it is well established that the manufacturing process of traditional US-type oral moist snuff is distinctively different from traditional Swedish snus, production methods for newer STPs labeled as snus were not reported in the literature included in this review.

To compare these new products with traditional Swedish snus, this Appendix provides quantitative information on components analyzed in traditional Swedish snus as well as in new products marketed as snus as reported in the more recent literature (2004 to 2009). Furthermore, a distinction between traditional Swedish snus and newer products marketed as snus from US-type oral moist snuff is made, where available data allowed direct comparison.

A II 2.2.2 Sodium Salts

There were no recently-published studies identified that analyzed sodium levels in new products marketed as snus. Only Lunell and Lunell (2005) compared extraction of sodium from *Catch Dry Mini*, a type of *Catch* snus, with a moisture content of 25%, with extraction from traditional Swedish snus products. These authors reported that the difference in sodium chloride content in the unused product compared to the used product was 4.73 $\pm$ 6.61 mg per portion (0.3 g) for *Catch Dry Mini* resulting in approximately 21 mg/g dry weight (wet weight multiplied by 1.33). These concentrations are in the same range as those reported for the traditional Swedish snus products tested in the same study.

A II 2.2.3 Alkaloids

Table A II-1a summarizes concentrations of nornicotine, anatabine, and anabasine in traditional Swedish snus (*General*) as well as in two new US products marketed as snus (*Camel Snus and Marlboro Snus*) as reported in a recent analysis of different STPs (Stepanov et al. 2008a).

Concentrations (mg/g dry weight) of nornicotine, anatabine, and anabasine in *General* were mostly lower than those detected in *Camel Snus* and *Marlboro Snus* but in the range of concentrations detected in four traditional US moist snuff products (Stepanov et al. 2008a).

When expressed as percentage of total nicotine content, nornicotine, anatabine, and anabasine levels in *General* snus were 1.3%, 2.2%, and 0.4%, respectively. In different types of *Camel* snus the levels were between 1.2 and 1.3%, 3.1 and 4.9%, and 0.4 and 0.7%, in different types of *Marlboro* snus between 2.3 and 3.8%, 9.9 and 14.6%, and 0.3 and 0.6%, respectively. In comparison, nornicotine, anatabine, and anabasine levels detected in traditional US-type moist snuff brands were between 0.6 and 1.1%, 2.2 and 6.2%, and 0.1 and 0.7% of their total nicotine content. Based on concentrations expressed in mg/g dry weight or as percentage of total nicotine content, nornicotine and anatabine levels in *Marlboro* snus were distinctly higher than those detected in *General* and *Camel* snus (Stepanov et al. 2008a).

A II 2.2.4 Nicotine, Free Nicotine, pH and Moisture

Table A II-1a summarizes concentrations of total nicotine, free nicotine (where available), pH and moisture levels in traditional Swedish snus (*General,* "general [sic] pouch"*,* and *Catch*) as well as several new products marketed as snus (*Catch Dry, du Maurier, Camel Snus,* and *Marlboro Snus*) as reported in more recent analyses of different STPs (Lunell and Lunell 2005; McNeill et al. 2006; Rickert et al. 2009; Stepanov et al. 2008a).

Based on these analyses, total nicotine concentrations in new products marketed as snus were generally higher than those in traditional Swedish snus, with *Camel Snus* being more in the range of US-type moist snuff products analyzed in the same studies (range, 19.6-31.2 mg/g dry weight) (McNeill et al. 2006; Rickert et al. 2009; Stepanov et al. 2008a). The exception was *Marlboro Snus Mild* with a total nicotine concentration of 12.8 mg/g dry weight, which was lower than concentrations detected in all other brands investigated (Stepanov et al. 2008a).

Based on data from Brunnemann and Hoffmann (1992), Swedish snus is thought to generally have a higher pH than most brands of US smokeless tobacco (Lunell and Lunell 2005). Data from the newer literature supports this statement. Measurements in US-type moist snuff products yielded a pH range of 6.97 to 8.23, with only two brands having a pH above 8, whereas the same studies determined the pH in traditional Swedish snus to be between 7.86 and 8.5 (Lunell and Lunell 2005; McNeill et al. 2006; Rickert et al. 2009; Stepanov et al. 2008a). By contrast, the pH of new products marketed as snus was lower than what has been measured for traditional Swedish snus. The lowest values were measured for four brands of *Marlboro Snus*, where the pH ranged between 6.47 and 6.85 and this was even lower than the lowest pH detected in US-type moist snuff products in the same studies (McNeill et al. 2006; Rickert et al. 2009; Stepanov et al. 2008a).

Accordingly, free nicotine in the *Marlboro Snus* samples was reported to range from 0.35 and 1.13 mg/g dry weight[1], whereas free nicotine was determined to be between 6.3 and 7.69 mg/g dry weight in traditional Swedish snus and between 6.09 and 9.16 mg/g dry

[1] Foulds and Furberg (2008) have therefore questioned if this product should be called snus and suggested that "the term should be reserved for moist, low toxin, medium/high nicotine delivery STPs that are qualitatively similar to the leading brands in Sweden."

weight in *Camel Snus* (McNeill et al. 2006; Stepanov et al. 2008a). Based on its lower pH, Lunell and Lunell (2005) concluded that US smokeless tobacco probably delivers nicotine less efficiently than Swedish snus. It should be noted that a recent study conducted at the Harvard School of Public Health concluded that levels of free nicotine in moist snuff products on the US market have increased between 2000 and 2006 (Alpert et al. 2008)[2]. The analysis of US-type moist snuff brands by Stepanov and colleagues (2008a) and McNeill and colleagues (2006) showed that free nicotine concentrations ranged from 2.4 to 7.14 mg/g dry weight, except for *Kodiak Wintergreen*, in which it was 12.1 mg/g dry weight and thus higher than in any of the other new and traditional US STPs measured in these studies. The pH of *Kodiak Wintergreen* was higher (pH 8.23) than what was determined in other US-type moist snuff products (Stepanov et al. 2008a), and its total nicotine concentration was 19.6 mg/g dry weight.

The moisture level in traditional Swedish snus is approximately 50%. The limited data on new products marketed as snus have identified differences in moisture content. For example, *Catch Dry Mini* was reported to have a moisture content of 25% moisture. Moisture levels measured in *Marlboro Snus Rich* and *Camel Snus Original* were reported to be 10.1% and 31.2%, respectively (Stepanov et al. 2008a).

A II 2.2.5 Nitrate and Nitrite

Table A II-1b summarizes concentrations of nitrate and nitrite in traditional Swedish snus (*General,* "general [sic] pouch") as well as in new products marketed as snus in the US and Canada (*Camel Snus, Marlboro Snus,* and *du Maurier*) as reported in three recent analyses of different STPs (McNeill et al. 2006; Rickert et al. 2009; Stepanov et al. 2008a).

The nitrate concentration in traditional Swedish snus as well as new products marketed as snus and measured by Stepanov and colleagues (2008a) were lower than those detected in traditional US-type moist snuff products analyzed in the same study. The latter ranged from 6.60 to 7.96 mg/g dry weight. As measured in this study, *Marlboro Snus* had the lowest nitrate concentrations, which were less than half of what was detected in *General*. The Canadian investigators Rickert and colleagues (2009), also analyzed nitrate concentrations in different STPs and detected significantly lower concentrations in *du Maurier* snus compared to US-type moist snuff brands, with the latter being in the range of 22.6 to 31.2 mg/g dry weight. It is unclear why the nitrate concentrations measured in similar US-type moist snuff brands in the Canadian study are considerably higher than those measured by the US investigators, Stepanov and colleagues (2008a).

Nitrite concentrations measured by Stepanov and colleagues (2008a) in *Camel Snus* and *Marlboro Snus* samples were similar to concentrations in *General* or not detected and below or at (for *Camel Snus Spice*) the GothiaTek® Standard limit of 7 µg/g dry weight. By contrast, concentrations in the traditional US-type moist snuff brands analyzed by the same authors exceeded this limit 1.5 to more than 7 times (range, 11-55

[2] Alpert HR, Koh H, and Connolly GN. 2008. Free nicotine content and strategic marketing of moist snuff tobacco products in the U.S.: 2000 - 2006. Tob Control 17:332-338.

μg/g dry weight). Similarly, McNeill and colleagues (2006) detected a nitrite concentration of 6.7 μg/g dry weight in one brand of US-type moist snuff, whereas the concentration in "general [sic] pouch" was below the detection limit of 0.2 μg/g.

A II 2.2.6 Other Components

Table A II-1b summarizes concentrations of chloride and other anions (formate, sulfate, and phosphate) as well as ammonia and propylene glycol in traditional Swedish snus (*General*) and new products marketed as snus in the US and Canada (*Camel Snus, Marlboro Snus,* and *du Maurier*) as reported in two recent analyses of different STPs (Rickert et al. 2009; Stepanov et al. 2008a).

Concentrations of chloride in *Camel Snus* and *Marlboro Snus* were approximately one-half to one-tenth of those detected in *General* (Stepanov et al. 2008a). By contrast, chloride concentrations in traditional US-type moist snuff analyzed in the same study were, with a range of 107 to 155 mg/g dry weight, up to twice as high as those in *General*.

Table A II-1a: Chemistry of Snus and New Products Marketed as Snus as Reported in the Literature (1)										
Brand	**STP Type Specified by Study Authors**	**Citation**	**Moisture (% w/w)**	**Dry Matter (%)**	**pH**	**Nicotine (mg/g)**	**Nicotine free (mg/g)**	**Nornicotine (mg/g)**	**Anatabine (mg/g)**	**Anabasine (mg/g)**
Traditional Swedish Snus										
General	Swedish snus	Stepanov et al. 2008a	48.5	NI	7.95	16.7	7.69	0.223	0.367	0.072
"general [sic] pouch"	Snus (Sweden)	McNeill et al. 2006	45.84	NI	7.86	15.2	6.3	NI	NI	NI
General	Snus	Lunell & Lunell 2005	NI	NI	8.4	18[#]	NI	NI	NI	NI
Catch Licorice	Snus	Lunell & Lunell 2005	NI	NI	8.5	14[#]	NI	NI	NI	NI
Catch Mini	Snus	Lunell & Lunell 2005	NI	NI	8.4	18[#]	NI	NI	NI	NI
New Products Marketed as Snus										
Catch Dry Mini	Snus	Lunell & Lunell 2005	NI	NI	7.3	21[#]	NI	NI	NI	NI
Du Maurier Freshmint	Swedish snus mint-flavored	Rickert et al. 2009	NI	70.8	7.39	23.1	NI	NI	NI	NI
Du Maurier Original	Swedish snus	Rickert et al. 2009	NI	73.9	7.39	18.1	NI	NI	NI	NI
Marlboro Snus Rich	New STP	Stepanov et al. 2008a	10.1	NI	6.83	17.8	1.08	0.438	2.60	0.111
Marlboro Snus Mild	New STP	Stepanov et al. 2008a	NI	NI	6.47	12.8	0.350	0.484	1.82	0.072
Marlboro Snus Spice	New STP	Stepanov et al. 2008a	NI	NI	6.85	17.9	1.13	0.411	2.17	0.097
Marlboro Snus Mint	New STP	Stepanov et al. 2008a	NI	NI	6.58	20.0	0.701	0.454	1.97	0.063

Table A II-1a: Chemistry of Snus and New Products Marketed as Snus as Reported in the Literature (1)

Brand	STP Type Specified by Study Authors	Citation	Moisture (% w/w)	Dry Matter (%)	pH	Nicotine (mg/g)	Nicotine free (mg/g)	Nornicotine (mg/g)	Anatabine (mg/g)	Anabasine (mg/g)
Camel Snus Original	New STP	Stepanov et al. 2008a	31.2	NI	7.46	28.2	6.09	0.353	1.39	0.164
Camel Snus Spice	New STP	Stepanov et al. 2008a	NI	NI	7.75	25.4	9.16	0.314	1.09	0.183
Camel Snus Frost	New STP	Stepanov et al. 2008a	NI	NI	7.59	23.7	6.4	0.313	0.741	0.103

Notes:
[#] Values given were on portion basis and had to be adjusted to g considering portion sizes (*General*: 8.84 mg nicotine/g; *Catch*: 7.04 mg nicotine/g; *Catch Mini*: 4.53 mg nicotine/0.5 g; *Catch Dry Mini:* 4.82 mg nicotine/0.3 g) and dry weight assuming 50% moisture (value multiplied by 2), except for *Catch Dry Mini* where 25% moisture was assumed (value multiplied by 1.33).
All amounts given as per dry weight. NI: Not investigated

Table A II-1b: Chemistry of Snus and New Products Marketed as Snus as Reported in the Literature (2)										
Brand	**STP Type Specified by Study Authors**	**Citation**	**Nitrite (μg/g)**	**Nitrate (mg/g)**	**Ammonia (mg/g)**	**Propylene Glycol (mg/g)**	**Formate (mg/g)**	**Chloride (mg/g)**	**Sulfate (mg/g)**	**Phosphate (mg/g)**
Traditional Swedish Snus										
General	Swedish snus	Stepanov et al. 2008a	4	4.62	NI	NI	4.89	75.7	7.55	0.344
"general [sic] pouch"	Snus (Sweden)	McNeill et al. 2006	ND*	NI	NI	NI	NI	NI	NI	NI
New Products Marketed as Snus										
Du Maurier Freshmint	Swedish snus mint-flavored	Rickert et al. 2009	NI	14.3	0.694	16.2	NI	NI	NI	NI
Du Maurier Original	Swedish Snus	Rickert et al. 2009	NI	14.0	0.657	16.6	NI	NI	NI	NI
Marlboro Snus Rich	New STP	Stepanov et al. 2008a	ND	1.71	NI	NI	1.89	7.92	7.45	1.28
Marlboro Snus Mild	New STP	Stepanov et al. 2008a	ND	1.54	NI	NI	1.56	7.28	6.86	1.28
Marlboro Snus Spice	New STP	Stepanov et al. 2008a	3	1.69	NI	NI	2.12	7.68	7.01	1.32
Marlboro Snus Mint	New STP	Stepanov et al. 2008a	3	1.58	NI	NI	1.51	7.41	6.63	1.31
Camel Snus Original	New STP	Stepanov et al. 2008a	ND	3.79	NI	NI	12.7	39.8	9.35	0.820
Camel Snus Spice	New STP	Stepanov et al. 2008a	7	3.79	NI	NI	14.7	39.7	8.42	0.725
Camel Snus Frost	New STP	Stepanov et al. 2008a	3	3.20	NI	NI	15.3	32.4	7.62	0.722

Notes:
All amounts given as per dry weight. ND: Not detected; NI: Not investigated; * Limit of detection: 0.2 μg/g

A II 2.2.7 Trace-Level Components

According to Rickert and colleagues (2009), it appears that some major international companies (e.g., British American Tobacco) that produce new products marketed as snus have adopted the GothiaTek® Standard limits established by Swedish Match for certain trace-level components.

A II 2.2.7.1. *N*-Nitroso Compounds

More recent studies that investigated new products marketed as snus have not presented analytical data on other *N*-nitroso compounds than TSNAs.

Tobacco-Specific *N*-Nitrosamines

Table A II-2 summarizes concentrations of TSNAs in traditional Swedish snus (*General,* "general [sic] pouch"*, Ettan, Catch, Göteborgs Rapé, and Grovsnus*) as well as in several new products marketed as snus (*Catch Dry, du Maurier, Camel Snus, Marlboro Snus,* and *Exalt*) as reported in more recent analyses of different STPs on the market in Sweden, the US, Canada, and the UK (Hatsukami et al. 2007; McNeill et al. 2006; Rickert et al. 2009; Rodu and Jansson 2004; Stepanov et al. 2006; Stepanov et al. 2008a).

Total TSNA concentrations as measured by different investigators in new products marketed as snus were in the same range as in traditional Swedish snus, with the exception of one report on the TSNA concentrations in *Exalt*. Rodu and Jansson (2004) reported that total TSNA concentrations in this Swedish Match product specifically manufactured for the US market were 5.8 μg/g dry weight, and noted that these higher TSNA levels suggested the influence of American taste expectations in product manufacturing. Stepanov and colleagues also analyzed *Exalt* samples as purchased in the US, and also in Sweden and reported total TSNA concentrations of 3.7 and 3.1 μg/g wet weight, which were higher than what was detected in *General* (2.0 μg/g wet weight) (Hatsukami et al. 2007; Stepanov et al. 2006). Stepanov and colleagues did not report the moisture content of the products investigated. If taking the high dry matter content (91%) for *Exalt* into consideration as it was reported by Rodu and Jansson (2004), the total TSNA concentrations measured by Stepanov and colleagues expressed as per dry weight are lower than those determined by Rodu and Jansson (2004). It is unclear if this indicates a true difference in TSNA content or is due to interlaboratory variabilities in analytical methods.

Similar to traditional Swedish snus, all new products marketed as snus had total TSNA concentrations that were below the GothiaTek® Standard limit of 10 μg/g dry weight. Furthermore, as for traditional Swedish snus, the combined NNK and NNN concentrations in most new products marketed as snus were below or close to the WHO recommended limit of 2 μg/g dry weight (WHO 2009). Exceptions were *Exalt* as well as *Marlboro Snus Mint*, where NNN concentrations of more than 3 μg/g dry weight were detected (Rodu and Jansson 2004; Stepanov et al. 2008a).

However, if compared with the TSNA content detected in US-type moist snuff as measured in the same studies discussed above, considerable differences become apparent. Total TSNA concentrations in the US-type moist snuff were between 4.5 and 14.6 μg/g dry weight and were thus higher than what was reported in traditional Swedish snus and most new products

marketed as snus (Rickert et al. 2009; Rodu and Jansson 2004; Stepanov et al. 2008a). NNK and NNN concentrations ranged from 0.4 to 2.26 µg/g dry weight and from 2.4 to 6.86 µg/g dry weight, respectively. NAB concentrations were reported to range from not quantifiable and 0.5 µg/g dry weight. NAT concentrations ranged from 1.1 to 6 µg/g dry weight.

Table A II-2: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Tobacco-Specific Nitrosamines

Brand	STP Type Specified by Study Authors	Citation	NNK (µg/g)	NNN (µg/g)	NAB (µg/g)	NAT (µg/g)	Total TSNAs (µg/g)
Traditional Swedish Snus							
General	Traditional Snus	Stepanov et al. 2008a	0.464	1.66	0.008	0.969	3.1
General	Swedish snus	Hatsukami et al. 2007/ Stepanov et al. 2006#	0.36 #	1.96 #	0.12 #	1.58 #	4 #
"general [sic] pouch"	Snus (Sweden)	McNeill et al. 2006	NR	NR	NR	NR	0.478†
General	Moist snuff, Sweden 2003	Rodu and Jansson 2004	0.4	1.1	0.1	0.6	2.1
Ettan	Moist snuff, Sweden 2003	Rodu and Jansson 2004	0.3	1.1	0.1	0.6	2.0
Catch Licorice	Moist snuff, Sweden 2003	Rodu and Jansson 2004	0.4	1.0	0.0	0.6	2.0
Göteborgs Rapé	Moist snuff, Sweden 2003	Rodu and Jansson 2004	0.4	1.1	0.0	0.6	2.2
Grovsnus	Moist snuff, Sweden 2003	Rodu and Jansson 2004	0.5	1.1	0.1	0.6	2.2
New Products Marketed as Snus							
Du Maurier Freshmint	Swedish snus mint-flavored	Rickert et al. 2009	NQ	1.214	NQ	0.905	2.119
Du Maurier Original	Swedish snus	Rickert et al. 2009	0.456	1.212	NQ	0.831	2.499
Marlboro Snus Rich	New STP	Stepanov et al. 2008a	0.259	1.27	ND	0.455	1.98
Marlboro Snus Mild	New STP	Stepanov et al. 2008a	0.229	1.52	ND	0.234	1.98
Marlboro Snus Spice	New STP	Stepanov et al. 2008	0.257	1.56	ND	0.246	2.06
Marlboro	New STP	Stepanov	0.215	3.28	ND	0.221	3.72

Table A II-2: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Tobacco-Specific Nitrosamines

Brand	STP Type Specified by Study Authors	Citation	NNK (µg/g)	NNN (µg/g)	NAB (µg/g)	NAT (µg/g)	Total TSNAs (µg/g)
Snus Mint		et al. 2008					
Camel Snus Original	New STP	Stepanov et al. 2008	0.27	1.15	0.012	0.297	1.73
Camel Snus Spice	New STP	Stepanov et al. 2008	0.157	1.27	0.015	0.305	1.75
Camel Snus Frost	New STP	Stepanov et al. 2008a	0.267	1.2	0.009	0.204	1.68
Exalt	New Product	Rodu and Jansson 2004	1.1	3.1	0.2	1.5	5.8
Exalt	Spit-free tobacco packet (purchased in Sweden)	Hatsukami et al. 2007/ Stepanov et al. 2006 #	0.27	2.3	0.13	0.98	3.7
Exalt	Spit-free tobacco packet (purchased in US)	Hatsukami et al. 2007/ Stepanov et al. 2006 #	0.24	2.1	0.05	0.68	3.1
Camel Snus Original	Spit-free tobacco packet	Hatsukami et al. 2007 #	0.16	0.79	0.008	0.19	1.15
Camel Snus Spice	Spit-free tobacco packet	Hatsukami et al. 2007 #	0.09	0.87	0.01	0.2	1.17
Camel Snus Frost	Spit-free tobacco packet	Hatsukami et al. 2007 #	0.16	0.83	0.006	0.13	1.12

Notes:
All amounts given as per dry weight, except:
Results on new products marketed as snus reported by Hatsukami et al. 2007/ Stepanov et al. 2006 reported TSNA concentrations as per wet weight. The moisture content of all new products was not given. The moisture content for General was assumed to be 50% and concentrations per wet weight (NNK 0.18 µg/g, NNN 0.98 µg/g, NAB 0.06 µg/g, NAT 0.79 µg/g, and total TSNAs 2.0 µg/g) were converted by multiplying by 2 to convert to concentrations per dry weight.
† Total TSNA = NNK + NNN + NAB
ND: Not detected; NQ: Not quantifiable; NR: Not reported

A II 2.2.7.2. Polycyclic Aromatic Hydrocarbons

Table A II-3 summarizes concentrations of PAHs in traditional Swedish snus (*General,* "general [sic] pouch") as well as in new products marketed as snus in the US and Canada (*Camel Snus, Marlboro Snus,* and *du Maurier*) as reported in three recent analyses of different STPs (McNeill et al. 2006; Rickert et al. 2009; Stepanov et al. 2008a).

In these studies, B[a]P concentrations in *General* as well as in all samples of *Marlboro Snus* and *du Maurier*, and most samples of *Camel Snus,* were either below the detection limit or up to 2 ng/g dry weight and thus below the limit of 5 ng/g dry weight recommended recently by the WHO (2009); one sample of *Camel Snus Original* was found to have a B[a]P concentration of 10.5 ng/g dry weight (McNeill et al. 2006; Rickert et al. 2009; Stepanov et al. 2008a). The B[a]P concentrations in these products were substantially lower than those measured in the same studies in US-type moist snuff products, which ranged between 19 and 83 ng/g dry weight (McNeill et al. 2006; Rickert et al. 2009; Stepanov et al. 2008a).

Stepanov and colleagues (2008a) also reported concentrations of seven additional PAHs in various STPs. B[b]F and B[k]F were generally not detected (in *General* nor in new products marketed as snus); however, these PAHs were detected in *Marlboro Snus Rich* and *Marlboro Snus Mild*, where the sum of B[b]F and B[k]F was nearly 3 ng/g dry weight. In comparison, levels in US-type moist snuff samples ranged between 28 and 57 ng/g dry weight. Similar to what was found for *General* samples, anthracene concentrations were below the detection limit in all *Marlboro Snus* and *Camel Snus* samples, whereas they were in the range of 323 to 1,060 ng/g dry weight in traditional US-type moist snuff. Concentrations of acenaphthylene, phenanthrene, fluoranthene, and pyrene in the US-type moist snuff samples were at least 10 times higher than those in *General,* while concentrations of phenanthrene, fluoranthene, and pyrene detected in *Marlboro Snus* and *Camel Snus* samples were slightly lower than those in *General*. Concentrations of acenaphthylene in the new products ranged from not detected to approximately twice of what was found in the *General* sample.

In a recent study, Stepanov and colleagues (2010) analyzed 23 PAHs in 17 brands of spitfree tobacco pouches (new products currently marketed as snus and produced by US companies: *Marlboro Snus, Camel Snus, Tourney, Grand Prix, Triumph, and Nordic Ice Snus*) and in 23 brands of US-type moist snuff (data not included in Table A II-3); no traditional Swedish snus products were included in this study. The authors concluded that, in agreement with their previous results (as reported above), “the levels of PAHs in spitfree tobacco pouches were very low”.

In their analysis of the results, Stepanov and colleagues (2010) reported that the mean sum of all PAHs in the newer products marketed as snus was 1.28 µg/g dry weight, which was approximately 11% of the average total PAHs in US-type moist snuff. The concentrations of B[a]P in the samples of new products marketed as snus were often below the limit of quantitation and the average was 12.3 ng/g dry weight, less than one-quarter of B[a]P concentrations detected in the US-type moist snuff samples (average 56 ng/g dry weight). The authors stated that the sum of PAHs that are classified as carcinogens in the new products marketed as snus averaged 1.18 µg/g dry weight “which is somewhat similar to moist snuff”. In their study, the average of the sum of PAHs classified as carcinogens in US-type moist snuff was 2.38 µg/g dry weight. The authors pointed out that the total amount (summing only the PAHs that are classified as carcinogens) was mainly due to a high naphthalene content, which seemed to be present at similar levels in all STP brands tested in this study. The average naphthalene level was 1.11 µg/g dry weight (range, 0.722-1.56 µg/g dry weight) in samples of new products marketed as snus and 1.73 µg/g dry weight (range, 0.886-2.27 µg/g dry weight) in US-type moist snuff samples. Naphthalene was also the major contributor to the sum of all

PAHs detected in samples of new products marketed as snus. The authors hypothesized that sources of naphthalene contamination could be common for US-type moist snuff and new products marketed as snus. The authors concluded that "when naphthalene was excluded from the calculations, the sum of the remaining carcinogenic PAHs in spitless tobacco was about 10% of that in moist snuff (0.066 vs 0.64 μg/g dry weight, respectively)." Traditional Swedish snus was not investigated in this study.

Table A II-3: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Polycyclic Aromatic Hydrocarbons

Brand	STP Type Specified by Study Authors	Citation	B[a]P (ng/g)	Acenaphthylene (ng/g)	Phenanthrene (ng/g)	Anthracene (ng/g)	Fluoranthene (ng/g)	Pyrene (ng/g)	B[b]F + B[k]F (ng/g)
Traditional Swedish Snus									
General	Swedish snus	Stepanov et al. 2008a	ND	1.70	55.3	ND	31.1	29.7	ND
"general [sic] pouch"	Snus (Sweden)	McNeill et al. 2006	1.99	NI	NI	NI	NI	NI	NI
New Products Marketed as Snus									
Du Maurier Freshmint	Swedish snus mint-flavored	Rickert et al. 2009	1.59	NI	NI	NI	NI	NI	NI
Du Maurier Original	Swedish snus	Rickert et al. 2009	2.08	NI	NI	NI	NI	NI	NI
Marlboro Snus Rich	New STP	Stepanov et al. 2008a	1.55	ND	14.8	ND	5.54	7.24	2.59
Marlboro Snus Mild	New STP	Stepanov et al. 2008a	2.06	ND	9.44	ND	4.42	4.43	2.93
Marlboro Snus Spice	New STP	Stepanov et al. 2008a	ND	ND	15.9	ND	5.38	6.24	ND
Marlboro Snus Mint	New STP	Stepanov et al. 2008a	1.02	3.15	14.6	ND	5.86	5.68	ND
Camel Snus Original	New STP	Stepanov et al. 2008a	10.5	3.95	41.7	ND	20.5	20.1	ND
Camel Snus Spice	New STP	Stepanov et al. 2008a	ND	4.14	33.7	ND	19.2	16.4	ND
Camel Snus Frost	New STP	Stepanov et al. 2008a	ND	4.99	40.7	ND	22.5	20.3	ND

Notes:
All amounts given as per dry weight. B[a]P: Benzo[a]pyrene; B[b]F: Benzo[b]fluoranthene; B[k]F: Benzo[k]fluoranthene; ND: Not detected; NI: Not investigated

A II 2.2.7.3. Aldehydes

Table A II-4 summarizes concentrations of aldehydes in traditional Swedish snus (*General*) as well as in new products marketed as snus in the US (*Camel Snus, Marlboro Snus*) as reported in one recent analysis of various STPs (Stepanov et al. 2008a).

Table A II-4: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Aldehydes

Brand	STP Type Specified by Study Authors	Citation	Formaldehyde (µg/g)	Acetaldehyde (µg/g)	Acrolein (µg/g)	Crotonaldehyde (µg/g)
Traditional Swedish Snus						
General	Swedish snus	Stepanov et al. 2008a	8.49	31.7	1.01	1.05
New Products Marketed as Snus						
Marlboro Snus Rich	New STP	Stepanov et al. 2008a	4.66	5.88	0.483	17.1
Marlboro Snus Mild	New STP	Stepanov et al. 2008a	4.09	3.33	0.591	18.4
Marlboro Snus Spice	New STP	Stepanov et al. 2008a	7.04	8.08	0.383	10.4
Marlboro Snus Mint	New STP	Stepanov et al. 2008a	5.35	10.5	0.726	4.83
Camel Snus Original	New STP	Stepanov et al. 2008a	1.51	6.64	0.31	0.552
Camel Snus Spice	New STP	Stepanov et al. 2008a	4.11	13.3	4.42	3.37
Camel Snus Frost	New STP	Stepanov et al. 2008a	3.02	16.4	3.31	3.56

Notes:
All amounts given as per dry weight.

Concentrations of formaldehyde and acetaldehyde in *General* was in the range of what was detected in traditional US-type moist snuff investigated in the same study (range, 6.58-10.6 µg/g dry weight and 17.1-72.3 µg/g dry weight, respectively) and slightly lower in *Marlboro Snus* and *Camel Snus*. Acrolein concentrations were in the same range as in US-type moist snuff (range, 2.58-7.85 µg/g dry weight) in *General* and in the same range or below in *Marlboro Snus* and *Camel Snus.* Similarly, crotonaldehyde concentrations were similar in General as compared to new products marketed as snus and US-type moist snuff (range, 0.98-6.35 µg/g dry weight), except for *Marlboro Snus*. Stepanov and colleagues (2008a) noted that crotonaldehyde levels in *Marlboro Snus*

were “relatively elevated” and recommended that the manufacturers “identify and eliminate the source of contamination”.

A II 2.2.7.4. Heavy Metals

Table A II-5 summarizes concentrations of some heavy metals in traditional Swedish snus (“general [sic] pouch”) as well as in new products marketed as snus in Canada (*du Maurier*) as reported in two recent analyses of different STPs on the UK and the Canadian market (McNeill et al. 2006; Rickert et al. 2009).

All heavy metal concentrations determined in these studies were below or, for cadmium, at the GothiaTek® Standard limits. Lead and arsenic concentrations detected in one traditional Swedish snus sample were similar to those detected in US-type moist snuff brands investigated in the same studies (ranges, 0.302-0.45 μg/g dry weight for lead and 0.218-0.366 μg/g dry weight for arsenic); concentrations were slightly lower in the two types of *du Maurier* snus. Nickel and chromium concentrations in one traditional Swedish snus sample as well as those in *du Maurier* snus were similar compared to those detected in US-type moist snuff brands investigated in the same studies (ranges, 1.145-2.64 μg/g dry weight for nickel and 0.837-1.69 μg/g dry weight for chromium). The chromium concentration in *du Maurier Original* was slightly higher with 1.985 μg/g dry weight. Cadmium concentrations in *du Maurier* snus were similar compared to those detected in US-type moist snuff (range, 0.865-1.068 μg/g dry weight). Cadmium in traditional Swedish snus was not analyzed.

Table A II-5: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Heavy Metals

Brand	STP Type Specified by Study Authors	Citation	Cadmium (μg/g)	Chromium (μg/g)	Nickel (μg/g)	Lead (μg/g)	Arsenic (μg/g)
Traditional Swedish Snus							
“general [sic] pouch”	Snus (Sweden)	McNeill et al. 2006	NI	1.54	2.59	0.50	0.30
New Products Marketed as Snus							
Du Maurier Freshmint	Swedish snus mint-flavored	Rickert et al. 2009	0.994	1.575	1.446	0.242	0.175
Du Maurier Original	Swedish snus	Rickert et al. 2009	0.967	1.985	1.536	0.233	0.143

Notes:
All amounts given as per dry weight. NI: Not investigated.

A II 2.2.7.5. Radioisotopes

Data on radioisotopes in traditional Swedish snus or new products marketed as snus was not identified in the more recent studies.

A II 2.2.7.6. Other Trace-Level Components

Data on other trace-level components in traditional Swedish snus or new products marketed as snus was not identified in the more recent studies.

A II 2.2.8 Potentially Protective Compounds

In their recent study, Rickert and colleagues (2009) measured selenium, an essential trace element, in STPs on the Canadian market, including new products marketed as snus (*du Maurier*). The selenium concentrations in *du Maurier Freshmint* and *du Maurier Original* were 0.153 and 0.157 μg/g dry weight, respectively. In most of the investigated US-type long-cut moist snuff brands, selenium levels could not be detected. Selenium concentrations in fine-cut and pouched US-type moist snuff brands ranged from not quantifiable to 0.082 μg/g dry weight.

A II 2.3 Summary and Discussion of Chemical Properties

It is well established that traditional US-type oral moist snuff is manufactured distinctively different from traditional Swedish snus. However, no details on the production methods of new products marketed as snus were identified in the scientific literature included in this review, so it is not known how the production method for these STPs compares to the manufacturing of traditional Swedish snus.

Based on analytical results from the chemical composition of new products marketed as snus as published in the scientific literature, there is considerable variability between traditional Swedish snus and new products marketed as snus for free nicotine content, pH and moisture levels; some of the new products may deliver considerably less nicotine..

TSNA concentrations detected in traditional Swedish snus and new products marketed as snus are considerably lower than those detected in traditional US-type moist snuff products. This is likely mostly due to the main difference in manufacturing process between these products, i.e., heat-treatment versus fermentation.

Similar to TSNA concentrations, most analyzed PAHs (including B[a]P) concentrations in traditional Swedish snus and new products marketed as snus are lower than those reported for traditional US-type moist snuff. Again, this is likely due in most part to processing differences between the STP types. A recent study (Stepanov et al. 2010) identified a specific PAH, naphthalene, thought to stem from other sources because it was present at similar levels in new products marketed as snus and in US-type moist snuff and represented the main component of the total PAH content in snus.

While this Appendix and Chapter 2 report all components as per dry weight of tobacco, this expression does not allow comparison of the actual exposure to these agents per single dose or portion of the products, due to the variability in moisture content and portion sizes (Stepanov et al. 2008a). Furthermore, it is difficult to compare products

because these factors, together with differences in pH, influence the nicotine delivery of a product. This would be an important issue in a risk assessment, because patterns of use of these products might differ depending on their nicotine delivery, which may affect individual users' exposure to components and therefore any associated potential health risks. An approach suggested by Rickert and colleagues (2009) is to take these variabilities into account by basing comparisons between products on ratios of levels of components to a product's nicotine yield.

Appendix III

Summary Tables of Epidemiology and Toxicology Studies of Swedish Snus

Appendix A1

Descriptive Studies of Dental Effects and Periodontal Disease

APPENDIX A-1
DESCRIPTIVE STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (N=9)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Andersson and Axell 1989 Southern Sweden This study compared oral mucosal lesions and gingival recessions associated with the use of loose and portion-bag packed snuff. This study is also summarized in Appendix B.	Cross Sectional Subjects included 252 men recruited from construction workers, shipyard workers, and outpatients from a dental school who were snuff users. Subjects were examined for oral mucosal lesions during 1986-1987. Lesions on the site where snuff was regularly placed were graded on a four grade clinical scale with Degree 1 being the least severe and Degree 4 being the most severe. The presence of gingival recessions was also recorded. There were 184 men who exclusively used loose snuff and 68 men who exclusively used portion-bag snuff. Those with serious disease or medications that might influence the local reaction of the oral mucosa were excluded.	Gingival recessions were found in 44 of 247 subjects. Among users of loose snuff 42 (23.5%) subjects showed gingival recessions while only 2 (2.9%) cases were found among users of portion-bag snuff ($p<0.05$). The factor with the highest relative risk for the development of gingival recessions was the package form (loose vs. portion-bag) (RR=8.71, $p<0.009$). No other factors (number of sites where quid was placed, hours of daily use, grams of snuff daily, years with regular snuff habit, or age) were significantly associated with the development of gingival recessions. Subjects were found, on average, to keep loose snuff and portion bag snuff in the mouth for about the same number of hours daily. However, greater daily amounts of loose snuff (23.6 $\pm$ 12.2 grams/day) than portion-bag snuff (11.3 $\pm$ 4.9 grams/day) were used, and loose snuff had been used for more years (10.4 $\pm$ 8.4) than portion-bag snuff (3.1 $\pm$ 2.5).	The authors concluded that clinical changes of the gingival margin are less pronounced among those who use portion-bag snuff than among those who use loose snuff. A total of 14 different brands of snuff were used, although 92.1% used six brands (General loose, General portion-bag, Grovsnus loose, Grovsnus portion-bag, Ettan loose, Ettan portion-bag). Relative risks do not appear to be controlled for any confounding factors.

APPENDIX A-1
CROSS SECTIONAL STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (N=9) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Andersson and Axell 1989 (continued)	All subjects had no other current tobacco habit than snuff and reported using snuff daily for at least the prior three months. However, 103 loose snuff and 24 portion-bag users reported prior smoking habits, and 4 loose snuff and 36 portion-bag users reported prior use of other smokeless tobacco products. "Snuff" is defined as loose or portion-bag packed Swedish moist snuff in this paper.		

APPENDIX A-1
CROSS SECTIONAL STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (N=9) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Bergström et al. 2006 Sweden This study examined the relationship between use of Swedish snus and periodontal bone loss (as assessed by bone height).	Cross Sectional Subjects were 84 apparently healthy men (ages 26 to 54) who were current (n=25), former (n=21) or never-users (n=38) of snuff. Snuff users were categorized into 2 exposure groups: light exposure (less than 15 years) and heavy exposure (15 years or more). Subjects provided information on tobacco habits via a structured questionnaire. Periodontal bone height (the distance from the cement-enamel junction to the periodontal bone crest, or CEJ-PBC) in each dental quadrant was assessed by bitewing radiograph. Clinical and radiographic exams were also performed. "Snuff" was defined as Swedish moist snuff in this study.	After controlling for age, the association between snuff use and bone height was not statistically significant ($p>0.05$). The mean (95% CI) CEJ-PBC distance was 1.06 mm (0.95-1.16) for never-users; 1.00 mm (0.87-1.13) for current snuff users; and 1.12 mm (0.97-1.26) for former users. The mean CEJ-PBC distance did not differ significantly between users with light vs. heavy exposure, regardless of whether they were current or former users. In addition, there were no statistically significant differences between user groups with respect to clinical characteristics (periodontal pocket depth or percentage of sites exhibiting gingival bleeding on probing). The authors noted that the results were not markedly modified when smoking was entered into the analysis. The outcome was similar in all quadrants of the mouth, regardless of where the snuff was placed.	The authors concluded that use of Swedish moist snuff is not associated with periodontal bone loss. They speculated that the harmful effect of smoking on periodontal tissues is probably due to toxic tobacco smoke products other than nicotine. The authors noted that most current snuff users exhibited (to varying degrees) a typical mucosal lesion; the lesion was not usually present in former users. It appears that no subject was a current smoker, although 10 current and 8 former snuff users were former smokers. (All never-users of snuff had never smoked.)

APPENDIX A-1
CROSS SECTIONAL STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (N=9) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Ekfeldt et al. 1990 Jönköping, Sweden This study presents an "individual tooth wear index" and uses this index to investigate factors correlated with occlusal wear.	Cross Sectional The study population consisted of 585 randomly selected dentate individuals (306 women and 279 men) from the community of Jönköping, Sweden who in 1983 reached the age of 20, 30, 40, 50, 60, 70, or 80 years. The degree of incisal and occlusal wear was quantified for each individual tooth using an index. This index was used as a dependent variable to investigate several factors related to tooth wear, including the use of snuff. "Snuff" was not specifically defined in this paper. The variable "snuffer" used in the model was binary (yes/no).	Step-wise multiple linear regression analysis indicated that, with respect to increased incisal and occlusal wear, the use of snuff explained 1.2% of the variance (R^2=0.012; $p<0.01$).	The authors noted that snuff use was correlated significantly with increased incisal and occlusal wear. Of the five factors found in the model to be related to tooth wear, snuff use was ranked fourth in order of explanatory power—lower than number of teeth, sex, bruxism, and age; but higher than buffer capacity. The authors hypothesize that snuff tobacco contains a certain amount of a silica compound, which may have an abrasive effect on the teeth.

APPENDIX A-1
CROSS SECTIONAL STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (N=9) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Julihn et al. 2008	Cross Sectional Subjects included 358 male and 328 female 19-yr-olds with different socio-economic profiles enrolled at seven public dental clinics in suburban Stockholm that answered a questionnaire on general health, tobacco habits, oral hygiene habits, and their parents' socio-economic background. The clinical and radiographic examination included registration of plaque, bleeding on probing (GBI), supra- and subgingival calculus, caries, and restorations.	There were 80 subjects that reported that they were daily snuff users and 26 subjects were evaluated for incipient alveolar bone loss. The adjusted odds ratio for incipient alveolar bone loss for snuff users was not statistically significant (OR = 1.15, 95% CI 0.7 – 1.89). The only risk factors that were statistically significantly correlated with incipient bone loss were subgingival calculus and proximal restoration $\geq$1	Adolescents with subgingival calculus as well as proximal restorations are at higher relative risk of exhibiting incipient alveolar bone loss compared to those without. In contrast to incipient alveolar bone loss, immigrant background is significantly associated with subgingival calculus among Swedish adolescents.

APPENDIX A-1
CROSS SECTIONAL STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (N=9) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hirsch et al. 1991 Göteborg, Sweden This study evaluated the relationship between tobacco habits among teenagers (including snuff) and dental caries.	Cross Sectional The study population included 2,145 dental patients (ages 14 to 19) who presented at any of 9 public dental clinics in Göteborg in 1986 for a yearly checkup, and who answered a questionnaire regarding tobacco habits. There were 1,574 (73%) non-users of tobacco and 571 (27%) tobacco users. The 571 tobacco users were further classified as smokers (n=374, or 17% of the total population) and snuff dippers (n=197, or 9% of the total population). "Snuff" was not defined in this paper.	<u>Profile of Snuff Users</u> Grams/Week of Snuff Consumption (n=197) > 50, <100 (Low): 23% >100, <200 (Moderate): 53% >200 (High): 24% Duration (in years) of Snuff Use (n=197) <2 years: 50% 2-5 years: 30% >5 years: 20% <u>Snuff Dippers Vs. Non-Users of Tobacco</u> T-tests indicated significantly higher ($p<0.001$) numbers of decayed, missing and filled teeth, decayed filled proximal surfaces, and initially decayed proximal surfaces, for all groups of tobacco users, smokers, and snuff dippers when compared to non-users of tobacco. <u>Multiple Regression Analysis</u> Results showed a positive correlation between "decayed, missing and filled teeth" and years of snuff use ($p<0.05$).	The authors concluded that a correlation exists between tobacco habits and increased caries prevalence; however, they state that no definitive conclusion can be made because dietary and oral habits have to be further elucidated. The authors noted that the number of tobacco users increased with age. Snuff use in this population was lower than expected, which the authors attributed to the fact that Göteborg is largely middle class and snuff dipping is more common among groups with lower socioeconomic status.

APPENDIX A-1
CROSS SECTIONAL STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (N=9) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Johansson et al. 1994 Northern Sweden This study compared dietary intake and the levels of traditional cardiovascular risk factors in edentulous middle-aged individuals and individuals of the same age and sex who still had natural teeth (*i.e.*, dentate).	Cross Sectional Subjects included 1,287 men and 1,330 women aged 25-64 years from the MONICA study (Monitoring Trends and Determinants in Cardiovascular Disease). Data were collected from a mailed questionnaire (January to April 1986 and 1990), blood analyses, registrations of blood pressure and anthropometric measures. 415 subjects were edentulous and 2,202 subjects were dentate. "Snuff" was not specifically defined in this paper. Those who had used snuff at least once daily were considered "regular snuff dippers.	Regular use of snuff did not differ between dentate and edentulous men and women.	The authors caution that the design of this study does not allow any conclusions on causality but merely on covariations between these variables. The authors noted that edentulous men and women were more often regular smokers, but not snuff users, than dentate individuals of the same age and sex.

APPENDIX A-1
CROSS SECTIONAL STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (N=9) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Modeer et al. 1980 Stockholm, Sweden This study examined the effects of smoking and oral use of snuff on oral health in Swedish schoolchildren.	Cross Sectional The study population consisted of 232 schoolchildren (119 boys and 113 girls) from the outskirts of Stockholm who received their dental treatment at the same Public Dental Service. Their mean age was 13.5 years. The children answered questions regarding smoking, snuff-taking, and toothbrushing habits prior to a clinical exam to assess oral hygiene, as measured by the Plaque Index of Silness and Loe (not described) and the Gingival Index of Loe and Silness (not described). "Snuff" was not specifically defined in this paper. None of the girls took snuff regularly but 11% of the boys did.	The mean consumption of snuff was 5 pinches per day. Snuff was present in the oral cavity for an average of 3.5 hours. Step-wise logistic regression indicated that snuff-taking was significantly correlated with both the Gingival Index ($p<0.001$) and the Gingival Index in the upper front jaw ($p<0.001$) after controlling for plaque index.	The authors concluded that snuff usage was significantly correlated with gingival index after controlling for plaque. They speculated that snuff usage may influence gingival tissue directly whereas smoking affects plaque accumulation. The authors found that the effect of snuff on gingival tissue was not solely related to the location of the substance, as the use of snuff was also found to be a predictor of gingivitis in general. They stated that the effect of snuff was remarkable in spite of the short duration.

APPENDIX A-1
CROSS SECTIONAL STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (N=9) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Montén et al. 2006 Göteborg, Sweden This study evaluated the potential association between use of smokeless tobacco and periodontal conditions in adolescents.	Cross Sectional The subjects were part of an epidemiologic study of 19-year-olds living in Göteborg. This study compared the prevalence of various periodontal conditions among a subsample of males who used snuff but did not smoke (n=33) and males who had never used tobacco (n=70). Subjects provided information on tobacco and oral hygiene habits and underwent clinical and radiographic examination. Multivariate logistic regression was used to identify factors associated with gingival recession. Outcomes were the prevalence of periodontal conditions (plaque score, gingivitis, probing pocket depth, clinical attachment loss, alveolar bone level, and gingival recessions). "Snuff" was defined as Swedish moist snuff in this paper.	Snuff users consumed a mean of 2.6 boxes/week (each box = 50 g of snuff). There were no significant differences between snuff users and never-tobacco users with respect to mean number of teeth, plaque score, number of sites with gingivitis, probing pocket depth, clinical attachment loss, or alveolar bone level. However, the prevalence of gingival recession was greater among snuff-users (42%) than among never-tobacco users (17%) (p=0.006). Multivariate logistic regression indicated that subjects with gingival recessions had significantly increased odds of using snuff (OR=3.7; 95% CI: 1.40-9.87) after adjusting for plaque, gingivitis, and tooth-brushing. The odds ratio associated with snuff use was higher (OR=5.1; 95% CI:1.67-15.55) when the analysis was restricted to the maxillary anterior tooth region (the typical location for the placement of snuff among Swedish users).	The authors concluded that, in this population of Swedish adolescents, use of snuff was not associated with the prevalence of periodontal disease, except for a significantly higher prevalence of gingival recessions. The study involved a relatively small number of subjects (only 30 were current snus users). The odds ratios were adjusted for plaque, gingivitis, and tooth-brushing.

APPENDIX A-1
CROSS SECTIONAL STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (N=9) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Wickholm et al. 2004 Stockholm, Sweden This study compared the prevalence of periodontal disease in four mutually exclusive groups of tobacco users.	Cross Sectional Study subjects were derived from a random sample of 3,273 residents in the Stockholm area; 1,674 participated. Subjects provided a lifetime history of tobacco use; they were then examined by a periodontist for evidence of periodontal disease (as assessed by plaque index, gingival index, amount of calculus, number of teeth with deep pockets and gingival recessions). "Snuff" was not specifically defined in this paper. There were four mutually exclusive groups of tobacco users: nonusers of tobacco, exclusive cigarette smokers, exclusive snuff users, or mixed users. Cumulative lifetime tobacco use was expressed in pack-years or can-years.	6.2% of men and 0.3% of women reported having used only snuff in their lifetimes. All groups of tobacco users had a higher prevalence of each outcome measure of periodontal disease than never-users of tobacco; the highest prevalence was seen among exclusive cigarette smokers and mixed users. There was a significant association between smoking and periodontal disease (compared to never-smoking), but there was no significant association between current snuff use and periodontal disease (compared to never use). There was an indication of association with former snuff use: the odds ratio associated with former snuff use was elevated, but not statistically significant (OR=2.55; 95% CI:0.71-5.95), after adjustment for gender, age, and education. The proportion of subjects with unhealthy periodontal conditions increased with increasing pack-years of smoking, but not with increasing can-years of snuff use.	The authors concluded that current use of snuff is not significantly associated with periodontal disease. Smoking was independently associated with periodontal disease. Mixed use of cigarettes and snuff was not associated with a lower prevalence of periodontal disease than exclusive smoking.

Appendix A2

Case-Control Studies of Periodontal Disease

APPENDIX A-2
CASE-CONTROL STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (N=1)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Kallestal and Uhlin 1992 Vasterbotten, Sweden This study investigated and identified factors connected with loss of buccal attachment in adolescents.	Case-control study (population-based), subjects drawn from a cross-sectional study. Cases (n=71) were 18-year-olds with buccal attachment loss (≥ 1mm in one or more sites) who had participated 2 years earlier in a cross-sectional study of periodontal conditions in adolescents. There were 2 sub-groups of cases, one identified as having buccal attachment loss in 1987 and the other with attachment loss in the years 1987 to 1989. Controls (n=66) were 18-years-olds with no attachment loss at the time of the prior investigation. "Snuff" was not defined in this study; instead, the study examined smokeless tobacco and does not specify whether "smokeless tobacco" refers to snuff, chewing tobacco, or both. The number of subjects using smokeless tobacco was not specified.	Statistical analyses were performed to detect factors related to buccal attachment loss. The interview included questions on the use of smokeless tobacco, how often it was used, and where in the mouth it was placed. The authors presented no quantitative data on the consumption of smokeless tobacco; however, they stated that cases and controls did not differ in their use of smokeless tobacco.	The authors concluded that factors associated with the anatomy of the buccal alveolar process are related to buccal attachment loss in populations where the level of oral hygiene is high. The authors apparently chose to collect data on smokeless tobacco use based on the results of a 1985 study (Offenbacher and Weathers) involving 14-year old boys in Atlanta, Georgia. In that study, gingival recessions were found more often in boys who used smokeless tobacco and had gingival inflammation. Consequently, the authors hypothesize that the failure to find a relationship between use of smokeless tobacco and buccal attachment loss may be due to the low level of gingivitis in the study population.

Appendix B

Descriptive Studies of Oral Mucosal Lesions

APPENDIX B
CROSS SECTIONAL STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (N=19)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Andersson and Axell 1989 Southern Sweden This study compared oral mucosal lesions and gingival recessions associated with the use of two different smokeless tobacco products, loose snuff and portion-bag packed snuff. This study is also described in Appendix A-1.	Cross Sectional study Subjects included 252 men recruited from construction workers, shipyard workers, and outpatients from a dental school who were snuff users. Subjects were examined for oral mucosal lesions during 1986-1987. Lesions on the site where snuff was regularly placed were graded on a four grade clinical scale with degree 1 being the least severe and grade 4 being the most severe. The presence of gingival recessions was also recorded. There were 184 men who exclusively used loose snuff and 68 men who exclusively used portion-bag snuff. Those with serious disease or medication that might influence the local reaction of the oral mucosa were excluded. A total of 14 different brands of snuff were used, although 92.1% used six brands (General loose, General portion-bag, Grovsnus loose, Grovsnus portion-bag, Ettan loose, Ettan portion-bag).	<u>Distribution of Oral Mucosal Lesion Severity</u> Loose Snuff Degree 1: 5.4% (10/184) Degree 2: 17.9% (33/184) Degree 3: 70.7% (130/184) Degree 4: 6.0% (11/184) Portion-bag Snuff Degree 1: 19.1% (13/68) Degree 2: 45.6% (31/68) Degree 3: 35.3% (24/68) Degree 4: 0.0% (0/68) Subjects were found, on average, to keep loose snuff and portion bag snuff in the mouth for about the same number of hours daily. However, greater daily amounts of loose snuff (23.6 ± 12.2 grams/day) than portion-bag snuff (11.3 ± 4.9 grams/day) were used, and loose snuff had been used for more years (10.4 ± 8.4) than portion-bag snuff (3.1 ± 2.5). Users of loose snuff had a significantly higher proportion of degree 3 and 4 (more severe) lesions ($p<0.001$), and the most severe lesions (degree 4) were only found in users of loose snuff. These effects were still seen after stratifying for previous smoking habits.	The authors concluded that the use of portion-bag snuff is associated with less severe oral mucosal lesions and a lower frequency of gingival recessions than is use of loose snuff.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Andersson and Axell 1989 (continued) Southern Sweden	All subjects had no other current tobacco habit than snuff and reported using snuff daily for at least the prior three months. However, 103 loose snuff and 24 portion-bag users reported prior smoking habits, and 4 loose snuff and 36 portion-bag users reported prior use of other smokeless tobacco products. "Snuff" is defined as loose or portion-bag packed Swedish moist snuff in this paper.	The most important risk factor for more severe lesions was the package form (RR 3.39). Also significantly associated with more severe lesions was the placing of snuff in one (vs. more than one) location (RR 2.91), increased hours of daily snuff use (RR 1.13), and increased grams of snuff per day (RR 1.05).	

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Andersson and Warfvinge 2003 Sweden The study evaluated how variations in pH and nicotine concentration of snuff affect the oral mucosa (clinically and histologically), salivary pH, and daily nicotine intake. [The group selected for this study came from the study population described in Andersson and Axell 1989.]	Cross-Sectional study Subjects were 20 healthy volunteers selected from a population of 104 habitual users of loose snuff (Brand A) who had participated in a previous study and who had a clinical thickening of the mucosa, classified as degree 3 or 4 lesions. These 20 subjects were studied during use of their regular brand (Brand A: pH 8.6, 0.8% nicotine), after 12 weeks use of a snuff with a lower pH (Brand B: pH 8.0, 0.8% nicotine), and after another 12 weeks use of a snuff with both lower pH and lower nicotine concentration (Brand C: pH 8.0, 0.4-0.5% nicotine). A clinical exam of the oral mucosa was conducted at baseline. The investigators assessed consumption of snuff, oral soft tissue changes, salivary pH, and nicotine intake at weeks 4, 12, 16, and 24. Severity of clinical lesions was assessed on a 4-point scale. Biopsies were taken from clinically observed lesions after usage of each of the three brands of snuff and histological changes were analyzed. "Snuff" is defined as loose Swedish oral moist snuff in this paper.	<u>Distribution of Oral Mucosal Lesion Severity</u> Recruitment, Brand A (pH 8.6, 0.8% nic) Degree 1: 0% (0/20) Degree 2: 0% (0/20) Degree 3: 80% (16/20) Degree 4: 20% (4/20) Week 4, Brand B (pH 8.0, 8.0% nic) Degree 1: 0% (0/20) Degree 2: 15% (3/20) Degree 3: 80% (16/20) Degree 4: 5% (1/20) Week 12, Brand B Degree 1: 0% (0/20) Degree 2: 35% (7/20) Degree 3: 65% (13/20) Degree 4: 0% (0/20) Week 16, Brand C (pH 8.0, 0.4-0.5% nic) Degree 1: 5% (1/20) Degree 2: 45% (9/20) Degree 3: 50% (10/20) Degree 4: 0% (0/20) Week 24, Brand C Degree 1: 10% (2/20) Degree 2: 55% (11/20) Degree 3: 35% (7/20) Degree 4: 0% (0/20)	The authors concluded that nicotine is one of the substances in snuff that has a biological effect on the oral mucosa. There also seems to be a synergistic effect between the pH and the nicotine concentration in the snuff. The subjects in this study were heavy snuff users (they consumed 43-49 g/day snuff, about twice the average amount consumed by Swedish snuff users). Average salivary pH was higher during snuff use than in the morning ($p<0.001$); it was also higher shortly after snuff was removed than during use. The degree of clinical oral mucosal changes was correlated with salivary cotinine levels ($p<0.01$) and nicotine dose ($p<0.01$). As the pH and nicotine concentrations became lower, the clinical and histological changes were significantly less pronounced. The mucosal samples displayed structural changes typical of lesions induced by Swedish snuff. There was no dysplasia.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Andersson et al. 1989 Southern Sweden The study identified histological tissue changes in the oral mucosa and compared these changes in users of loose can-packed and portion-bag-packed moist snuff. [The group selected for this study came from the study population described in Andersson and Axell 1989.]	Cross-Sectional study Of the 252 biopsies obtained from snuff users recruited from populations of construction workers, shipyard workers, and outpatients from a dental school, 14 matched pairs of loose and portion-bag users were analyzed for histological changes related to the package form. The pairs were selected based on use by the same brand (but different package form) of tobacco, placement in the same site, and use of similar grams/day and hours of daily use. These groups differed only by duration of use: 10.3 ± 8 years (loose) versus 4.4 ± 2.8 years (portion-bag). "Snuff" is defined as loose can- packed and portion-bag packed Swedish moist snuff in this paper.	<u>Distribution of Oral Mucosal Lesion Severity</u> Loose Snuff Degree 1: 0.0% (0/14) Degree 2: 14.3% (2/14) Degree 3: 85.7% (12/14) Degree 4: 0.0% (0/14) Portion-bag Snuff Degree 1: 14.3% (2/14) Degree 2: 50.0% (7/14) Degree 3: 35.7% (5/14) Degree 4: 0.0% (0/14)	The authors concluded that, based on comparable snuff habits, loose snuff may cause clinically more pronounced changes (Degree 3) accompanied by histologic Type 1 changes. Portion-bag snuff, is associated with less pronounced changes (Degree 1-2) and shows more histologically Type 2 (or very discrete) changes. Subjects were questioned on brand of snuff used, however, brand specific information was not provided. All 28 cases displayed some degree of non-specific inflammation. The authors were unable to detect any clear-cut difference in inflammation between the loose and portion-bag snuff users. In 14 matched pairs of loose and portion-bag snuff users, cases of hyperplasia and increased mitotic rate were evenly distributed between the two groups. No unequivocal cases of dysplasia were recorded. Loose snuff was found to be associated with a higher frequency of clinical degree 3-4 lesions than portion-bag packed snuff. Loose snuff users also showed predominantly histologic Type 1 changes (increased epithelial thickness with vacuolated cells and frequent chevron type changes), while portion-bag users showed more histologic Type 2 changes (variably thickened surface layer with keratinization).

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Andersson et al. 1990 Southern Sweden This study analyzed the impact of different patterns of Swedish snuff consumption on oral histologic changes. [The group selected for this study came from the study population described in Andersson and Axell 1989.]	Cross-Sectional study Of the 252 biopsies obtained from snuff users recruited from populations of construction workers, shipyard workers, and outpatients from a dental school, two groups were selected for this study. Group 1 consisted of 8 pairs of loose snuff users and focused on histopathology associated with many vs. few years of consumption when daily use within the pairs was similar. Group 2 consisted of a total of 25 cases and examined histopathology associated with low vs. high daily consumption of loose or portion-bag packed snuff. "Snuff" is defined as loose and portion-bag packed Swedish moist snuff in this paper. 184 subjects exclusively used loose and 68 subjects exclusively used portion-bag packed snuff.	<u>Distribution of Oral Mucosal Lesion Severity</u> Loose Snuff - Many Years of Use Degree 1: 0.0% (0/8) Degree 2: 25.0% (2/8) Degree 3: 75.0% (6/8) Degree 4: 0.0% (0/8) Loose Snuff - Few Years of Use Degree 1: 12.5% (1/8) Degree 2: 0.0% (0/8) Degree 3: 87.5% (7/8) Degree 4: 0.0% (0/8) <u>Low Daily Consumption</u> Loose Snuff Degree 1: 60% (3/5) Degree 2: 20% (1/5) Degree 3: 20% (1/5) Portion-bag Snuff Degree 1: 60% (3/5) Degree 2: 40% (2/5) Degree 3: 0.0% (0.5) <u>High Daily Consumption</u> Loose Snuff Degree 1: 0.0% (0/8) Degree 2: 0.0% (0/8) Degree 3: 87.5% (7/8) Degree 4: 12.5% (1/8) Portion-bag Snuff Degree 1: 0.0% (0/7) Degree 2: 57.1%% (4/7) Degree 3: 42.8% (3/7) Degree 4: 0.0% (0/7)	The authors concluded that many years of loose snuff use does not *per se* result in tissue changes that differ significantly from changes seen in subjects with only a few years of loose snuff use. In comparison with low consumption, high daily consumption of portion-bag packed or loose snuff results in more pronounced surface epithelial changes. The authors speculated that daily but not intermittent use of snuff causes a mixed tissue reaction of injury and repair. The capacity for tissue repair appears most influenced by daily consumption levels, rather than duration of use. Of the 16 cases comprising Group 1, no cases suggestive of dysplasia were found. The authors state that the different types of surface changes were evenly and seemingly randomly distributed among subjects with long and short histories of use. In the analysis of Group 2, one case was considered clinical grade 4, suggestive of dysplasia. Histological differences between the loose and portion-bag users with high daily were difficult to identify. However, among those with low daily consumption, portion-bag snuff tended to cause less severe changes.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Andersson et al. 1991 Southern Sweden This study examined whether histopathological findings supported the clinical four-point scale used for subgrouping snuff dipper's mucosal lesions. [The group selected for this study comes from the study population described in Andersson and Axell 1989.]	Cross-Sectional study Of the 252 biopsies obtained from snuff users recruited from populations of construction workers, shipyard workers, and outpatients from a dental school, 70 were examined for this study. Ten cases were selected for each clinical grade (1-4) for a total of 40 cases for loose snuff users and 30 cases for portion-bag snuff users (no clinical grade 4 cases were present among portion-bag snuff users). "Snuff" is defined as loose packed and portion-bag packed moist snuff in this paper.	The distribution of oral mucosal lesion severity is not provided, since cases were selected on the basis of their clinical grade. Surface layer changes were subtle in Degree 1 lesions and surface thickening became more pronounced in Degrees 2-4 lesions. Type 2 changes were most frequent in Degrees 1 and 2. Atrophy, hyperplasia, mitoses, and basal cell hyperplasia were more frequent in higher clinical degree lesions. Among portion-bag users, surface changes were less common in those with Degree 1 lesions. However, the pattern of tissue changes among portion-bag users and loose snuff users with Degree 2 or 3 lesions were comparable.	The authors concluded that the four different clinical degrees employed to register snuff dipper's lesions are justified because they generally correspond to a fairly consistent set of tissue changes. The authors also noted that (with the exception of Degree 1) within each clinical grade portion-bag and loose snuff users show similar histologic patterns. It was emphasized, however, that there is no clear cut difference between clinical degrees, either clinically or histologically, and thus, an overlap between degrees is logical and sometimes occurs.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Andersson et al. 1994 Southern Sweden This study investigated whether potential differences in nicotine uptake or metabolism accounted for differences in the oral mucosa of users of loose and portion-bag packed moist snuff. [Many of the individuals in this study were from the study population described in Andersson and Axell 1989.]	Cross-Sectional study A total of 54 habitual users (men) of smokeless tobacco were selected for this study: 22 loose snus users, 23 portion-bag users and 9 users of chewing tobacco (45 total snuff users). Those selected used no other forms of tobacco. Changes in the oral mucosa were registered according to a four-point scale (Degree 1-4). The 45 snuff users were selected from the 252 men originally studied by Andersson and Axell (1989). All 45 snuff users used the same brand and had similar daily snuff consumption. "Snuff" is defined as oral moist snuff, or snus, in loose or portion-bag form in this paper. Swedish smokeless tobacco was examined, which included chewing tobacco.	<u>Distribution of Oral Mucosal Lesion Severity</u> Loose Snuff Degree 1: 4.0% (1/22) Degree 2: 23.0% (5/22) Degree 3: 73.0% (16/22) Degree 4: 0.0% (0/22) Portion-bag Snuff Degree 1: 9.0% (2/23) Degree 2: 48.0% (11/23) Degree 3: 39.0% (9/23) Degree 4: 0.0% (0/23)	The authors concluded that the clinical severity of buccal mucosal changes did not correlate with nicotine or tobacco-specific nitrosamine content of the snuff or with biological markers for nicotine uptake among users. The authors speculated that the higher pH of the loose snuff may contribute to the greater severity of mucosal lesions seen in loose snuff users. Portion-bag users showed predominantly Degree 1 and 2 lesions, while loose snus users showed more Degree 3 lesions. No Degree 4 lesions were reported among subjects in either group. No difference was observed in biomarkers for nicotine uptake or in the metabolic pattern among users of portion-bag and loose snuff. This was observed despite the greater amounts of nicotine and tobacco-specific nitrosamines that could be extracted experimentally from loose versus portion-bag snuff.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Andersson et al. 1995 Sweden This study is an investigation of oral mucosal changes and nicotine regulation that occurs among users of portion-bag snus when switching from an ordinary snus product to a low-nicotine product. [Studies 1 and 2 appear to use individuals from the study population described in Andersson and Axell 1989.]	Cross-Over Study Study 1 Subjects were 24 habitual users of normal Brand A snus (nicotine content 0.8%-0.9%) were followed for 12 weeks. During weeks 1 and 2, participants continued to use Brand A snus, *ad libitum*. At the start of week 3, participants switched to Brand B snus (nicotine content 0.4%-0.5%) and continued to use it, *ad libitum* for 10 weeks. Consumption data, soft tissue changes, nicotine intake, and nicotine metabolites were measured. Lesions were registered according to the degree of clinical severity on a 4-point scale. Study 2 A total of 18 individuals who had switched from Brand A to Brand B snus in Study 1 were evaluated for two weeks, after at least one year after switching. Consumption data, soft tissue changes, nicotine intake, and nicotine metabolites were measured. Lesions were registered according to the degree of clinical severity on a 4-point scale. "Snuff" is defined as portion-bag Swedish oral moist snuff or snus, in this paper. Subjects of Study 2 had no other tobacco habit.	<u>Distribution of Oral Mucosal Lesion Severity</u> <u>Study 1</u> Regular Nicotine Snus Degree 0: 0.0% (0/24) Degree 1: 17.0% (4/24) Degree 2: 46.0% (11/24) Degree 3: 37.0% (9/24) 10 Weeks After Switching to Low Nicotine Snus Degree 0: 4.0% (1/24) Degree 1: 17.0% (4/24) Degree 2: 75.0% (18/24) Degree 3: 4.0% (1/24) <u>Study 2</u> Degree 0: 0.0% (0/24) Degree 1: 28.0% (5/24) Degree 2: 55.0% (10/24) Degree 3: 17.0% (3/24)	The authors concluded that snus users do not compensate for reduced nicotine delivery following switching to a reduced-nicotine product. Although an obvious change in mucosal lesion severity was seen after changing to the low-nicotine snus, the authors concluded that it is unclear whether the severity of oral mucosal changes were associated with the lower nicotine content. There was a slight, but statistically significant, increase in daily amount of snus intake in Study 1 when switching from Brand A to Brand B snus (+15%, $p<0.001$). A statistically significant decrease in daily nicotine intake after switching to Brand B snus was observed (-43%, $p<0.001$). In Study 1, there was a decrease in Degree 3 lesions during Weeks 4-12 (during consumption of Brand B snus). After switching to Brand B snus in Study 1, there was a reduced degree of whiteness and mucosal thickening. The predicted probability of developing a Degree 2 (or higher) lesion when consuming Brand A or Brand B snus was less with Brand B snus if consumption was <20 grams/day, but similar at levels of snus consumption above 20 grams/day. The probability of inducing a Degree 3 (or higher) lesion was about three times as large when consuming Brand A snus as when consuming Brand B snus at any level of snus consumption.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Axell 1976 Central Sweden This study investigated the prevalence of 60 types of oral mucosal lesions in an adult Swedish population.	Cross-Sectional study Of 30,118 individuals, aged 15 years or above, from the total adult population of Uppsala County in central Sweden, 20,333 adults (10,036 males and 10,297 females) were examined for the prevalence of various types of oral lesions in 1973-1974. "Snuff" is not defined in this paper.	<u>Prevalence of Snuff Dipper's Lesion</u> Total: 8.04% Male: 15.94% Female: 0.19% A total of 1,466 individuals were identified as having Snuff Dipper's lesion (1,459 males; 7 females). Snuff dippers were reported to comprise 14.2% of the total males, <0.1% of the total females, and 7.1% of the total population examined. These lesions were characterized by authors as "most often whitish, but there may also be more subtle changes without color changes and with only slight wrinkling."	The author reports that almost without exception, snuff dipping gives rise to characteristic lesions of the oral mucosa. However, no direct evaluation of the presence of this type of lesion exclusively in snuff dippers was presented. Prevalence of the lesions were first calculated in the various demographic groups and thereafter weighted together, yielding prevalence for males, females and total population.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Axell 1987 Central Sweden The study investigated the prevalence of oral white lesions based on a new classification in adults. [This study uses the same study population described in Axell 1976.]	Cross-Sectional study Of 30,118 individuals, aged 15 and above, from the total adult population of Uppsala County in Central Sweden, 20,333 adults (10,036 males and 10,297 females) were examined between 1973 and 1974 for a survey of the prevalence of various types of oral lesions. Weighted prevalences were calculated for 14 demographic groups (for age and sex strata and for the total population). "Snuff" is not defined in this paper.	<u>Prevalence of Snuff Dipper's Lesion</u> Total: 8.0% Male: 15.9% Female: 0.2% A highly significant difference between sexes was observed ($p<0.001$).	The author concluded that snuff dipper's lesion is a defined clinical entity with a specific etiology that is distinct from tobacco-associated leukoplakia. The author notes that, unlike leukoplakia (white patch), the changes seen in snuff dippers are yellowish, brownish, or involve no color change. The author further points out that while the precancerous potential of leukoplakia in Scandinavia is approximately 4%, the precancerous nature of snuff dipper's lesion is more doubtful. Among 200,000 snuff dippers in Sweden, only one case per year of oral cancer may be found. No carcinogenicity data are presented in this report.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Axell and Hedin 1982 Central Sweden The study investigated whether an association existed between excessive oral melanin pigmentation and different tobacco habits. [This is the same study population described in Axell 1976.]	Cross-Sectional study Of 30,118 individuals, aged 15 and above, from the total adult population of Uppsala County in Central Sweden, 20,333 adults (10,036 males and 10,297 females) were examined between 1973 and 1974 for a survey of the prevalence of various types of oral lesions. Tobacco habits were classified into seven categories, including snuff dipping. "Snuff" is not defined in this paper.	<u>Prevalence of Oral Melanin Pigmentation</u> Any tobacco habit 18.9% Snuff dipping 4.7% No tobacco consumption 3.0%	The authors concluded that snuff dipping did not significantly elevate the prevalence of oral melanin pigmentation. Snuff dippers were more frequently pigmented in the anterior labial alveolar mucosa of the maxilla and the buccal mucosa than those with no tobacco habit. However, the authors note that no melanin pigmentation was seen at the site where the quid of snuff was placed. The authors speculated that the absence of hyperpigmentation at the site of snuff use may be due to differences in epithelial keratinization in this area. Snuff dipping was not associated with a statistically significant increase in the incidence of oral melanin pigmentation when compared to those with no tobacco habits.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Axell and Henricsson 1985 Central Sweden The study investigated whether an association existed between recurrent aphthous ulcers (RAU) and different tobacco habits. [This is the same study population described in Axell 1976.]	Cross-Sectional study The study authors examined 20,333 people aged 15 years and older who participated in an epidemiological survey of oral mucosal lesions in the general population of Uppsala County in central Sweden. All persons answered a questionnaire on tobacco habits and whether they had experienced RAU. Tobacco habits were classified into eleven categories, including snuff dipping. Those with mixed habits were excluded. "Snuff" is not defined in this paper. A total of 877 subjects were solely snuff users (4.3%).	<u>Prevalence of Recurrent Aphthous Ulcers</u> Any tobacco habit 13.6% Snuff dipping 15.0% No tobacco consumption 21.7%	The authors concluded that the suppression of recurrent aphthous ulcers occurred in those with any tobacco habit and was only moderate among snuff users. The authors speculated that increased keratinization of the oral mucous membrane may resist RAU formation in the mouth by preventing antigenic bacterial substances from penetrating through the epithelium. This could prevent immune system stimulation. Snuff dipping was associated with a statistically significant decrease in frequency of RAU compared to no tobacco consumption ($p<0.001$). All groups practicing any of the examined tobacco habits showed lower frequencies of RAU than non-tobacco users.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Axell et al. 1976 Sweden This study describes the histopathological appearance of snuff dipper's lesions. [This study examines a subpopulation of individuals from the study population described by Axell 1976.]	Cross-Sectional study Of 20,000 individuals participating in an epidemiological survey of oral mucosal lesions, approximately 1,200 snuff dippers were identified. Of the snuff dippers, 114 males (aged 20-88) underwent biopsy. Snuff dipper's lesion was diagnosed when there was a lesion of the oral mucosa in a location that was at the exact site of regular snuff placement. Lesions were graded using a four-point scale (Degree 1, 2, 3, or 4). Another gradation (Degree X) was assigned to patients who had stopped using snuff between the initial examination and the biopsy. All patients with clinical lesions of Degree 4 were subjected to biopsy (n=36). Individuals with lesions of other degrees were biopsied at random (Degree 1, n=4; Degree 2, n=17; Degree 3, n=51, and Degree X, n=6). "Snuff" is not defined in this paper. Snuff brands used by these subjects included Ettan, Grovsnus, Roda Lacket, and Svenskt.	None of the 114 biopsies showed changes interpreted as cellular atypia or epithelial dysplasia. All but one biopsy showed an increased total epithelial thickness, which was more pronounced in Degree 3 and 4 lesions. In lesions with lower clinical grades, the epithelial surface appeared intermediate between undisturbed keratinization and vacuolization.	The authors concluded that increased epithelial thickness, especially in the presence of a vacuolated surface layer, was the only histological feature that correlated with severity of clinical appearance of the lesions. Neither the degree of inflammation nor amorphous changes were correlated with clinical grading of the lesions. Acanthosis was found in all clinical groups, and was increased in degrees 3 and 4 lesions. Epithelial hyperplasia, seen in 30 cases, did not correlate with clinical grading. Inflammatory reactions (slight in most cases, moderate in 16 cases, and severe in 11 cases) also showed no correlation with clinical grading. Amorphous, weakly eosinophilic, PAS-positive, and van Gieson yellow areas were seen in only 9 subjects and did not correlate with clinical grade. With the exception of the presence of amorphous areas in connective tissue of users of Ettan and Roda Lacket brands, no correlation was seen between brand and either clinical or histological appearance.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Frithiof et al. 1983 Stockholm The authors examined oral lesions clinically and histologically (via light- and electron-microscopy) to investigate the effects of snuff on the oral mucosa.	Cross-Sectional study Subjects included 21 male snuff users (range 31-79 years of age; mean 55 years) who were referred to the dental school at Karolinska Institute for treatment of snuff-induced lesions of the oral mucosa. "Snuff" was not defined specifically in this paper, but appears to refer to Swedish snuff.	<u>Clinical Findings (prevalence not reported)</u> ▪ Snuff-induced lesions had a characteristic whitish appearance, frequently with brown discoloration. ▪ Some lesions had dark red pinpricks surrounded by elevated, swollen, whitish zone. ▪ Net-like whitish tissue in combination with reddish areas. ▪ Texture was wrinkled and swollen. ▪ Firmer than surrounding normal tissue. ▪ In some cases desquamating epithelium and ulcerations were observed. ▪ Gingival retraction (9.5%). ▪ Upon stopping, the lesion was markedly normalized in structure and color after one week. After 14 days, only remnants of patches remained and the mucosa had regained most of its soft consistency and normal color. <u>Light Microscopy Findings</u> ▪ Epithelial hyperplasia (100%) Hyperorthokeratinization (57.1%) Hyperparakeratinization (42.9%) Surface layer contained enlarged vacuolated cells with nuclear remnants. ▪ Acanthosis (100%). ▪ Inflammatory reaction (76.2%).	The authors concluded that the daily use of snuff in a limited area of the mucobuccal fold results in a characteristic lesion. Clinical healing can occur within 2 to 3 weeks of cessation of use, even after decades of use. The authors acknowledge that it was not possible from this study to determine the period of time required for a snuff-induced lesion to develop. Even if lesions are induced by longstanding use of snuff, little is known about whether the chemical or the mechanical irritation is the main inducing factor. The authors suggest that snuff-induced lesions should be totally excised if dysplasia or cellular atypia are found. However, they concede that the premalignant significance of the mild dysplasia found in this study is questionable and may be due to inflammatory infiltration. The authors speculate that the use of dentures, poor oral hygiene, undernourishment, vitamin deficiencies, iron deficiency, habitual use of alcohol, and irregular daily life patterns are possible confounding factors (not controlled for in most studies), which may be present in many snuff users. Furthermore, they note that differences in habits and the composition of snuff brands makes it "difficult to assess the general probability of malignification of snuff-induced lesions."

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Frithiof et al. 1983 (continued) Stockholm		▪ Mild epithelial dysplasia characterized by drop-shaped rete processes, reduction of cellular adhesion in basal and spinous cell layers, and slight cellular pleomorphism (23.8%). ▪ Carcinoma or carcinoma-in-situ (0%). <u>Electron Microscopy Findings (11/21 examined)</u> ▪ Cells in surface layers partly keratinized and contained nuclear remnants (100%). ▪ Increased amounts of tonofilaments in spinous and basal layers (100%). ▪ Odland bodies, small round keratohyaline granules in spinous layer (81.8%). ▪ Lamina densa of basal layer doubled in 45.5%, discontinuous in 55.5%. ▪ Cytoplasmic processes from basal cells (36.4%). ▪ Inflammatory cells and filamentous material of unknown composition found in connective tissue of some specimens.	

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hirsch et al. 1982 Sweden The aim of this investigation was to study the clinical, histomorphological and histochemical characteristics of oral lesions "induced by exposure to snuff."	Cross-Sectional study The study population included 50 male patients who were all "habitual snuff-dippers." Subjects' ages ranged from 15-84 years (mean age 41.3 ± 17.6). Biopsies of upper vestibular mucosa and submucosa were obtained for histomorphological and histochemical examination. Lesions were graded on a four grade clinical scale with Degree 1 being the least severe and Degree 4 being the most severe. Snuff habits and information on smoking and drinking habits were obtained through a questionnaire. A total of 68% of snuff users were also social drinkers. Half of these were smokers as well. Among non-drinkers, 8% were smokers and snuff dippers and 24% used snuff only. "Snuff" is defined as wet snuff in this paper. Eight different brands of snuff were used by study participants	<u>Distribution of Oral Mucosal Lesion severity</u> Degree 1: 20% (10/50) Degree 2: 18% (9/50) Degree 3: 22% (11/50) Degree 4: 40% (20/50) Younger patients were usually found to have lesions of clinical Degrees 1, 2, and 3, while significantly more older patients had Degree 4 lesions. Patients with Degree 4 lesions had been snuff-dippers significantly longer than the rest of the patients. Patients with Degree 3 and 4 lesions also used snuff approximately twice as long per day as patients with Degree 1 and 2 lesions. Increased epithelial thickness was seen in 94% of specimens. Most exhibited slight or moderate parakeratinization, vacuolated cells in the superficial epithelium, and 80% had varying degrees of stromal inflam-mation. The clinical Degree 4 lesions had these changes to a greater extent. Salivary gland inflammation and degeneration were most prevalent in Degree 3 and 4 lesions. Slight dysplasia was observed in 9/50 patients (18%) and was distributed across all four clinical grades.	The authors concluded that a correlation between snuff habits and the clinical degree of the oral lesion was found. A correlation between snuff habits and certain superficial and deeply located cell changes was also seen. The investigators noted that the most marked degenerative changes were seen in the salivary glands and speculated that this may lead to epithelial changes. They postulated that decreased saliva production could lessen the protection of the epithelium. No significant differences with regard to clinical degree of lesion and histological appearances could be found either between patients with multiple habits and those who used only snuff, or between patients who used different brands of snuff and those who used one brand only.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Larsson et al. 1991 Sweden This clinical follow-up study assessed the possible reversibility of oral mucosal changes associated with the use of Swedish moist snuff. [This study included individuals included in the study population described by Andersson and Axell 1989.]	Cross-Sectional study From a sample of 252 Swedish men (184 used loose packed moist snuff and 68 used portion-bag packed moist snuff on a daily basis), 29 loose snuff users (aged 21-70 years) were selected for this study based on the observation of histopathological changes that differed from those typically seen in snuff users (*i.e.*, increased mitotic rate, increased cell density, loss of cell cohesion). All 29 had used snuff for 3 to 40 years prior to changing their habits. New biopsies were taken from the same mucosal areas as the original biopsies at least 6 months after either quitting, changing to portion bags, reducing the use of snuff and/or reducing placement of the quid in a single spot. The study group was compared to 5 loose snuff users (aged 29 to 58 years) that were selected based on a daily consumption of at least 25 grams for 12 hours or more daily, and for 7 to 29 years and who also changed their snuff habits. "Snuff" is defined as Swedish moist snuff, in the loose or portion-bag packed form, in this study.	<u>Distribution of Oral Mucosal Lesion Severity</u> Group 1 (increased mitotic rate and cell density, and loss of cohesion, n=7) *Initial* ▪ Degree 3: 57.1% (4/7) ▪ Degree 4: 42.9% (3/7) *At follow-up (4 quit, 2 reduced habit, 1 continued unchanged)* ▪ Degree 0: 57.1% (4/7) ▪ Degree 1: 28.6% (2/7) ▪ Degree 3: 14.3% (1/7) Those who quit had normal tissue at re-biopsy. Abnormal histopathology remained only in the individual who did not change his habit. Group 2 (increased mitotic rate and cell density, n=20) *Initial* ▪ Degree 2: 5.0% (1/20) ▪ Degree 3: 85.0% (17/20) ▪ Degree 4: 10.0% (2/20) *At follow-up (11 quit, 9 reduced habit)* ▪ Degree 0: 55.0% (11/20) ▪ Degree 1: 15.0% (3/20) ▪ Degree 2: 25.0% (5/20) ▪ Degree 3: 5.0% (1/20) Those who quit had normal tissue at re-biopsy. Abnormal histopathology (few mitoses but no increase in cell density) was seen in only 1 individual (had reduced his habit).	The authors concluded that tissue changes, clinically and histologically, were reversible following cessation of snuff use. The authors also noted that none of the initially abnormal findings (increased mitotic rate, increased cell density, loss of cell cohesion) represented dysplasia since dysplasia is not considered reversible. Based on the initial findings, the 29 loose snuff users were arbitrarily subdivided into four subgroups for re-biopsy analysis. At follow-up, 69% (20/29) of subjects and 60% (3/5) of comparison subjects changed their habit, either by quitting, changing to portion bags, or changing the mucosal placement of the snuff. Reversibility was found in 69% of subjects and 60% of comparison subjects.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Larsson et al. 1991 (continued) Sweden		Group 3 (increased cell density only, n=1) *Initial* ▪ Degree 2: 100% (1/1) *At follow-up (continued unchanged)* ▪ Degree 2: 100.0% (1/1) Abnormal histopathology remained. Group 4 (increased cell density and loss of cohesion, n=1) *Initial* ▪ Degree 2: 100% (1/1) *At follow-up (quit)* ▪ Degree 0: 100.0% (1/1) Normal tissue and no abnormal histopathology at follow-up. Controls (no abnormal histopathology initially, n=5) *Initial* ▪ Degree 3: 100% (5/5) *At follow-up (3 either quit, changed to portion bags, or changed quid placement, 2 changed habits only slightly)* ▪ Degree 0: 60% (3/5) ▪ Degree 3: 40% (2/5) Normal tissue in 3 whom either quit, changed to portion bags, or changed quid placement. All cases that discontinued their snuff habit exhibited normal mucosa at re-biopsy. Of the seven that reduced their use of snuff, all showed reduced epithelial changes.	

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Martensson 1978 Sweden [Translated from Swedish] This study is a case series of 10 oral tobacco users with changes in the oral mucosa.	Cross-Sectional study In the department of "Tooth and Jaw Diseases" of the Karolinska Hospital, Sweden, the author examined 10 male patients (ages 26-80) who were snuff or chewing tobacco users with changes in their mucous membrane. The author states he "recently" examined these patients; therefore, presumably the examinations took place just prior to 1978. Mucous membrane lesions were excised and examined by a pathologist. "Snuff" and chewing tobacco are described as being made up of finely ground tobacco with between 2 and 5% nicotine. All 10 patients were "pure" users of snuff or chewing tobacco who had never smoked. Patients reported using tobacco or snuff for several years.	Clinical observation revealed thickened and pleated mucous membranes that were colored gray-white and occasionally somewhat brownish. Pathological examination of excised material from the lesions revealed changes ranging from hyperkeratosis to more or less atypical phenomena. One case was of an 80-year old man with squamous cell carcinoma of the gums of the upper jaw. This patient had used snuff tobacco in precisely that location for many years. The patient also had a partial dental prosthesis, deficient oral hygiene, and laryngeal cancer with glandular metastases in the throat.	The author makes no specific conclusions about the ability of snuff to cause oral mucosal lesions. The author points out, however, that in all patients the expressed changes in the mucous membranes were located where the chewing portion was placed.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Mornstad et al. 1989 Sweden The authors investigated the influence of habits of snuff dipping and different brands of wet snuff on the clinical appearance of snuff dipper's lesion in Swedish users. [This study contains individuals identified in the study population described by Axell 1976.]	Cross-Sectional study The individuals in this study were drawn from an epidemiological survey of oral mucosal lesions in 20,333 individuals from a region 100 kilometers west of Stockholm (Axell 1976). In that study, around 1,600 individuals were identified as snuff dippers and 1,466 individuals had snuff dipper's lesions (1,459 males and 7 females). The female users were excluded from this study. Snuff dipper's lesion was diagnosed when the oral mucosal lesion was found at the site of snuff use. Lesions were clinically graded for severity (Degree 1 through Degree 4). At least 10 different brands of snuff were used by the participants. The brands Ettan, Grovsnus, or Roda Lacket made up 94.2% of total usage.	<u>Distribution of Oral Mucosal Lesion Severity</u> (Derived from cross-tabulation of age versus severity of lesion.) Degree 1: 14.4% (208/1449) Degree 2: 29.2% (423/1449) Degree 3: 51.5% (746/1449) Degree 4: 5.0% (72/1449) Severity of lesions was positively correlated with longer years of use, higher daily amounts of snuff used, greater contact time between snuff and the oral mucosa, and to some extent with the age of the snuff user (up to 74 years). While younger users consumed more snuff, older users held snuff in the mouth for longer periods. 77.3% used the snuff in one mouth location, while 22.7% changed locations, which resulted in less severe lesions (but this was not statistically significant). Among the 3 most commonly used brands, Ettan brand snuff caused more severe lesions than Roda Lacket or Grovsnus. There was no statistically significant difference in severity between the Roda Lacket and Grovsnus brands.	The authors concluded that mucosal lesion severity is correlated with years of use, amount used, the time the quid is in contact with the mucosa, to some extent age, and the brand of snuff used. The authors state that it is still unknown which ingredients in snuff are responsible for tissue injuries, although there appears to be a correlation between pH, severity of lesion, and subjective feeling in the mouth. Only Ettan, Grovsnus, and Roda Lacket were tested for relationships between snuff brands and clinical appearances.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Roosaar et al. 2006 Sweden The purpose of this study was to document the natural course of snus-induced lesions (SILs) over several decades, with particular emphasis on the development of oral cancer.	Clinical follow-up study Subjects were 1,115 men who were identified in as having SILs in 1973-1974 during a population-based prevalence survey of oral mucosal lesions among 20,333 Swedish adults. They were followed for 27-29 years through linkage to death, population, migration and cancer registries. At study entry, information was obtained on type of tobacco used at entry and in the past, including quantities and brands. In 1993, a sample of the men (n=183) was selected for repeat interviews and clinical re-examination (performed by a single examiner who knew that the participant had an SIL in 1973-1974, but was unaware of the degree and site). Existing lesions were graded from 1 (superficial; no obvious thickening) to 4 (heavily wrinkled/thickened). A standardized incidence ratio was estimated for oral cancer, with the expected number of cancers calculated by multiplying the observed person-time in age, sex, and calendar year strata by cancer incidence rates in comparable strata of the Swedish population. "Snuff" is defined in this study as moist Swedish snus.	Three incident cases of oral cancer were observed during follow-up, corresponding to a standardized incidence ratio of 2.3 (95% CI:0.5-6.7). None of the oral cancers occurred at the site of the original SIL. Two occurred in individuals who were also daily smokers. Among men re-examined in 1993, there was a strong relationship between the current level of snus use (both hours/day and grams/day) and the severity of the lesions. The lesions reversed if snus use was discontinued, and they also tended to regress among long-time users who did not change their snus habits.	The authors concluded that oral cancers rarely occur at the site of lesions observed in the distant past. SILs are probably no more than markers of current or recent snus consumption. The authors speculated that the regression of SILs over time among men who had not decreased their snus use could reflect changes in commercially available snus over the years (e.g., the introduction of portion bags). This is the first long-term follow-up study that provides data on the course of these lesions. It provides evidence that is supportive of what has been seen in analytic studies: that use of snus is not associated with development of oral cancer at the site of SILs. This study cohort was prospective in nature, was population-based, and had a long follow-up. In addition, the follow-up of subjects through record linkage was almost complete. The subset of men who was reexamined in 1993 (n=183) was compared to the initial cohort (n=1,115) with respect to age, tobacco habits, residence, degree of lesion, and alcohol consumption. There were some minor differences (e.g., location of residence), but none that were considered to be important.

APPENDIX B
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Salonen et al. 1990 Southwestern Sweden This study is a survey of the prevalence of different oral mucosal lesions and an analysis of the relationship between identified lesions and tobacco habits.	Cross-Sectional study Subjects were randomly selected, from each age strata in the total adult population of the Northern Medical Care District of Alvsborg County in southwestern Sweden, during November 1983-December 1984 to participate in a survey and dental examination of total oral health status. From an initial group of 920 individuals who were examined, complete information from the survey of tobacco habits was available on 918 subjects (448 men and 470 women). "Snuff" is not defined in this paper. Among the 918 subjects, there were 58 men who were snuff dippers only (0 women) and 21 men who both smoked and used snuff (0 women).	A total of 63 men and 0 women had snuff dipper's lesion. The authors reported the prevalence to be 14.5% in males and 7.2% overall. The prevalence figures are reported to have been weighted to reflect a higher sampling fraction among the highest age strata. Among the 58 subjects who used only snuff, there were 92 sites with lesions described as "snuff dipper's lesion," 8 sites with excessive melanin pigmentation, and 10 sites with fibroepithelial polyps.	The authors drew no specific conclusions regarding the exclusive use of snuff and oral mucosal lesions.

Appendix C1

Descriptive Studies of Head and Neck Cancer

APPENDIX C-1
DESCRIPTIVE STUDIES OF HEAD AND NECK CANCERS AMONG SWEDISH SNUS USERS (N=2)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Ahlbom 1937 Stockholm, Sweden *(translated from German)* This study examined the relationship between three types of tobacco use (pipe smoking, cigar and cigarette smoking, and snuff and chewing tobacco in the mouth) and the location of oral cancer tumors.	Descriptive study Subjects included male patients (generally between ages 60 and 80) at the Swedish Radium Institute between 1931 and 1936 for outer oral cavity carcinomas (n=68), inner oral cavity carcinomas (n=78), lip cancer (n=312), and "carcinomas of the pharynx, larynx and esophagus" (n=87). Most subjects had consumed tobacco continuously for a prolonged period (30-40 years). "Snuff" is not defined in this paper. "Snuff and chewing tobacco in the mouth" was reported by 70% of outer oral cavity carcinoma cases, 28% of inner oral cavity carcinoma cases, and 37% of the lip cancer cases.	<u>Distribution of Tobacco Habits by Cancer Site in Male Patients</u> **Carcinoma of the outer oral cavity (98% of 68 cases used tobacco)** Snuff and chewing tobacco: 70% Pipe smokers: 23% Cigar and cigarette smokers: 7% **Carcinoma of the inner oral cavity (96% of 78 cases used tobacco)** Snuff and chewing tobacco: 28% Pipe smokers: 35% Cigar and cigarette smokers: 39% **Lip cancer (86% of 312 cases used tobacco)** Snuff and chewing tobacco: 37% Pipe smokers: 57% Cigar and cigarette smokers: 6% **Carcinomas of the pharynx, larynx, and esophagus (99% of 87 cases used tobacco)** Snuff and chewing tobacco: 16% Pipe smokers: 20% Cigar and cigarette smokers: 64%	**The author stated that chewing tobacco was a relatively larger factor than pipe smoking for lip carcinoma in this group of subjects. The author made no specific conclusions regarding snuff use and cancers at other sites.** The author refers to the "predisposing effect of chewing tobacco. . .", and states that, "in most cases the tobacco or snuff was in the same place in the mouth every day, at times even during the night, for a period of 30-40 years." In at least 70% of the cases, the carcinomas developed at the exact location where the tobacco had been placed. Numerous other risk factors were considered in this paper. The author concluded that outdoor work, poor oral hygiene, tooth decay and pyorrhea alveolaris were important contributing causes for lip and oral cavity cancer. Heavy tobacco and alcohol consumption and syphilis were important predisposing factors for squamous cell carcinoma in the upper aerodigestive tract in men.

APPENDIX C-1
DESCRIPTIVE STUDIES OF HEAD AND NECK CANCERS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Axell et al. 1978 Sweden This study examined the relationship between snuff and tobacco use and location of oral cancer tumors. [Additional histologic details for 23 cases is provided in Sundström et al. 1982.]	Descriptive study 950 cases of squamous cell carcinoma of the oral cavity (excluding salivary glands, tongue, and floor of mouth) were identified from the cancer records of the National Board of Health and Welfare for the years 1962 through 1971. After limiting the analysis to males whose medical records were available for examination, 375 cases remained. The records were examined for information on tobacco habits (ongoing snuff user, earlier snuff habit, snuff-taking denied, alternative tobacco habit, and no information about tobacco habit), the usual placement of snuff in the mouth, and tumor location (documented, probable, or improbable correspondence with usual site of snuff placement). "Snuff" was defined as Swedish snuff in this paper. There were 49 ever-users of snuff.	Information about tobacco habits was found in the medical records of 176 cases (47% of the total cases). Records indicated that 49 of the oral cancer cases had "ongoing or earlier" snuff habits; in 33 of these cases, there was "documented or probable" correspondence between the location of snuff placement in the mouth and the location of the cancer. Percentages of cancer cases with a "documented" or "probable" association with region where snuff is usually placed: ▪ 67.3% for verified snuff users ▪ 16.7% for those who denied snuff use ▪ 6.4% for smokers of cigarettes, pipes and cigars ▪ 14.6% for who stated no tobacco use.	**The authors concluded that snuff is a factor contributing to the occurrence of cancer on and around the forward-facing surfaces of the alveolar ridge in the oral cavity's frontal parts. However, the risk for the individual snuff taker of getting oral cancer as a consequence of his snuff usage is very slight.** The authors estimate that the incidence rate of oral cavity cancer is about 0.5 cases per 100,000 male snuff takers per year in Sweden. By comparison, the risk of lung cancer is about 60-70 per 100,000. Thus, from a cancer standpoint, the authors concluded that snuff use should be regarded as a considerably less risky tobacco habit than smoking. The authors stated that the proportion of snuff-related tumors increased with increasing age, with the largest number of oral cancer cases occurring in those aged 71 to 80 years.

Appendix C2

Case-Control Studies of Head and Neck Cancer

APPENDIX C-2
CASE-CONTROL STUDIES OF HEAD AND NECK CANCERS AMONG SWEDISH SNUS USERS (N=4)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Lagergren et al. 2000 Sweden This study investigated the role of tobacco smoking, alcohol intake, and use of oral snuff in the etiology of head and neck cancer. Results on adenocarcinoma of the gastric cardia are presented in Appendix E-1.	Case-control study (population-based) Cases were patients from the population of Sweden who were newly diagnosed with esophageal adenocarcinoma (n=189) or esophageal squamous cell carcinoma (n=167) between 1995 and 1997. Controls were 820 individuals randomly selected from age and sex strata to resemble the age and sex distribution among the esophageal adenocarcinoma subjects.	<u>Oral Snuff Usage</u> **Esophageal adenocarcinoma** Never used Ever used **Esophageal squamous-cell carcinoma** Never used Ever used <u>Duration of Usage</u> **Esophageal adenocarcinoma** 1-10 years 11-25 years >25 years *p for trend=0.31* **Esophageal squamous-cell carcinoma** 1-10 years 11-25 years >25 years *p for trend=0.18*	<u>Odds Ratios (95% CI)</u> 1.0 (reference) 1.2 (0.7-2.0) 1.0 (reference) 1.4 (0.9-2.3) 0.9 (0.4-2.2) 0.8 (0.3-1.8) 1.9 (0.9-4.0) 1.2 (0.5-2.5) 0.9 (0.4-2.1) 2.0 (0.9-4.1)	**The authors concluded that there was no statistically significant association between snuff dipping and the risk of either type of esophageal tumor.** Snuff users were defined as those taking a quid of snuff at least once per week for 6 months or more. Odds ratios were adjusted for age, gender, tobacco smoking, alcohol use, education level, body mass index, reflux symptoms, intake of fruit of vegetables, energy intake, and physical activity. The authors state that those using 15-35 quids per week experienced a statistically significant 2-fold increase in the risk of esophageal adenocarcinoma when compared to never-users; however, the lower confidence interval is not greater than 1.0 and therefore does not meet the definition of statistical significance. In this study, neither tobacco smoking nor alcohol consumption was found to be linked to esophageal adenocarcinoma, but both (particularly hard liquor) appeared to be strong risk factors for esophageal squamous cell carcinoma. The Swedish snuff used in this study is produced through a heat processing system instead of fermentation. The authors note that fermentation may increase the concentration of tobacco-specific carcinogens and therefore these results may not be generalizable to all types of snuff or smokeless tobacco.
			1.9 (0.9-4.1) *p for trend=0.18*	

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX C-2
CASE-CONTROL STUDIES OF HEAD AND NECK CANCERS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Lagergren et al. 2000 (continued)		Intensity of Usage **Esophageal adenocarcinoma** 1-14 quids/week 15-35 quids/week >35 quids/week **Esophageal squamous-cell carcinoma** 1-14 quids/week 15-35 quids/week >35 quids/week	Odds Ratios (95% CI) (continued.) 1.0 (0.4-2.3) 2.0 (1.0-4.3) 0.8 (0.3-2.0) *p for trend=0.53* 1.2 (0.5-2.5) 2.1 (1.0-4.4) 1.0 (0.4-2.4) *p for trend=0.27*	

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX C-2
CASE-CONTROL STUDIES OF HEAD AND NECK CANCERS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Lewin et al. 1998 Southern Sweden This study investigated the role of tobacco smoking, alcohol intake, use of moist oral snuff, dietary factors, occupational exposures, and oral hygiene in the etiology of head and neck cancer.	Case-control study (population-based) Cases were 545 men (40-79 years old) included in population registries with incident cancer of the head and neck (squamous cell carcinoma of the oral cavity, oro- and hypopharynx, larynx, and esophagus). Cases lived in the Stockholm county or southern healthcare region of Sweden from January 1988 through January 1990. Controls were 641 randomly selected men stratified by region (Stockholm and the southern region) and age (40-54 yrs, 55-64 yrs and 65-79 yrs). Referents were selected from continuously updated registers of the base population. "Snuff" was defined as moist oral snuff in this paper. 83 cases and 91 controls reported "ever-use" of snuff. Ever-users were those who had ever regularly used 1 package (50 grams) per week; current users were those who used snuff 1 year prior to the time of interview.	Head and Neck Cancer **Oral snuff usage** Never used Ever used Current users Ex-users **Age at start** Never used <25 years ≥25 years **Duration of usage** Never used <30 years ≥30 years **Total consumption** Never used <125 kg ≥125 kg **Intensity of usage** Never used ≤50 g/week >50 g/week	Relative Risk Estimates (95% CI) 1.0 (reference) 1.1 (0.7-1.5) 1.0 (0.6-1.6) 1.2 (0.7-1.9) 1.0 (reference) 1.0 (0.6-1.6) 1.1 (0.7-1.8) 1.0 (reference) 1.0 (0.7-1.6) 1.1 (0.6-2.0) 1.0 (reference) 1.0 (0.7-1.6) 1.1 (0.6-2.0) 1.0 (reference) 0.8 (0.5-1.3) 1.6 (0.9-2.6)	**The authors concluded that use of Swedish oral snuff was not associated with significantly increased risk of head and neck cancer.** In this study, tobacco smoking and alcohol intake had a strong interactive effect on the risk of head and neck cancer. Relative risk estimates were adjusted for age, region of residence, alcohol use, and smoking using logistic regression analysis. Adjustment for other factors (duration of smoking, a number of dietary factors, oral hygiene) had little or no effect. None of the risk estimates for head and neck cancer associated with oral snuff usage, age at start, duration of use, total consumption, or intensity of use were statistically significant. In addition, the authors presented relative risk estimates for cancers of specific sites (oral cavity, larynx, esophagus, pharynx) associated with oral snuff use; none of these were significantly elevated. In analyses with never-users of tobacco as the reference category, some elevated risks of oral cancer were seen for ever-users and ex-users of snuff (it is unclear whether these risk estimates were adjusted for any potential confounders). The authors note that precision was very low in these analyses because the numbers of subjects was very small (9 cases and 10 controls). Cancer cases in this study included cancers of the pharynx, larynx, and esophagus, in addition to oral cancer in aggregate. When broken out by sub-site, there was no significant association between oral snuff use and increased risk of cancer of the oral cavity; the larynx; the esophagus; or the pharynx.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX C-2
CASE-CONTROL STUDIES OF HEAD AND NECK CANCERS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Lewin et al. 1998 (cont.)		Oral Snuff Usage	Relative Risks (95% CI)	
		Oral Cavity		
		Never used	1.0 (reference)	
		Ever used	1.4 (0.8-2.4)	
		Current users	1.0 (0.5-2.2)	
		Ex-users	1.8 (0.9-3.7)	
		Larynx		
		Never used	1.0 (reference)	
		Ever used	0.9 (0.5-1.5)	
		Current users	1.0 (0.5-1.9)	
		Ex-users	0.8 (0.4-1.7)	
		Esophagus		
		Never used	1.0 (reference)	
		Ever used	1.2 (0.7-2.2)	
		Current users	1.1 (0.5-2.4)	
		Ex-users	1.3 (0.6-3.1)	
		Pharynx		
		Never used	1.0 (reference)	
		Ever used	0.7 (0.4-1.3)	
		Current users	0.7 (0.3-1.5)	
		Ex-users	0.8 (0.3-1.9)	

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX C-2
CASE-CONTROL STUDIES OF HEAD AND NECK CANCERS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Rosenquist et al. 2005 Sweden This study investigated the relationship between smoking, alcohol consumption, and snuff use and oral and oropharyngeal squamous cell carcinoma (OOSCC).	Case-control study (population-based) Cases were 132 individuals (91 men) with OOSCC born in Sweden (with no previous cancer diagnosis except skin cancer) who were identified at the ENT departments of two university hospitals where almost all patients with oral cancer who live in southern Sweden are treated. Controls were 320 individuals (215 men) born in Sweden (with no previous cancer diagnosis except skin cancer) who were selected from the Swedish Population Register by stratified random sampling. Controls (3 per case) were matched to cases by age (±3 years), sex, and county. "Snuff" was defined as Swedish moist snuff in this paper. 13 cases and 31 controls were current users; 7 cases and 34 controls were ex-users. Among current snuff users, mucosal changes at the site of snuff placement were classified according to clinical severity using a 4-point scale.	Oral Snuff Use **Oral Snuff Use** Never used Current user Ex-user **Type of Snuff** Never Used Fermented Non-fermented **Duration** Never used <30 years ≥30 years **Exposure Time** Never used ≤10 hr/day >10 hr/day **Consumption** Never used 1-14 g/day >14 g/day	Odds Ratios (95% CI) 1.0 (reference) 1.1 (0.5-2.5) 0.3 (0.1-0.9)** 1.0 (reference) 0.7 (0.3-1.4) 0.6 (0.2-1.9) 1.0 (reference) 0.6 (0.3-1.3) 0.8 (0.2-2.8) 1.0 (reference) 0.7 (0.3-1.5) 0.5 (0.2-1.6) 1.0 (reference) 0.9 (0.3-2.5) 1.7 (0.5-5.7)	**The authors concluded that use of Swedish snuff is not associated with increased risk of OOSCC, probably due to its low levels of TSNAs.** Odds ratios presented were adjusted for alcohol consumption and tobacco smoking, as well as the matching characteristics of age, sex, and county. Regardless of the way snuff exposure was assessed (current or ex; duration; exposure in hours per day; or consumption in grams per day), snuff was not associated with significantly increased risk of OOSSC. All 44 subjects who currently used snuff had clinical lesions. Use of snuff for more than 10 hours per day was associated with more pronounced lesions (p=0.01), but other measures of use (amount consumed daily, duration of use, or location of quid placement) were not associated with increased severity of lesions. Thus, this study provides additional evidence that, although oral mucosal lesions are common among snuff users, they are not likely to transform to cancer. Approximately 3/4 of the snuff users were considered to have been exposed to fermented snuff, meaning that they had been snuff users prior to 1984 when the fermentation process was abolished. It appears that some of the snuff users were also smokers, as the odds ratios were adjusted for smoking. Both tobacco smoking and heavy alcohol consumption were shown to be significant risk factors for OOSCC in this study.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX C-2
CASE-CONTROL STUDIES OF HEAD AND NECK CANCERS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Schildt et al. 1998b Northern Sweden This study investigated whether Swedish moist snuff, cigarette smoking, or consumption of alcoholic beverages leads to an increased risk of oral cancer. [This study includes individuals from the same study population as Schildt et al. 1998a.]	Case-control study (population-based) Cases were 354 (117 females, 237 males) patients with histologically verified squamous cell oral cancer diagnosed in the 4 most northern counties of Sweden during 1980-1989 and reported to the Cancer Registry. After exclusions, there were 354 subjects (117 females, 237 males) in the analysis. Controls were 354 subjects (117 females, 237 males) drawn from the National Population Registry matched for age, sex, county of residence, and vital status. "Snuff" was defined as moist snuff in this paper. 67 cases and 72 controls were active or ex-users of snuff.	<u>Oral Snuff Use</u> **Oral Snuff Use** Never user Active user Ex-user Ever-user **Oral snuff use among never- smokers** Never-users of snuff Ex-users of snuff Active snuff users **Lifetime use** ≤156.0 kg >156.0 kg	<u>Odds Ratios (95% CI)</u> 1.0 (reference) 0.7 (0.4-1.1) 1.5 (0.8-2.9) 0.9 (0.6-1.4) 1.0 (reference) 1.8 (0.9-3.5) 0.7 (0.4-1.2) 0.8 (0.4-1.5) 1.1 (0.5-2.0)	**The authors stated that oral snuff was not a risk factor for oral cancer in this study.** Odds ratios presented were not adjusted for potential confounding factors, other than the matching characteristics of gender, age and county. There were few snuff users who had never smoked (42 active users and 13 ex-users had never smoked). Active snuff users did not experience any significantly increased risk regardless of smoking status. The authors state that an increased risk was found for lip cancer among ex-snuff users when this cancer was examined alone (OR=1.8, 95% CI: 0.9-3.7), but this was not statistically significant, nor did the analysis adjust for smoking. No difference in risk was found among different snuff brands used (authors do not state what these brands were). In a multivariate analysis looking at many risk factors for oral cancer, the odds ratio for snuff use was 0.8 (95% CI:0.5-1.3) after adjustment for all the other factors in the model. This analysis indicated that the most important risk factors were beer and liquor consumption, followed by light beer and smoking; however, none of these was statistically significant.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix C3

Cohort Studies of Head and Neck Cancer

APPENDIX C-3
COHORT STUDIES OF HEAD & NECK CANCER AMONG SWEDISH SNUS USERS (N=4)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Boffetta et al. 2005 Norway This study investigated the effect of smokeless tobacco on risk of cancer of the following organs: oral cavity and pharynx, esophagus, stomach, pancreas, lung, kidney, and urinary bladder. Results on pancreatic, stomach, lung, and kidney and bladder cancers can be found in appendices D, E-2, G and F respectively.	Cohort study Subjects were drawn from two sources: a systematic sample of the general adult population of Norway identified from the 1960 census, and relatives of Norwegian migrants to the U.S. Subjects provided data on lifestyle habits (including use of smokeless tobacco) in a questionnaire in 1964 and 1967. They were followed until date of diagnosis of cancer, date of emigration, date of death, or December 31, 2001, whichever occurred first. Follow-up was carried out by linkage with nationwide residence, mortality, and cancer incidence registries. These analyses are based on 10,136 men for whom data on snus use were available. 31.7% had used snus regularly: there were 1,999 regular current users; 1,216 regular former users; and 6,921 never or occasional users.	Oral Snuff Usage **Oral/Pharyngeal Cancer** Never user of snus Ever users of snus Former users of snus Current users of snus **Esophageal Cancer** Never users of snus Ever users of snus Former users of snus Current users of snus	Relative Risk (95% CI) 1.00 (reference) 1.10 (0.50-2.41) 1.04 (0.31-3.50) 1.13 (0.45-2.83) 1.00 (reference) 1.40 (0.61-3.24) 1.90 (0.69-5.27) 1.06 (0.35-3.23)	**The authors concluded that use of snus was associated with a modest, nonsignificant increase in risk of oral/pharyngeal and esophageal cancer.** Relative risks were adjusted for age and smoking of cigarettes, cigars, and pipes. The authors stated that different approaches to control for the potential confounding effect of tobacco smoking resulted in risk estimates that were similar to those reported here. This study has several weaknesses. The relative risks were not adjusted for alcohol consumption. Tobacco habits were assessed only at study enrollment, which is problematic, given the long duration of follow-up (more than 30 years). There was no information on amount or duration of snus use, so dose-response analyses were not possible.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX C-3
COHORT STUDIES OF HEAD & NECK CANCER AMONG SWEDISH SNUS USERS (N=4) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Luo et al. 2007 Sweden This study evaluated the association between oral, lung, and pancreatic cancer. Oral cancer was defined as ICD-7 codes 140, 141, 143, 144. Results on pancreatic cancer and lung cancer are presented in Appendices D and G, respectively.	Retrospective cohort study Subjects were 279,897 male Swedish construction workers who underwent regular preventive health check-ups and had at least one visit from 1978-1992, when information on smoking and snus was obtained through personal interviews with nurses. Subjects were followed until date of first cancer diagnosis, death, emigration, or December 31, 2004, whichever occurred first. Follow-up was carried out through linkage with nationwide death, emigration, and cancer incidence registries. Adjusted relative risks were derived from Cox proportional hazards regression models. Categories of use included various smoked tobacco as well as pure snuff use (type of snuff not specified, but assumed to be Swedish). Some analyses were restricted to the 125,576 men who were never-smokers at cohort entry. 31% of the subjects were current or former snus users. There were 258 cases of oral cancer (60 among never-smokers).	<u>Oral Snuff Usage</u> **Risk of Oral Cancer Among all Cohort Members** Never-users of tobacco Ever-users of snus **Risk of Oral Cancer Among 125,576 Never-Smokers** Never-users of tobacco Ever-users of snus Ex-users of snus Current users of snus Amount snus consumed 1-9 g/day ≥10 g/day	<u>Relative Risk (95% CI)</u> 1.00 (reference) 0.7 (0.5-0.9)** 1.00 (reference) 0.8 (0.4-1.7) 0.7 (0.1-5.0) 0.9 (0.4-1.8) 0.7 (0.2-2.8) 0.9 (0.4-2.0) *p for trend =0.8*	**The authors stated that there was no excess of oral cancer among snus users.** The study cohort was large, there was a high prevalence of snus use, the follow-up time was long (20 years on average), and the follow-up was almost complete. Relative risks adjusted for age and body mass index (and also for smoking among all cohort members). The authors suggest that the reduced risk of oral cancer among snus users may be due to residual negative confounding. The authors state that, with only 10 cases of oral cancer among ever-users of snus in the never-smoker stratum, risk estimates may be liable to chance variation. Tobacco habits were assessed only at study entry; changes in tobacco habits over time could influence the results. However, the authors report that 12% of 17,634 never-smoking snus users were later recorded as former or current smokers, and that 7% of 39,469 never-users of tobacco were later recorded as former or current smokers; thus they concluded that "misclassification of smoking status affected our reported estimates no more than trivially."

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX C-3
COHORT STUDIES OF HEAD & NECK CANCER AMONG SWEDISH SNUS USERS (N=4) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Roosaar et al. 2008 Sweden This study evaluated and compared the effects of snus and smoking on cancer incidence within the following 3 groups: 1) oral & pharyngeal cancer (ICD7: 140-148); 2) smoke-related cancers[1]; and 3) any cancer (ICD7: 140-209). The effect of snus on the risk of death from any cancer was also evaluated. Results on smoke-related cancers and any cancer are presented in Appendix H.	Cohort study Subjects were identified from a cohort established in 1973-74 and followed up for mortality and cancer incidence between 1973 and 2002 using national registers. Subjects were 9,860 males from Uppsala County, central Sweden, who filled out a questionnaire about tobacco and alcohol consumption, and all underwent a clinical examination of the oral cavity. 867 men (9%) were ever daily snus users (but never daily smokers), 5,309 (53%) were ever daily smokers (but never ever daily snus users) and 692 (7%) were both ever daily snus users and ever daily smokers.	**Oral Snuff and Smoking Usage** **Oral/Pharyngeal Cancer** Snus use Never daily use Ever daily use Smoking <70 years never daily use <70 years ever daily use ≥70 years never daily use ≥70 years ever daily use Restricted to never smokers Snus use Never daily use Ever daily use	**Hazard Ratio (95% CI)** 1.0 (ref) 3.1 (1.5-6.6)* 1.0 (ref) 0.5 (0.1-1.4) 1.0 (ref) 5.6 (1.6-19.6)* 1.0 (ref) 2.3 (0.7-8.3)	**The authors conclude that their results are inconsistent with claims that the use of snus is without demonstrable risk. Relative risks are consistently lower than those associated with smoking.** Models were adjusted for alcohol consumption, area of residence, calendar period and smoking or snus use. The follow up time of the cohort was long (29 years). The authors state that the residual negative confounding from smoking dose is an important concern for those who both smoke and use snus. The authors state that the snus-related relative risks for the oral & pharyngeal category was based on no more than 11 and 5 exposed cases, respectively, leaving the risk estimates liable to possible chance variation. Since tobacco habits were assessed only at study entry (1973) it is possible that these habits could have changed after inclusion into the cohort and influenced the study results. The authors concluded, however, that "since smoking is rarely taken up after age 25, the analyses that were restricted to never-smokers should not have been seriously affected by changes in smoking habits." Additionally, there was no information on the amount or duration of snus use, so dose-response analyses were not possible.

[1] including oral & pharyngeal (ICD7: 140-148), oesophageal & gastric (ICD7: 150-151), pancreatic (ICD7: 157), laryngeal and pulmonary (ICD7: 161-162), kidney, bladder & other urinary organs (ICD7: 180-181)

* denotes statistically significant increase in risk

** denotes statistically significant decrease in risk

APPENDIX C-3
COHORT STUDIES OF HEAD & NECK CANCER AMONG SWEDISH SNUS USERS (N=4) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Zendehdel et al. 2008 Sweden This study investigated the effects of tobacco smoking and snus habits on esophageal and stomach cancer incidence. Esophageal cancer (ICD7 code 150) was broken down into adenocarcinoma and squamous cell carcinoma. Stomach cancer (ICD7 code 151) was subdivided into cardia (151.1) and noncardia (all other 151) cancer. Results on stomach cancer presented in Appendix E-2.	Retrospective cohort study Subjects were 336,381 male Swedish construction workers who underwent preventative health check-ups and provided information on smoking and snus habits between 1971 and 1993. Subjects were followed until date of any diagnosis of cancer, death, emigration or December 31, 2004. Almost complete follow-up was carried out through linkage with nationwide death, emigration, and cancer incidence registries. Overall, 58% of the workers were current or former smokers at time of entry. The prevalence of snus use was 28% overall while 12% of the subjects were never-smoking snus users.	**Oral Snuff and Smoking Usage** **Esophageal Cancer** Ever-smokers Adenocarcinoma Squamous cell carcinoma Current smokers Adenocarcinoma Squamous cell carcinoma In the entire cohort: Snus users, adjusted only for BMI and attained age Adenocarcinoma Squamous cell carcinoma Snus users, additionally adjusted for smoking intensity Adenocarcinoma Squamous cell carcinoma Among never-smokers: Users of snus only Adenocarcinoma Squamous cell carcinoma	Relative Risk (95% CI) 2.3 (1.4-3.7)* 5.2 (3.1-8.6)* 2.9 (1.8-4.8)* 7.6 (4.5-12.7)* 1.0 (0.6-1.5) 1.1 (0.8-1.5) 1.0 (0.6-1.5) 1.0 (0.8-1.4) 0.2 (0.0-1.9) 3.5 (1.6-7.6)* (See Zendehdel et al. 2008 for additional analyses)	**The authors concluded that "although some uncertainty remains regarding the causality and the strength of association as well as the generalizability to other populations than Swedish men … Scandinavian snus cannot be considered to be without a carcinogenic risk." The authors also state that they "found little evidence of any net positive effect of snus use through its presumed reduction in smoking dose."** The study cohort was large, there was a high prevalence of snus use, the follow-up time was long (22.2 years on average), and the follow-up was almost complete. All relative risks were adjusted for attained age and BMI. For some analyses, the relative risks were adjusted for smoking, including smoking intensity; however there was no information on the amount or duration of snus use, so dose-response analyses were not possible. Unavailability of alcohol and lifestyle information is a serious limitation. Since tobacco habits were assessed only at study entry it is possible that these habits could have changed after inclusion into the cohort and influenced the study results. The authors confirmed that differential misclassification is a valid concern since roughly twice as many repeat visitors who reported being never-smoking snus users at study entry reported ever smoking during repeat visit(s) compared to never-users of any tobacco at study entry. The authors note, however, that this misclassification is an unlikely explanation for their findings. The analyses of some cancer subtypes for never-smoking snus users were based on small numbers (1 and 10 snus-exposed cases of adenocarcinoma and squamous cell carcinoma respectively). Chance could have played a role.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix D

Cohort Studies of Pancreatic Cancer

APPENDIX D
COHORT STUDIES OF PANCREATIC CANCER AMONG SWEDISH SNUS USERS (N=3)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Boffetta et al. 2005 Norway This study investigated the effect of smokeless tobacco on risk of cancer of the following organs: oral cavity and pharynx, esophagus, stomach, pancreas, lung, kidney, and urinary bladder. Results on oral, pharyngeal and esophageal cancer can be found in Appendix C-3 while stomach, lung, and kidney and bladder cancers can be found in appendices E-2, G and F respectively.	Cohort study Subjects were drawn from two sources: a systematic sample of the general adult population of Norway identified from the 1960 census, and relatives of Norwegian migrants to the U.S. Subjects provided data on lifestyle habits (including use of smokeless tobacco) in a questionnaire in 1964 and 1967. They were followed until date of diagnosis of cancer, date of emigration, date of death, or December 31, 2001, whichever occurred first. Follow-up was carried out by linkage with nationwide residence, mortality, and cancer incidence registries. These analyses are based on 10,136 men for whom data on snus use were available. 31.7% had used snus regularly: there were 1,999 regular current users; 1,216 regular former users; and 6,921 never or occasional users.	Oral Snuff Usage **Risk of Pancreatic Cancer Among All Snus Users Regardless of Smoking Status** Never user of snus Ever users of snus Former users of snus Current users of snus **Risk of Pancreatic Cancer Among Ever-Users of Snus According to Smoking Status** Never users of snus Never smokers Former smokers Current smokers	Relative Risk (95% CI) 1.00 (reference) 1.67 (1.12-2.50)* 1.80 (1.04-3.09)* 1.60 (1.00-2.55) Relative Risk (95% CI) 1.00 (reference) 0.85 (0.24-3.07) 1.37 (0.59-3.17) 1.86 (1.13-3.05)*	**The authors concluded that this study provides evidence that smokeless tobacco products may be carcinogenic to the pancreas. However, they also stated that the increase in risk of pancreatic cancer was restricted to current tobacco smokers.** Relative risks among all snus users were adjusted for age and smoking of cigarettes, cigars, and pipes. Relative risks among ever-users of snus according to smoking status were adjusted for age and (among current smokers) amount of tobacco smoking. The authors state that different approaches to control for the potential confounding effect of tobacco smoking resulted in risk estimates that were similar to those reported here. Residual confounding by tobacco smoking or other potential risk factors for pancreatic cancer (such as heavy alcohol intake and a diet poor in fruits and vegetables) cannot be completely ruled out. Using a model with a continuous term for amount of tobacco smoking, the relative risk of pancreatic cancer for ever use of snus was 1.66 (95% CI:1.06-2.62). This study has several weaknesses. Tobacco habits were assessed only at study enrollment, which is problematic, given the long duration of follow-up (more than 30 years). There was no information on amount or duration of snus use, so dose-response analyses were not possible.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX D
COHORT STUDIES OF PANCREATIC CANCER AMONG SWEDISH SNUFF USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Luo et al. 2007 Sweden This study evaluated the association between oral, lung, and pancreatic cancer. Pancreatic cancer was defined as ICD-7 code 157. Results on oral cancer and lung cancer are presented in Appendices C-3 and G, respectively.	Retrospective cohort study Subjects were 279,897 male Swedish construction workers who underwent regular preventive health check-ups and had at least one visit from 1978-1992, when information on smoking and snus was obtained through personal interviews with nurses. Subjects were followed until date of first cancer diagnosis, death, emigration, or December 31, 2004, whichever occurred first. Follow-up was carried out through linkage with nationwide death, emigration, and cancer incidence registries. Adjusted relative risks were derived from Cox proportional hazards regression models. Categories of use included various smoked tobacco as well as pure snuff use (type of snuff not specified, but assumed to be Swedish). Some analyses were restricted to the 125,576 men who were never-smokers at cohort entry. 31% of the subjects were current or former snus users. There were 468 cases of pancreatic cancer (83 among never-smokers).	<u>Oral Snuff Usage</u> **Risk of Pancreatic Cancer Among all Cohort Members** Never-users of tobacco Ever-users of snus **Risk of Pancreatic Cancer Among 125,576 Never-Smokers** Never-users of tobacco Ever-users of snus Ex-users of snus Current users of snus Amount snus consumed 1-9 g/day ≥10 g/day	<u>Relative Risk (95% CI)</u> 1.00 (reference) 0.9 (0.7-1.2) 1.00 (reference) 2.0 (1.2-3.3)* 1.4 (0.4-5.9) 2.1 (1.2-3.6)* 1.9 (0.8-4.3) 2.1 (1.1-3.8)* *p for trend =0.01*	**The authors stated that snus use was independently associated with increased risk of pancreatic cancer among never-smokers.** The study cohort was large, there was a high prevalence of snus use, the follow-up time was long (20 years on average), and the follow-up was almost complete. Relative risks adjusted for age and body mass index (and also for smoking among all cohort members). However, the authors did not adjust the risk estimates for pancreatitis, a recognized risk factor for pancreatic cancer. The excess risk of pancreatic cancer was seen only among never-smokers. A significant dose-response trend was seen among never-smokers. The authors stated that the apparent specificity for the pancreas as the target organ is biologically plausible. Tobacco habits were assessed only at study entry; changes in tobacco habits over time could influence the results. However, the authors report that 12% of 17,634 never-smoking snus users were later recorded as former or current smokers, and that 7% of 39,469 never-users of tobacco were later recorded as former or current smokers; thus they concluded that "misclassification of smoking status affected our reported estimates no more than trivially."

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX D
COHORT STUDIES OF PANCREATIC CANCER AMONG SWEDISH SNUFF USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Heuch et al. 1983 Norway This study investigated the effects of tobacco chewing or use of snuff on the risk of pancreatic cancer. [Updated and extended by Boffetta et al. 2005]	Prospective cohort study Subjects were 16,713 individuals from three distinct sources: a probability samples of males from the general adult Norwegian population as recorded in the 1960 census, relatives of Norwegian migrants to the U.S and male and female spouses and siblings of individuals interviewed in a case-control study of gastrointestinal cancer. Subjects provided data on lifestyle habits (including use of snuff) in questionnaires in 1964 and 1967-1968. They were followed until date of diagnosis of cancer, date of emigration, date of death, or December 31, 1978, whichever occurred first. Follow-up was carried out by linkage with nationwide residence, mortality, and cancer incidence registries. These analyses are based on 11,959 men and 2,519 women in the age interval 45-74.	<u>Oral Snuff Usage (regular use vs. never used)</u> **All cases of pancreatic cancer** Among all individuals with chewing data **Histologically-verified cases only** Among all individuals with chewing data Among men with alcohol, cigarette and chewing data Among men with alcohol, cigarette and chewing data, with adjustment for alcohol use and cigarette smoking	<u>Relative Risk (p-value)</u> 1.34 (0.21) 2.20 (0.045)* 2.31 (0.067) 2.85 (0.060)	**The authors state that their point estimates indicate that chewing of tobacco or use of snuff may be an important risk factor but that further evaluation of this relationship should wait until more data are available.** The authors do not indicate the prevalence of snus users in this cohort; however they do note that few women had been chewing tobacco or using snuff, and that the data almost fully reflect results among men only. In one subanalysis, the relative risk was adjusted for cigarette smoking and alcohol use in addition to adjustments for region, urban/rural place of residence, age and sex. This relative risk was elevated but although borderline, not statistically significant. Strengths of this study include its large sample size and prospective study design, however the number of cases available in general and to a study of the joint effects of the various risk factors was much smaller than the number of cases with information on each separate factor; limiting the reliability of adjusted relative risks.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix E1

Case-Control Studies of Stomach Cancer

APPENDIX E-1
CASE-CONTROL STUDIES OF STOMACH CANCER AMONG SWEDISH SNUS USERS (N=3)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hansson et al. 1994 Northern and Central Sweden This study examined the influence of tobacco (primarily cigarette and pipe smoking) and alcohol on the risk of gastric cancer.	Case-control study (population-based) Cases were 338 subjects with newly diagnosed, histologically confirmed gastric cancer. Cases included males and females between ages 40-79, born in Sweden, and living in one of 5 counties from February 1989 through January 1992. Controls were 679 randomly selected subjects obtained from continuously updated population registries and frequency matched to cases by age and gender (approximately 2 controls for each case). "Snuff" is not specifically defined in this paper. The exact number of snuff users was not presented.	Snuff Dipping	Odds Ratio (95% CI) 0.70 (0.47-1.06) *(Reference group was not specified.)*	**The authors found no statistically significant association between snuff dipping and risk of gastric cancer.** The number of snuff users is not explicitly stated in the paper, although the authors state that there were 50 cases and 82 controls who had never smoked cigarettes but who used other kinds of tobacco (smoking cigars or pipes, chewing snuff or tobacco). The odds ratio for gastric cancer associated with snuff dipping was adjusted for age, gender, socio-economic status, vegetable intake, and other tobacco use. No details on snuff use (quantity, frequency, etc.) were provided in this paper.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX E-1
CASE-CONTROL STUDIES OF STOMACH CANCER AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Lagergren et al. 2000 Sweden This study investigated the role of tobacco smoking, alcohol intake, and use of oral snuff in the etiology of head and neck cancer. Results on esophageal cancer are presented in Appendix C-2.	Case-control study (population-based) Cases were patients from the population of Sweden who were newly diagnosed with adenocarcinoma of the gastric cardia (n=262) between 1995 and 1997. Controls were 820 individuals randomly selected from age and sex strata to resemble the age and sex distribution among the esophageal adenocarcinoma subjects.	**Gastric cardia adenocarcinoma** <u>Oral Snuff Usage</u> Never used Ever used <u>Duration of Usage</u> 1-10 years 11-25 years >25 years <u>Intensity of Usage</u> 1-14 quids/week 15-35 quids/week >35 quids/week	<u>Odds Ratios (95% CI)</u> 1.0 (reference) 1.2 (0.8-1.8) 1.0 (0.5-1.8) 1.1 (0.6-2.0) 1.1 (0.6-2.2) *p for trend =0.45* 1.2 (0.6-2.1) 1.3 (0.7-2.5) 1.3 (0.7-2.4) *p for trend=0.30*	**The authors concluded that there was no statistically significant association between snuff dipping and the risk of gastric cardia adenocarcinoma.** Snuff users were defined as those taking a quid of snuff at least once per week for 6 months or more. Odds ratios were adjusted for age, gender, tobacco smoking, alcohol use, education level, body mass index, reflux symptoms, intake of fruit of vegetables, energy intake, and physical activity. In this study, tobacco smoking significantly increased the risk of gastric cardia adenocarcinoma. The Swedish snuff used in this study is produced through a heat processing system instead of fermentation. The authors note that fermentation may increase the concentration of tobacco-specific carcinogens and therefore these results may not be generalizable to all types of snuff or smokeless tobacco.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX E-1
CASE-CONTROL STUDIES OF STOMACH CANCER AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Ye et al. 1999 Northern and Central Sweden This study examined the effects of smoking, use of smokeless tobacco, alcohol intake and risk of gastric cancer by sub-site and histologic type.	Case-control study (population-based) Cases included 561 subjects with new, histologically confirmed gastric cardia cancer (n=90) and distal stomach cancer (260 cases of intestinal type; 164 cases of diffuse type). There were 47 cases with other histologic types of cancer that were excluded from the analysis. Cases included males and females, aged 40-79, born in Sweden and living in one of 5 counties from February 1989 through January 1995. Controls were 1,164 randomly selected subjects obtained from continuously updated population registries and frequency matched to cases by age and gender (approximately 2 controls for each case). "Smokeless tobacco" included chewing tobacco and snuff. Ever-users of snuff included 192 controls, 15 cardia cancer cases, and 63 distal stomach cancer cases.	<u>Snuff Dipping</u> **Cardia cancer** Never-users Ex-users Current users Ever-users **Distal stomach cancer-intestinal** Never-users Ex-users Current users Ever-users **Distal stomach cancer-diffuse** Never-users Ex-users Current users Ever-users	<u>Odds Ratios (95% CI)</u> 1.0 (reference) 0.8 (0.3-1.9) 0.5 (0.2-1.1) 0.6 (0.3-1.2) 1.0 (reference) 0.9 (0.5-1.6) 0.8 (0.5-1.3) 0.8 (0.5-1.2) 1.0 (reference) 0.7 (0.3-1.6) 0.6 (0.3-1.2) 0.7 (0.4-1.2)	**The authors found no evidence that snuff dipping increased the risk of gastric cancer (of any sub-site or histologic type).** Users of smokeless tobacco, including chewing tobacco and snuff, were defined as those practicing the habit at least once a week for 6 months or more. Few subjects had ever chewed tobacco and none of the female subjects had ever used moist snuff. Therefore, analyses of the effects of smokeless tobacco were restricted to snuff use among males. Among gastric cardia cases, there were 9 current snuff users and 6 ex-users of snuff; among distal stomach cancer cases, there were 37 current snuff users and 26 ex-users of snuff; and among controls, there were 118 current snuff users and 74 ex-users of snuff. Odds ratios of the risk of gastric cancer at different levels, durations and frequencies of snuff use were adjusted for age, residence area, BMI, socio-economic status, and smoking. Current smokers who had ever used snuff had an OR of 1.0, significantly smaller than that for smokers who did not use snuff ($p<0.05$).

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix E2

Cohort Studies of Stomach Cancer

APPENDIX E-2
COHORT STUDIES OF STOMACH CANCER AMONG SWEDISH SNUS USERS (N=2)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Boffetta et al. 2005 Norway This study investigated the effect of smokeless tobacco on risk of cancer of the following organs: oral cavity and pharynx, esophagus, stomach, pancreas, lung, kidney, and urinary bladder. Results on oral, pharyngeal and esophageal cancer can be found in Appendix C-3 while pancreatic, lung, and kidney and bladder cancers can be found in appendices D, G and F respectively.	Cohort study Subjects were drawn from two sources: a systematic sample of the general adult population of Norway identified from the 1960 census, and relatives of Norwegian migrants to the U.S. Subjects provided data on lifestyle habits (including use of smokeless tobacco) in a questionnaire in 1964 and 1967. They were followed until date of diagnosis of cancer, date of emigration, date of death, or December 31, 2001, whichever occurred first. Follow-up was carried out by linkage with nationwide residence, mortality, and cancer incidence registries. These analyses are based on 10,136 men for whom data on snus use were available. 31.7% had used snus regularly: there were 1,999 regular current users; 1,216 regular former users; and 6,921 never or occasional users.	Oral Snuff Usage **Stomach Cancer** Never user of snus Ever users of snus Former users of snus Current users of snus	Relative Risk (95% CI) 1.00 (reference) 1.11 (0.83-1.48) 1.29 (0.87-1.91) 1.00 (0.71-1.42)	**The authors concluded that use of snus was associated with a modest, nonsignificant increase in the risk of stomach cancer.** Relative risks were adjusted for age and smoking of cigarettes, cigars, and pipes. The authors stated that different approaches to control for the potential confounding effect of tobacco smoking resulted in risk estimates that were similar to those reported here. This study has several weaknesses. The relative risks were not adjusted for alcohol consumption. Tobacco habits were assessed only at study enrollment, which is problematic, given the long duration of follow-up (more than 30 years). There was no information on amount or duration of snus use, so dose-response analyses were not possible.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX E-2
COHORT STUDIES OF STOMACH CANCER AMONG SWEDISH SNUS USERS (Continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Zendehdel et al. 2008 Sweden This study investigated the effects of tobacco smoking and snus habits on esophageal and stomach cancer incidence. Esophageal cancer (ICD7 code 150) was broken down into adenocarcinoma and squamous cell carcinoma. Stomach cancer (ICD7 code 151) was subdivided into cardia (151.1) and noncardia (all other 151) cancer. Results on esophageal cancer presented in Appendix C-3.	Retrospective cohort study Subjects were 336,381 male Swedish construction workers who underwent preventative health check-ups and provided information on smoking and snus habits between 1971 and 1993. Subjects were followed until date of any diagnosis of cancer, death, emigration or December 31, 2004. Almost complete follow-up was carried out through linkage with nationwide death, emigration, and cancer incidence registries. Overall, 58% of the workers were current or former smokers at time of entry. The prevalence of snus use was 28% overall while 12% of the subjects were never-smoking snus users.	<u>Oral Snuff and Smoking Usage</u> **Stomach Cancer** Ever-smokers Cardia Noncardia Current smokers Cardia Noncardia In the entire cohort: Snus users, adjusted only for BMI and attained age Cardia Noncardia Snus users, additionally adjusted for smoking intensity Cardia Noncardia Among never-smokers: Users of snus only Cardia Noncardia	<u>Relative Risk (95% CI)</u> 2.1 (1.5-3.0)* 1.3 (1.2-1.6)* 2.3 (1.6-3.3)* 1.4 (1.2-1.6)* 1.0 (0.7-1.3) 1.1 (1.0-1.3) 1.0 (0.8-1.4) 1.1 (1.0-1.3) 0.9 (0.4-2.0) 1.4 (1.1-1.9)* (See Zendehdel et al. 2008 for additional analyses)	**The authors concluded that "although some uncertainty remains regarding the causality and the strength of association as well as the generalizability to other populations than Swedish men … Scandinavian snus cannot be considered to be without a carcinogenic risk." The authors also state that they "found little evidence of any net positive effect of snus use through its presumed reduction in smoking dose."** The study cohort was large, there was a high prevalence of snus use, the follow-up time was long (22.2 years on average), and the follow-up was almost complete. All relative risks were adjusted for attained age and BMI. For some analyses, the relative risks were adjusted for smoking, including smoking intensity; however there was no information on the amount or duration of snus use, so dose-response analyses were not possible. Unavailability of alcohol and lifestyle information is a serious limitation. Since tobacco habits were assessed only at study entry it is possible that these habits could have changed after inclusion into the cohort and influenced the study results. The authors confirmed that differential misclassification is a valid concern since roughly twice as many repeat visitors who reported being never-smoking snus users at study entry reported ever smoking during repeat visit(s) compared to never-users of any tobacco at study entry. The authors note, however, that this misclassification is an unlikely explanation for their findings.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix F

Cohort Studies of Kidney and Bladder Cancers

APPENDIX F
COHORT STUDIES OF KIDNEY AND BLADDER CANCERS AMONG SWEDISH SNUS USERS (N=1)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Boffetta et al. 2005 Norway This study investigated the effect of smokeless tobacco on risk of cancer of the following organs: oral cavity and pharynx, esophagus, stomach, pancreas, lung, kidney, and urinary bladder. Results on oral, pharyngeal and esophageal cancer can be found in Appendix C-3 while stomach, lung, and pancreatic cancer can be found in appendices E-2, G and D respectively.	Cohort study Subjects were drawn from two sources: a systematic sample of the general adult population of Norway identified from the 1960 census, and relatives of Norwegian migrants to the U.S. Subjects provided data on lifestyle habits (including use of smokeless tobacco) in a questionnaire in 1964 and 1967. They were followed until date of diagnosis of cancer, date of emigration, date of death, or December 31, 2001, whichever occurred first. Follow-up was carried out by linkage with nationwide residence, mortality, and cancer incidence registries. These analyses are based on 10,136 men for whom data on snus use were available. 31.7% had used snus regularly: there were 1,999 regular current users; 1,216 regular former users; and 6,921 never or occasional users.	<u>Oral Snuff Usage</u> **Kidney Cancer** Never user of snus Ever users of snus Former users of snus Current users of snus **Bladder Cancer** Never users of snus Ever users of snus Former users of snus Current users of snus	<u>Relative Risk (95% CI)</u> 1.00 (reference) 0.72 (0.44-1.18) 1.17 (0.63-2.16) 0.47 (0.23-0.94)** 1.00 (reference) 0.83 (0.62-1.11) 0.98 (0.66-1.47) 0.72 (0.52-1.06)	**The authors concluded that use of snus was not associated with any increase in the risk of kidney or bladder cancer.** Relative risks were adjusted for age and smoking of cigarettes, cigars, and pipes. The authors stated that different approaches to control for the potential confounding effect of tobacco smoking resulted in risk estimates that were similar to those reported here. This study has several weaknesses. The relative risks were not adjusted for alcohol consumption. Tobacco habits were assessed only at study enrollment, which is problematic, given the long duration of follow-up (more than 30 years). There was no information on amount or duration of snus use, so dose-response analyses were not possible.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix G

Cohort Studies of Lung Cancer

APPENDIX G
COHORT STUDIES OF LUNG CANCER AMONG SWEDISH SNUS USERS (N=3)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Boffetta et al. 2005 Norway This study investigated the effect of smokeless tobacco on risk of cancer of the following organs: oral cavity and pharynx, esophagus, stomach, pancreas, lung, kidney, and urinary bladder.	Cohort study Subjects were drawn from two sources: a systematic sample of the general adult population of Norway identified from the 1960 census, and relatives of Norwegian migrants to the U.S. Subjects provided data on lifestyle habits (including use of smokeless tobacco) in a questionnaire in 1964 and 1967. They were followed until date of diagnosis of cancer, date of emigration, date of death, or December 31, 2001, whichever occurred first. Follow-up was carried out by linkage with nationwide residence, mortality, and cancer incidence registries. These analyses are based on 10,136 men for whom data on snus use were available. 31.7% had used snus regularly: there were 1,999 regular current users; 1,216 regular former users; and 6,921 never or occasional users.	**Risk of All Types of Lung Cancer Regardless of Smoking Status** Never user of snus Ever users of snus Former users of snus Current users of snus **Risk of All Types of Lung Cancer Among Ever-Users of Snus According to Smoking Status** Never users of snus Never smokers Former smokers Current smokers **Risk of Lung Adenocarcinoma Regardless of Smoking Status** Never user of snus Ever users of snus Former users of snus Current users of snus	Relative Risk (95% CI) 1.00 (reference) 0.80 (0.61-1.05) 0.80 (0.54-1.19) 0.80 (0.58-1.11) Relative Risk (95% CI) 1.00 (reference) 0.96 (0.26-3.56) 0.64 (0.24-1.68) 0.68 (0.51-0.90)** Relative Risk (95% CI) 1.00 (reference) 0.83 (0.42-1.65) 0.86 (0.30-2.43) 0.81 (0.36-1.85)	**The authors concluded that use of snus was associated with no increase in the relative risk of lung cancer (all histological types and adenocarcinoma).** Relative risks among all snus users were adjusted for age and smoking of cigarettes, cigars, and pipes. Relative risks among ever-users of snus according to smoking status were adjusted for age and (among current smokers) amount of tobacco smoking. The authors stated that different approaches to control for the potential confounding effect of tobacco smoking resulted in risk estimates that were similar to those reported here. This study has several weaknesses. The relative risks were not adjusted for alcohol consumption. Tobacco habits were assessed only at study enrollment, which is problematic, given the long duration of follow-up (more than 30 years). There was no information on amount or duration of snus use, so dose-response analyses were not possible.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX G
COHORT STUDIES OF LUNG CANCER AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Bolinder et al. 1994 Sweden This study evaluated examine whether long-term exposure to smokeless tobacco is associated with excess risk of dying from cardiovascular disease. Data were also collected on mortality due to lung cancer. [Subjects were selected from the same overall study population as Bolinder et al. 1992. This paper was one of 6 papers that were the basis of Bolinder's 1997 dissertation.] Results on all cancers, cardiovascular disease, and stroke are presented in Appendices I, J-3 and K-2, respectively.	Cohort study Subjects were 84,781 Swedish male construction workers identified between 1971 and 1974, and who were alive on January 1, 1974. They were followed for cause-specific mortality (ischemic heart disease, stroke, all cardiovascular disease, lung cancer, and all cancer) from 1974 through 1985 with the aid of the Swedish National Cause of Death Register. The classification of tobacco habits was aimed at isolating subjects in groups with a single type of tobacco exposure. Smokeless tobacco users were subjects who reported only present smokeless tobacco use and no former or present smoking (n=6,297). Smokeless tobacco is not defined in this paper, but is assumed to be Swedish snus as the cohort population is Swedish men.	<u>Death due to Lung Cancer by Use or Non-Use of Smokeless Tobacco</u> **Among ages 35-54 at study entry** Nonusers Smokeless tobacco users **Among ages 55-65 at study entry** Nonusers Smokeless tobacco users	<u>Relative Risk (95% CI) of Death</u> 1.0 (reference) 1.2 (0.2-9.1) 1.0 (reference) 0.8 (0.1-3.9)	**The authors stated that no excess risk of death due to cancer was observed in smokeless tobacco users when compared to nonusers.** There were only 3 deaths from lung cancer in this study so Relative risks reported here are adjusted only for age. However the authors report that adjustment for area of domicile, BMI, blood pressure, diabetes, and history of heart symptoms and use of blood pressure medication did not affect the estimates.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX G
COHORT STUDIES OF LUNG CANCER AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Luo et al. 2007 Sweden This study evaluated the association between oral, lung, and pancreatic cancer. Lung cancer was defined as ICD-7 code 162. Results on oral cancer and pancreatic cancer are presented in Appendices C-3 and E, respectively.	Cohort study Subjects were 279,897 male Swedish construction workers who underwent regular preventive health check-ups and had at least one visit from 1978-1992, when information on smoking and snus was obtained through personal interviews with nurses. Subjects were followed until date of first cancer diagnosis, death, emigration, or December 31, 2004, whichever occurred first. Follow-up was carried out through linkage with nationwide death, emigration, and cancer incidence registries. Adjusted relative risks were derived from Cox proportional hazards regression models. Categories of use included various smoked tobacco as well as pure snuff use (type of snuff not specified, but assumed to be Swedish). Some analyses were restricted to the 125,576 men who were never-smokers at cohort entry. 31% of the subjects were current or former snus users. There were 2,216 cases of lung cancer (154 among never-smokers).	Oral Snuff Usage **Risk of Lung Cancer Among all Cohort Members** Never-users of tobacco Ever-users of snus **Risk of Lung Cancer Among 125,576 Never-Smokers** Never-users of tobacco Ever-users of snus Ex-users of snus Current users of snus **Amount snus consumed** 1-9 g/day >10 g/day	Relative Risk (95% CI) 1.00 (reference) 0.7 (0.6-0.7)** 1.00 (reference) 0.8 (0.5-1.3) 0.9 (0.3-3.0) 0.8 (0.4-1.3) 1.0 (0.5-2.1) 0.7 (0.4-1.3) *p for trend =0.2*	**The authors stated that there was no excess of lung cancer among snus users.** The study cohort was large, there was a high prevalence of snus use, the follow-up time was long (20 years on average), and the follow-up was almost complete. Relative risks adjusted for age and body mass index (and also for smoking among all cohort members). The authors suggest that the reduced risk of lung cancer among snus users may be due to residual negative confounding. Tobacco habits were assessed only at study entry; changes in tobacco habits over time could influence the results. However, the authors report that 12% of 17,634 never-smoking snus users were later recorded as former or current smokers, and that 7% of 39,469 never-users of tobacco were later recorded as former or current smokers; thus they concluded that "misclassification of smoking status affected our reported estimates no more than trivially."

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix H

Other Cancers

APPENDIX H
COHORT STUDIES OF OTHER CANCERS AMONG SWEDISH SNUS USERS (N=6)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Bolinder et al. 1994 Sweden This study evaluated examine whether long-term exposure to smokeless tobacco is associated with excess risk of dying from cardiovascular disease. Data were also collected on mortality due to all cancer and lung cancer. [Subjects were selected from the same overall study population as Bolinder et al. 1992. This paper was one of 6 papers that were the basis of Bolinder's 1997 dissertation.] Results on lung cancer, cardiovascular disease, and stroke are presented in Appendices H, J-3 and K-2, respectively.	Cohort study Subjects were 84,781 Swedish male construction workers identified between 1971 and 1974, and who were alive on January 1, 1974. They were followed for cause-specific mortality (ischemic heart disease, stroke, all cardiovascular disease, lung cancer, and all cancer) from 1974 through 1985 with the aid of the Swedish National Cause of Death Register. The classification of tobacco habits was aimed at isolating subjects in groups with a single type of tobacco exposure. Smokeless tobacco users were subjects who reported only present smokeless tobacco use and no former or present smoking (n=6,297). Smokeless tobacco is not defined in this paper, but is assumed to be Swedish snus as the cohort population is Swedish men.	<u>Death Due to All Cancers by Use or Non-Use of Smokeless Tobacco</u> **Among all subjects** Nonusers of smokeless tobacco Smokeless tobacco users **Among ages 35-54 at study entry** Nonusers Smokeless tobacco users **Among ages 55-65 at study entry** Nonusers Smokeless tobacco users	<u>Relative Risk (95% CI) Of Death</u> 1.0 (reference) 1.1 (0.9-1.4) 1.0 (reference) 1.2 (0.8-1.9) 1.0 (Reference) 1.0 (0.8-1.3)	**The authors stated that no excess risk of death due to cancer was observed in smokeless tobacco users when compared to nonusers.** The study did not examine specific types of cancer, with the exception of lung cancer, probably due to relatively small numbers of cancers (there were 96 total cancers among 6,297 Swedish smokeless tobacco users). Relative risks reported here are adjusted only for age. However the authors report that adjustment for area of domicile, BMI, blood pressure, diabetes, and history of heart symptoms and use of blood pressure medication did not affect the estimates.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX H
COHORT STUDIES OF OTHER CANCERS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Fernberg et al. 2007 Sweden The purpose of this study was to investigate the role of tobacco smoking, oral moist snuff use, and BMI on the incidence of several subtypes of leukemia and multiple myeloma (MM).	Cohort study Subjects were 336,381male construction workers in Sweden who underwent periodic preventive health check-ups. Subjects were followed from entry into the cohort (1969-1992) until emigration, death, date of cancer diagnosis, or December 31, 2004, whichever occurred first. Incidence of leukemia and MM was ascertained through the year 2004 by record linkage with nationwide cancer, migration, and death registries. Information on tobacco use was collected at the first health check-up by self-administered questionnaire or nurse interview. The mean age at entry was 34.3 years and average follow-up was 22.2 person-years. 12% of the male subjects were pure snuff dippers (defined as moist snuff). Among male snuff users, there were 4 cases of ALL; 10 of AML; 12 of CML; and 26 of MM.	<u>Oral Snuff Usage -- Men</u> **Acute Lymphocytic Leukemia** Never tobacco user Pure snuff dipper **Acute Myelogenous Leukemia** Never tobacco user Pure snuff dipper **Chronic Myelogenous Leukemia** Never tobacco user Pure snuff dipper **Multiple Myeloma** Never tobacco user Pure snuff dipper	<u>Incidence Rate Ratios (95% CI)</u> 1.0 (reference) 1.24 (0.39-4.01) 1.0 (reference) 0.81 (0.41-1.60) 1.0 (reference) 1.17 (0.60-2.28) 1.0 (reference) 0.92 (0.61-1.40)	**The authors concluded that exclusive use of snuff was not associated with increased risk of leukemia (ALL, AML, or CML) or multiple myeloma.** Analyses of snuff use were restricted to pure users of snuff. Incidence rate ratios were adjusted for age and body mass index. This study did not include cases of chronic lymphocytic leukemia. Data on tobacco use were obtained only at the first health check-up and not reassessed during follow-up. Subjects may have changed their tobacco habits during the long follow-up period.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX H
COHORT STUDIES OF OTHER CANCERS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Fernberg et al. 2006 Sweden The purpose of this study was to investigate the role of tobacco use and BMI on the development of malignant lymphomas, specifically non-Hodgkin's lymphoma (NHL) or Hodgkins disease (HD).	Cohort study Subjects were 335,612 construction workers (17,691 women) in Sweden who underwent periodic preventive health check-ups. Subjects were followed from entry into the cohort (1971-1992) until emigration, death, date of cancer diagnosis, or December 31, 2000, whichever occurred first. Incidence of NHL and HD was ascertained through the year 2000 by record linkage with nationwide cancer, migration, and death registries. The mean age at entry was 44.6 years and average follow-up was 19.1 person-years. 28% of the male subjects had ever used snuff (defined as moist snuff). Among male snuff users, there were 66 cases of NHL and 15 cases of HD; there was only 1 female who used snuff, and no cases of NHL or HD.	<u>Oral Snuff Usage -- Men</u> **Non-Hodgkin's Lymphoma** Never tobacco user Ever snuff dipper 1-30 years snuff dipping >30 years snuff dipping **Hodgkin's Disease** Never tobacco user Ever snuff dipper 1-30 years snuff dipping >30 years snuff dipping <u>Oral Snuff Usage -- Women</u> **Non-Hodgkin's Lymphoma** Never tobacco user Ever snuff dipper **Hodgkin's Disease** Never tobacco users Ever snuff dipper	<u>Incidence Rate Ratios (95% CI)</u> 1.0 (reference) 0.77 (0.59-1.01) 0.81 (0.60-1.11) 0.69 (0.41-1.15) 1.0 (reference) 0.88 (0.49-1.58) 0.70 (0.36-1.37) 3.78 (1.23-11.60)* 1.0 (reference) 1.36×10^{-15} (~0) 1.0 (reference) 8.72×10^{-16} (~0)	**The authors found no link between snuff use and risk of NHL. With respect to HD, the overall analysis did not show snuff use to be associated with significant increased risk. However, being a snuff dipper for more than 30 years was associated with significantly increased risk of HD among men.** Incidence rate ratios were adjusted for age, body mass index, and use of other tobacco categories. In this study, the outcome of NHL included chronic lymphocytic leukemia. Data on tobacco use were obtained only at the first health check-up and not reassessed during follow-up. Subjects may have changed their tobacco habits during the long follow-up period. The authors note that the novel finding of an increased risk of HD with long-term snuff dipping in men must be verified by additional studies. It was based on only 4 cases, limiting the statistical power of the finding.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX H
COHORT STUDIES OF OTHER CANCERS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Odenbro et al. 2007 Sweden This study examined whether tobacco use was associated with any of three types of melanoma, including cutaneous malignant melanoma (CMM) melanoma *in situ* (MIS), and intraocular malignant melanoma (IMM).	Cohort study Subjects were 339,802 male construction workers in Sweden who were seen at outpatient health clinics. Subjects entered the cohort with their first clinic visit (between 1971-1975 or 1978-1992). Exposure information was obtained prospectively by self-administered questionnaire and personal interviews. Subjects were followed until date of melanoma diagnosis, death, emigration, or December 31, 2004, whichever occurred first. Follow-up was carried out by linkage with nationwide death, migration, and cancer incidence registries. Categories of use included various smoked tobacco as well as pure snuff use (type of snuff not specified, but assumed to be Swedish). 70% of the subjects had ever used some tobacco product; 10% were pure snuff users. There were 96 cases of melanoma among pure snuff users.	<u>Oral Snuff Usage</u> **All Melanoma** Tobacco nonuser Pure snuff user 1-29 years ≥30 years **CMM** Tobacco nonuser Pure snuff user 1-29 years ≥30 years **MIS** Tobacco nonuser Pure snuff user 1-29 years ≥30 years **IMM** Tobacco nonuser Pure snuff user 1-29 years ≥30 years	<u>Incidence Rate Ratio (95% CI)</u> 1.00 (reference) 0.65 (0.52-0.82)** 0.71 (0.55-0.90)** 0.51 (0.27-0.98)** *p for trend <0.001* 1.00 0.63 (0.48-0.81)** 0.70 (0.53-0.92)** 0.47 (0.22-1.00) *p for trend <0.001* 1.00 0.64 (0.36-1.14) 0.67 (0.37-1.23) 0.39 (0.05-2.88) *p for trend =0.08* 1.00 1.14 (0.43-3.07) 1.17 (0.33-4.10) 1.05 (0.23-4.79) *p for trend =0.75*	**The authors concluded that snuff use was associated with decreased risk of CMM and MIS.** This study was large, the follow-up was long (22.6 years on average), and follow-up was almost complete. It appears that the tobacco use data were updated in some manner for most subjects. It would be important to update tobacco use data in a study with such a long follow-up time, as subjects may change tobacco habits over time. The risk estimates are for exclusive use of snuff only. The incidence rate ratios were adjusted for age, sunlight exposure, birth cohort, and body mass index. (The authors did not have actual data on sun exposure; instead they accounted for recreational sun exposure by adjusting for birth cohort and adjusted for occupational sun exposure by creating a sun exposure matrix.) The authors noted that the biological mechanisms behind these findings are unclear.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX H
COHORT STUDIES OF OTHER CANCERS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Odenbro et al. 2005 Sweden This study examined the effect of tobacco use on the risk of cutaneous squamous cell carcinoma (CSCC).	Cohort study Subjects were 337,311 male construction workers in Sweden who were seen at outpatient health clinics. Subjects entered the cohort with their first clinic visit. Exposure information was obtained by self-administered questionnaire. Subjects were followed until date of CSCC diagnosis, death, emigration, or December 31, 2000, whichever occurred first. Follow-up was carried out by linkage with nationwide death, migration, and cancer incidence registries. Categories of use included cigarette smoking, cigar smoking, pipe smoking, and snuff dipping. (Snuff was not specifically defined.) Snuff dippers were categorized by length of use (<30 years or ≥30 years).	Oral Snuff Usage **Snuff Usage** Nontobacco User Snuff Dipper **Years of Snuff Dipping** <30 ≥30	Incidence Rate Ratio (95% CI) 1.00 (reference) 0.64 (0.44-0.95)** 0.79 (0.46-1.38) 0.58 (0.34-0.99)**	**The authors concluded that tobacco smoking is not associated with increased risk of CSCC. Furthermore, snuff use is associated with a decreased risk of CSCC.** This study was large (337,311 subjects), the follow-up time was long (30 years), and the follow-up was almost complete. However, it is unclear whether the investigators reassessed tobacco habits after study enrollment. It would be important to do so in a study with such a long follow-up time, as subjects may change tobacco habits over time. 28% of the subjects had ever used snuff. 13% had only ever used snuff. There were 29 cases of CSCC among snuff dippers. The incidence rate ratios were adjusted for age and for all other categories of tobacco use. The authors did not have data on recreational sun exposure, and thus could not adjust their risk estimates for this important risk factor. They note that occupational sun exposure was not linked to CSCC risk in this cohort.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX H
COHORT STUDIES OF OTHER CANCERS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Roosaar et al. 2008 Sweden This study evaluated and compared the effects of snus and smoking on cancer incidence within the following 3 groups: 1) oral & pharyngeal cancer (ICD7: 140-148); 2) smoke-related cancers[1]; and 3) any cancer (ICD7: 140-209). The effect of snus on the risk of death from any cancer was also evaluated. Results on oral & pharyngeal cancer are presented in Appendix C-3.	Cohort study Subjects were identified from a cohort established in 1973-74 and followed up for mortality and cancer incidence between 1973 and 2002 using national registers. Subjects were 9,860 males from Uppsala County, central Sweden, who filled out a questionnaire about tobacco and alcohol consumption, and all underwent a clinical examination of the oral cavity. 867 men (9%) were ever daily snus users (but never daily smokers), 5,309 (53%) were ever daily smokers (but never ever daily snus users) and 692 (7%) were both ever daily snus users and ever daily smokers.	<u>Oral Snuff and Smoking Usage</u> **Smoke-Related Cancer** Snus use Never daily use Ever daily use Smoking Never daily use Ever daily use Restricted to never smokers Snus use Never daily use Ever daily use **Any cancer** Snus use Never daily use Ever daily use Smoking Never daily use Ever daily use Restricted to never smokers Snus use Never daily use Ever daily use	<u>Hazard Ratio (95% CI)</u> 1.0 (ref) 1.1 (0.8-1.4) 1.0 (ref) 2.2 (1.8-2.7)* 1.0 (ref) 1.6 (1.1-2.5)* 1.0 (ref) 1.00 (0.87-1.15) 1.0 (ref) 1.26 (1.13-1.40)* 1.0 (ref) 1.1 (0.9-1.4)	**The authors conclude that their results are inconsistent with claims that the use of snus is without demonstrable risk. Relative risks are consistently lower than those associated with smoking.** Models were adjusted for alcohol consumption, area of residence, calendar period and smoking or snus use. The follow up time of the cohort was long (29 years). The authors state that the residual negative confounding from smoking dose is an important concern for those who both smoke and use snus. Since tobacco habits were assessed only at study entry (1973) it is possible that these habits could have changed after inclusion into the cohort and influenced the study results. The authors concluded, however, that "since smoking is rarely taken up after age 25, the analyses that were restricted to never-smokers should not have been seriously affected by changes in smoking habits." Additionally, there was no information on the amount or duration of snus use, so dose-response analyses were not possible.

[1] including oral & pharyngeal (ICD7: 140-148), oesophageal & gastric (ICD7: 150-151), pancreatic (ICD7: 157), laryngeal and pulmonary (ICD7: 161-162), kidney, bladder & other urinary organs (ICD7: 180-181)

* denotes statistically significant increase in risk

** denotes statistically significant decrease in risk

Appendix I

Animal Studies of Carcinogenicity

APPENDIX I
ANIMAL STUDIES OF THE CARCINOGENIC EFFECTS OF SWEDISH SNUS (N=7)

CITATION	STUDY DESIGN	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hirsch and Thilander 1981	Six male and female Sprague-Dawley rats with surgically created test canals. Test canals were filled with 200 mg Swedish snuff twice a day, 5 days/week. Snuff remained in the test canals for an average of 6 hours for 9 months. Test groups: 2 Control 4 Swedish snuff "Snuff" was defined as Swedish snuff in this paper (brand name: Roda Lacket).	Only 6 animals were evaluated over 9 months. No tumors were identified. Mucosal lesions (hyperkeratosis; slight dysplastic lesions; hyperplasia) were present in the rats treated with snuff at 9 months.	The authors concluded that this experimental model was an effective means to study the effects of Swedish snuff on the oral mucosa. This study was designed to evaluate the applicability of an animal model involving a surgically-created test canal, not evaluate the effects of Swedish snuff.

APPENDIX I
ANIMAL STUDIES OF THE CARCINOGENIC EFFECTS OF SWEDISH SNUS (continued)

CITATION	STUDY DESIGN	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hirsch and Johansson 1983	Male and female Sprague-Dawley rats with surgically created test canals. Test canals were filled with 200 mg Swedish snuff twice a day, 5 days/week for duration of experiment. Estimated average exposure to snuff was 12 hr/day. Test duration ranged from 9-22 months (depending on test group). Test groups comprised: 15 Control 42 Swedish snuff 10 Highly alkaline Swedish snuff Animals were sacrificed after 9 months (standard Swedish snuff and control animals), 12 months (standard Swedish snuff and control animals), 18 months (control animals), or when moribund after 18-22 months of exposure (alkaline Swedish snuff animals). "Snuff" was defined as Swedish snuff in this paper (brand name: Roda Lacket).	Only one tumor of the oral cavity was observed among 67 rats. The single tumor (a squamous cell carcinoma) was observed in a rat from the standard Swedish snuff group. This tumor was detected after 8.5 months. Histopathological lesions of the oral mucosa and stomach (*e.g.*, hyperplasia and dysplasia) were observed at higher rates in test animals compared to control animals. There was no statistical analysis of the results.	The authors concluded that Swedish snuff was not carcinogenic in this animal model.

APPENDIX I
ANIMAL STUDIES OF THE CARCINOGENIC EFFECTS OF SWEDISH SNUS (continued)

CITATION	STUDY DESIGN	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hirsch et al. 1984	Female Sprague-Dawley rats with surgically created test canals. Test canals were filled with 200 mg Swedish snuff twice a day, 5 days/week for duration of experiment. Test duration was 18 months Test groups comprised: 10 Control (no Swedish snuff, no HSV-1 inoculation) 10 Swedish snuff 7 Swedish snuff + HSV-1 inoculation 7 HSV-1 inoculation "Snuff" was defined as Swedish snuff in this paper (brand name: Roda Lacket).	Squamous cell carcinoma of the oral cavity developed in 2/7 rats in Swedish snuff + HSV-1 group (no squamous cell carcinomas of the oral cavity seen in other test groups). Dysplasia observed in 3/10 rats in Swedish snuff group, 4/7 rats in Swedish snuff +HSV group (dysplasia not observed in other groups) Total number of malignant tumors + tumor-like abnormalities was statistically significantly ($p<0.05$) higher in rats exposed to Swedish snuff or Swedish snuff + HSV-1 than other groups.	The authors found that HSV-1 infection in combination with Swedish snuff exposure may be associated with development of squamous cell carcinomas of the oral cavity.

APPENDIX I
ANIMAL STUDIES OF THE CARCINOGENIC EFFECTS OF SWEDISH SNUS (continued)

CITATION	STUDY DESIGN	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hirsch et al. 1986	Female Sprague-Dawley rats with surgically created test canals. Test canals were filled with 200 mg Swedish snuff twice a day, 5 days/week for 13 months. Rats were sacrificed after 13 months (10 rats), one month later following no treatment for that period (10 rats), or 4 months after cessation of exposure (10 rats). Test groups comprised: 10 Control 30 Swedish snuff "Snuff" was defined as Swedish snuff in this paper (brand name: Roda Lacket).	Slight to moderate hyperplasia of the tongue and buccal mucosal epithelium was evident in all animals administered Swedish snuff. Mucosal lesions (slight to moderate hyperplasia and marked hyperorthokeratosis in some areas) were observed in test animals sacrificed after 13 months of exposure to Swedish snuff. The squamous epithelium showed mild focal atypia (40%) as well as focal ulcerations (20%) but the border between the stratum basale and the connective tissue was always well defined. The inflammatory reaction (mostly lymphocytic infiltrates) in the underlying connective tissue was slight (60%) or severe (40%) and a prominent fibrosis in the connective tissue was noted (100%). In rats given a treatment-free recovery period, mucosal lesions were less severe. The rats killed after 4 mos termination of the snuff exposure. exhibited only slightly hyperplastic epithelium of the gingival sulcus (70%), with	The authors found that Swedish snuff causes hyperplastic, reactive oral mucosal lesions. These lesions were less severe among rats sacrificed after a treatment-free period of 4 months, suggesting that mucosal lesions were reversible.

APPENDIX I
ANIMAL STUDIES OF THE CARCINOGENIC EFFECTS OF SWEDISH SNUS (continued)

CITATION	STUDY DESIGN	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
		little or no keratinization. The epithelial atrophy was less (30%) and only occasional ulcerations were seen	

APPENDIX I
ANIMAL STUDIES OF THE CARCINOGENIC EFFECTS OF SWEDISH SNUS (continued)

CITATION	STUDY DESIGN	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Larsson et al. 1989	Lewis and Sprague Dawley rats with surgically created test canals. Test canals were filled with 200 mg Swedish snuff twice a day, 5 days/week for duration of the experiment. Test duration was 30 months Test groups comprised: 8 SD rats – control 12 SD rats HSV-1 inoculation-only 13 SD rats Swedish snuff 15 SD rats HSV-1 inoculation + Swedish snuff 12 Lewis rats Nitroquinoline–N-oxide (NQO) 12 Lewis rats NQO + Swedish snuff Among rats exposed to NQO, the test canal was exposed to 100 mg NQO for 4 weeks before start of 30-month study. "Snuff" was not defined in this paper, but the authors did state it was a Swedish snuff.	Squamous cell carcinomas were observed in and near the test canal at the following rates: Control: 0/8 rats HSV-1 inoculation: 2/12 rats Swedish snuff: 2/13 rats HSV-1 inoculation + Swedish snuff: 1/15 rats NQO: 3/12 rats NQO + Swedish snuff: 2/12 rats There was a statistically significant increase in the total number of malignant tumors outside of oral cavity in group exposed to HSV-1 and Swedish snuff compared to other groups: HSV-1 + Swedish snuff (7 malig. tumors/15 rats) Controls (1 malig. tumor/8 rats) HSV-1 (2 malig. tumors/12 rats) Swedish snuff (3 tumors/13 rats)	The authors concluded that Swedish snuff does not promote the carcinogenicity of the tumor initiator NQO in the oral cavity. They also theorized that HSV-1 infection in combination with Swedish snuff exposure may have been associated with the development of malignant tumors outside of the oral cavity.

APPENDIX I
ANIMAL STUDIES OF THE CARCINOGENIC EFFECTS OF SWEDISH SNUS (continued)

CITATION	STUDY DESIGN	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Sand et al. 2002	Male Lewis and Sprague Dawley rats with surgically created test canals. Test canals were filled with 200 mg Swedish snuff twice a day, 5 days/week. The average exposure time was 12 hrs/day. Test groups comprised: Control Swedish snuff HSV-1 inoculation monthly HSV-1 inoculation + Swedish snuff Nitroquinoline–N-oxide (NQO) weekly for 5 weeks NQO + Swedish snuff Animals were sacrificed after approximately 23 months. "Snuff" was defined as "a commercially available Swedish brand."	The amount of countable subepithelial mast cells in the oral mucosa was significantly decreased only in the NQO group. The effect of Swedish snuff and HSV-1 was weak. Squamous cell carcinomas were observed in the head and neck region at the following rates: Control: 0/8 rats HSV-1: 2/12 rats Swedish snuff: 1/13 rats HSV-1 + snuff: 1/15 rats 4-NQO: 2/12 rats 4-NQO + snuff: 2/12 rats Dysplasia of the squamous epithelium on the lip and in the crevicular epithelium was seen in 4 rats: 1 HSV-1 1 HSV-1 + snuff 2 snuff only	The authors concluded that Swedish snuff (either alone or with HSV-1) has only minimal effects on mast cells, believed to be involved in the development of and defense against tumors. Only the carcinogenic substance NQO caused a significant decline in the mast cell population. The authors also concluded that mast cells play a role in the immunological cell defense against chemical carcinogens.

APPENDIX I
ANIMAL STUDIES OF THE CARCINOGENIC EFFECTS OF SWEDISH SNUS (continued)

CITATION	STUDY DESIGN	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Stenström et al. 2007	This study was designed to evaluate whether consumption of snus in the diet influenced the rate of development of gastric cancer in wild mice (WT) and a strain of transgenic mice (INS-GAS). INS-GAS mice are hyper-secretors of gastrin, which over time leads to gastric atrophy, intestinal metaplasia, dysplasia and gastric cancer. Mice were fed either control diets or a diet containing approximately 5-9% snus for 6 months. The investigators also evaluated whether concomitant infection with *Helicobacter pylori* (Hp) (which increases risk of gastric cancer) influenced the effect of the snus. There were six treatment groups: (1) WT, (2) WT + snus, (3) WT + snus + Hp, (4) INS-GAS, (5) INS-GAS + snus, and (6) INS-GAS + snus + Hp. After 6 months, animals were sacrificed and the investigators examined the stomach wall and intestines for histopathologic changes. "Snuff" was defined as Swedish snuff (brand name: General).	There was no gastric cancer in either the untreated WT mice or the snus-treated WT mice. Mild morphologic changes (without statistical difference) were seen in the stomachs of the snus-treated WT mice (compared to untreated). Gastric carcinoma *in situ* developed in 2 of 8 (25%) INS-GAS mice without snus consumption and 4 of 8 (50%) with snus consumption. Snus-treated INS-GAS mice had increased intestinal metaplasia, foveolar hyperplasia, oxyntic gland atrophy, epithelial defects, inflammation Hp infection markedly increased the rate of gastric carcinoma *in situ* in both the WT + snus + Hp group (9 of 17, or 53%) and the INS-Gas + snus + Hp group (12 of 12, or 100%).	The author concluded that this study supports the hypothesis that snus exposure accelerates gastric cancer development in the setting of hypergastrinemia and/or Hp infection. Few animals in some treatment groups in this study; investigators did not always indicate if differences reported were statistically significant. This study showed that consumption of snus was associated with an increase in gastric cancer among transgenic INS-GAS mice. However, this may have limited relevance to humans who use snus, as this strain of mice inevitably develops gastric cancer. The study failed to include a control group of either WT or INS-GAS mice that received only Hp infections. Thus it is not possible to draw informed conclusions about the interaction of snus use and Hp infection.

Appendix J1

Descriptive Studies of Cardiovascular Effects

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (N=11)

CITATION, LOCATION	STUDY TYPE, POPULATION, BRIEF DESCRIPTION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Bolinder 1997a This study examined the role of long-term exposure to nicotine on metabolic risk factors for cardiovascular disease in Swedish middle-aged men. [This study is Bolinder's Ph.D. dissertation; it included individuals from the same study population as Bolinder et al. 1997a, Bolinder et al. 1997b, and Bolinder and de Faire 1998.] See Appendix O-1 for results on body weight.	Descriptive study The study population included 151 healthy male firefighters aged 35-60 years. Blood samples were evaluated for biochemical cardiovascular risk factors and hematology. Biochemical parameters and other physiological indicators were used to calculate the atherogenic index, insulin resistance, and risk of future cardiovascular events. Study subjects were classified into major tobacco habit groups of smokeless tobacco users (n=29), smokers (n=33), and non-users of tobacco (n=42). Inter-group comparisons used only these three groups. The remaining subjects (n=47) included ex-tobacco users or those who had switched from one tobacco habit to the other. "Snuff" is also referred to as smokeless tobacco and is not defined in this paper, but appears to be Swedish snuff.	The atherogenic index, insulin resistance, and predicted risk of cardiovascular disease were increased but not significantly in users of smokeless tobacco compared to non users. By contrast, smokers had significantly greater values for these three indices than never-users of tobacco. Smokeless tobacco users did not differ significantly (after adjusting for potential confounders) from never-users of tobacco in any of the measured variables including serum lipids and lipoproteins, glucose and insulin, hemostatic factors, leukocytes, and hemoglobin. By contrast, smokers had a significantly different serum lipid profile, level of glucose and insulin, and hemostatic profile than never-users indicating an elevated cardiovascular risk.	**The author concluded that the risk of cardiovascular disease seems to be smaller in smokeless tobacco users than in smokers.** The authors caution, however, that the number of subjects in this study was small and that despite the lack of significant alterations in cardiovascular risk profile in smokeless tobacco users compared to never-users in this study, it can still be hypothesized that the moderate increases of most of the measured variables towards a slightly raised cardiovascular risk might reflect a truly negative influence of exposure.

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION, BRIEF DESCRIPTION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Bolinder and de Faire 1998 Sweden The goal of this study was to investigate whether the use of smokeless tobacco among healthy middle-aged men is associated with any alteration in blood pressure and heart rate during daytime and nighttime, compared with smokers and nonusers of tobacco. [This study includes individuals from the same study population as Bolinder et al. 1997a, and Bolinder et al. 1997b. This paper was one of 6 papers that were the basis of Bolinder's 1997a dissertation.]	Descriptive study The study population included 135 healthy male firefighters aged 35-60 years. Subjects received both a clinical blood pressure measurement and 24-hour ambulatory blood pressure recordings. Study subjects were classified into three major tobacco habit groups of smokeless tobacco users (n=47), smokers (n=29), and non-users of tobacco (n=59). Smokeless tobacco users in this analysis included both subjects who had never smoked but used smokeless tobacco (n=27) and ex-smokers who currently used smokeless tobacco (n=20). "Snuff" is also referred to as smokeless tobacco, and is not defined in this paper.	During ambulatory blood pressure monitoring, smokeless tobacco users (≥ 45 years old) and smokers exhibited significantly higher daytime and 24-hour systolic blood pressures compared to non-users of tobacco. The blood pressures of smokeless tobacco users showed a highly significant correlation with blood cotinine levels (the main nicotine metabolite). Heart rate (daytime and nighttime) was also significantly elevated in both smokeless tobacco users and smokers compared with nonusers.	**The authors concluded that the exposure to nicotine in smokeless tobacco causes significant effects on heart rate and blood pressure in healthy subjects. The authors speculate that long-term tobacco use may contribute to the development of sustained hypertension.** Adjustments for confounders (*i.e.*, age, BMI, waist-hip ratio, physical fitness and alcohol consumption) had no significant effect on these findings.

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION, BRIEF DESCRIPTION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Bolinder et al. 1997a Sweden This study investigated the possible influence of long-term exposure to smokeless tobacco on the atherosclerotic process in middle-aged men in Sweden. [This study includes individuals from the same study population as Bolinder et al. 1997b, and Bolinder and de Faire 1998. This paper was one of 6 papers that were the basis of Bolinder's 1997 dissertation.]	Descriptive study The study population included 143 healthy male firefighters aged 35-60 years old. Atherosclerotic development was determined using carotid ultrasonography of the right carotid artery. In addition, blood levels of biochemical risk factors for cardiovascular disease (serum lipids, serum lipoproteins, and plasma fibrinogen) were determined. Study subjects were classified into major tobacco habit groups of smokeless tobacco users who had never smoked (n=28), smokers (n=29), and never users of tobacco (n=40). Inter-group comparisons used only these three groups. The remaining subjects (n=46) included ex-tobacco users or those who had switched from one tobacco habit to the other. "Snuff" is also referred to as smokeless tobacco, and is defined in this paper as ground and moistened dark tobacco, buffered to a pH of about 8.5 with sodium carbonate.	Smokeless tobacco users did not differ significantly from never-users regarding any artery wall measurements or lumen diameters. Smokers, however, showed a statistically significant 5%-20% greater mean thickness of the carotid wall than never-users of tobacco after adjusting for age differences. Carotid plaques were not significantly increased in smokeless tobacco users (2/28; 7.1%) compared to non-users of tobacco (0/40; 0%) but were significantly increased among smokers (11/29; 37.9%; $p<0.001$). Further, the amount of cigarettes consumed per day and the number of years of smoking significantly correlated with the occurrence of plaques ($p=0.03$ and $p<0.001$, respectively). Biochemical cardiovascular risk factors showed a slight trend toward levels associated with increased risk in snuff users, but these did not differ significantly from never-users of tobacco. By contrast, smokers showed statistically significant adverse effects on the levels of all biochemical parameters associated with cardiovascular risk that were measured. There was an apparent interaction between increased serum cholesterol and smoking on the carotid intima media thickness, but this was not found to be true for smokeless tobacco users.	**The authors concluded that smokeless tobacco does not appear to be associated with an acceleration of atherosclerosis similar to that observed in smokers. The authors also concluded that the data did not support an ability of smokeless tobacco to aggravate atherogenesis in individuals with raised levels of cardiovascular risk factors in a manner similar to that seen in smokers.**

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION, BRIEF DESCRIPTION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Bolinder et al. 1997b Sweden This study examined the influence of long-term nicotine exposure on clinical measures of physical fitness and cardiovascular response. [This study includes individuals from the same study population as Bolinder et al. 1997a and Bolinder and de Faire 1998. This paper was one of 6 papers that were the basis of Bolinder's 1997 dissertation.]	Descriptive study The study population included 144 healthy male firefighters aged 35-60 years. Heart rate, blood pressure, and oxygen uptake at rest and during exercise at gradually increasing workloads were determined. Study subjects were classified into major tobacco habit groups. The study included smokeless tobacco users (n=48), smokers (n=31), and non-users of tobacco (n=65). Smokeless tobacco users in this analysis included those who had previously smoked but had switched to smokeless tobacco. The smokeless tobacco users had used this product for a median of 24-25 years. "Snuff" is not defined in this paper, but appears to refer to Swedish snuff.	In smokeless tobacco users, no significant differences were observed for maximal oxygen uptake or maximal work compared with non-users. Further, no significant relationship was seen between the quantity of smokeless tobacco used and maximal workload. In smokers, both maximal workload and oxygen uptake were significantly lower (i.e., clinically worse) by approximately 15% compared with non-users. In addition, smokers showed a significant negative relationship between the amount of tobacco used and maximal workload. Use of smokeless tobacco < 2 hours prior to the test led to a heart rate on average 6 beats/min. higher, a systolic blood pressure 10-15 mmHg higher, and a diastolic blood pressure 6 mmHg higher, than was found in those who had their last intake of smokeless tobacco > 2 hours prior to the test. These differences were seen both at rest and at work, but were not always statistically significant and did not affect the achieved level of maximal oxygen uptake or workload.	**The authors concluded that long-term use of smokeless tobacco does not significantly influence exercise capacity in healthy, physically well-trained subjects. The authors also concluded that nicotine exposure does not appear to be of major importance in reducing physical performance in healthy subjects.** The authors speculate that acute nicotine exposure is likely to explain the higher heart rate and blood pressure in individuals exposed to smokeless tobacco < 2 hours before exercise testing when compared to those not recently exposed. Statistical analyses were adjusted for age, BMI, waist/hip ratio, alcohol consumption, level of physical training and physical demands of the job.

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION, BRIEF DESCRIPTION	RESULTS		AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Bolinder et al. 1992 Sweden The aim of this study was to investigate the relationship between tobacco consumption habits and general health status. [This study includes individuals from the same study population as Bolinder et al. 1994**.** This paper was one of 6 papers that were the basis of Bolinder's 1997 dissertation.] Data on gastrointestinal and body weight effects observed in this study are summarized in Appendices L-1 and O-1 respectively.	Descriptive study (cross-sectional study) Subjects in this population survey were 97,586 male construction workers (16-65 years of age) who received health examinations during 1971 through 1974. Physical examinations included blood pressure and heart rate measurements and included a questionnaire about tobacco use and health status. Information was also acquired on sick leave and the allocation of disability pensions. Of the 97,586 subjects examined, 59,864 were excluded because of use of more than 1 type of tobacco product or because they were ex-smokers. The remaining subjects (n=37,722; 1,370 of whom were disability pensioners) were grouped for analysis by tobacco habit: non-users (n=23,885), smokeless tobacco users who had never been regular smokers (n=5,014), and smokers of = 15 cigarettes per day who had never been regular users of smokeless tobacco (n=8,823). "Snuff" is referred to as smokeless tobacco, and is defined as mainly moist snuff in this paper.	<u>Health Hazard Evaluated By Use Of Smokeless Tobacco</u> A number of endpoints associated with cardiovascular disease were significantly more prevalent among the smokeless tobacco users than in non-users of tobacco. <u>Measured Effect of Cardiovascular Disease Risk Factors for Ages 45-55</u> **Cardiovascular diagnosis** Non-user Smokeless tobacco **Hypertension** Non-user Smokeless tobacco **Diastolic BP>90** Non-user Smokeless tobacco **Systolic BP>160** Non-user Smokeless tobacco <u>Measured Effect of Cardiovascular Disease Risk Factors for Ages 56-65</u> **Cardiovascular diagnosis** Non-user Smokeless tobacco	<u>Odds Ratios (95% CI):</u> 1.0 (reference) 1.6 (0.7-3.5) 1.0 (reference) 3.0 (1.9-4.9)* 1.0 (reference) 1.8 (1.5-2.1)* 1.0 (reference) 1.7 (1.3-2.1)* 1.0 (reference) 1.5 (1.1-1.9)*	**The authors concluded that an increased cardiovascular risk is associated with the use of smokeless tobacco. They also note that the most significant result of this study was that there was a higher prevalence of elevated blood pressure (diastolic > 90 mmHg, systolic > 160 mmHg) among smokeless tobacco users, compared to both smokers and non-users.** The authors also note that the higher risk of early retirement due to cardiovascular disease or hypertension among smokeless tobacco users supports the view that nicotine might have an important role in causing cardiovascular damage or hypertension, but caution that the number of cases of disability attributed to hypertension may be too small to be conclusive.

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION, BRIEF DESCRIPTION	RESULTS		AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Bolinder et al. 1992 (continued)		**Diastolic BP>90**	1.0 (reference)	
		Non-user	1.3 (1.1-1.4)*	
		Smokeless tobacco		
		Systolic BP>160	1.0 (reference)	
		Non-user	1.2 (1.1-1.4)*	
		Smokeless tobacco		
		Measured Effect of Cardiovascular Disease Symptoms		
		Breathlessness on slight effort	1.0 (reference)	
		Non-user	1.4 (1.3-1.6)*	
		Smokeless tobacco		
		Chest pain Walking up hill	1.0 (reference)	
		Non-user	1.2 (1.1-1.4)*	
		Smokeless tobacco		
		Pain in the leg while walking	1.0 (reference)	
		Non-user	1.3 (1.1-1.5)*	
		Smokeless tobacco		
		White finger symptoms	1.0 (reference)	
		Non-user	1.4 (1.3-1.6)*	
		Smokeless tobacco		
			* Denotes statistically significant increase in risk.	

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION, BRIEF DESCRIPTION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Eliasson et al. 1995 Northern Sweden This study examined the influence of cigarette smoking and use of smokeless tobacco on potential cardiovascular risk factors. See Appendix O-1 for results on body weight.	Descriptive study Subjects included 1,583 participants of the MONICA study (Monitoring Trends and Determinants in Cardiovascular Disease), who were selected from a group of 2000 (1000 men and 1000 women) aged 25-64 years. Between January 1990 and April 1990 subjects underwent blood sampling for plasma fibrinogen levels and fibrinolytic activity (tissue plasminogen activator [tPA] activity and plasminogen activator inhibitor type 1 [PAI-1] activity). A subset of these subjects (n=754) underwent oral glucose tolerance testing. Subjects were classified into five categories of tobacco use. Snuff dippers were defined as regular users of moist snuff who did not use other types of tobacco (n=92 men and 12 women). The women snuff dippers were excluded from this analysis. "Snuff" is also referred to as smokeless tobacco, and is defined in this paper as a form of moist oral snuff.	Snuff dipping did not significantly affect fibrinogen levels, tPA activity, PAI-1 activity, fasting glucose levels, or insulin levels in response to a glucose challenge. Current smokers had a significantly higher level of plasma fibrinogen when compared to snuff dippers ($p<0.001$).	**The authors concluded that the use of smokeless tobacco, as moist oral snuff, did not appear to affect fibrinogen levels, fibrinolytic activity or insulin levels.** The authors speculated that if a high fibrinogen level mediates the atherothrombotic effects of smoking, then the failure of smokeless tobacco to raise fibrinogen levels implies that smokeless tobacco carries less risk for cardiovascular events.

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION, BRIEF DESCRIPTION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Eliasson et al. 1991 Sweden This study evaluated cardiovascular risk factors among healthy young males who were habitual snuff-users, and compared them with the same risk factors in non-tobacco users and cigarette smokers.	Descriptive study This study used young male volunteers recruited from university students, teachers, and blue-collar workers. All subjects were ≤ 31 years old and weighed ≤ 28 kg. All subjects underwent a physical exam (including blood pressure, blood chemistry, and hematology) completed a questionnaire about habits. All testing was completed after an overnight fast and abstention from tobacco and abstention from alcohol for 24 hours. Subjects included never-users of tobacco (n=18), users of at least 50 g of moist snuff per week for 2 years (n=21; 5 of whom were ex-smokers), and smokers of at least 10 cigarettes per day for 2 years (n=19; 1 of whom had used snuff previously). "Snuff" is also referred to as smokeless tobacco and is defined as moist oral snuff in this paper.	Serum insulin levels were significantly higher in snuff-users than in non-tobacco-users. No differences in pulse rate or blood pressure were found between snuff-users and non-tobacco-users. In addition, no difference in serum lipids or blood glucose between these two groups was detected.	**The authors concluded that the use of smokeless tobacco in the form of moist snuff does not appear to have any significant impact on cardiovascular risk factors in healthy young men, with the possible exception of elevated serum insulin levels. They also note higher fibrinogen levels among snuff users, although significance was borderline.** The authors noted that considerable differences in life style were observed across the groups, with lower levels of physical activity and higher levels of alcohol and coffee consumption among tobacco users. The authors speculate that these differences may have contributed to the differences in insulin levels seen between groups. The authors also noted that the timing of use of tobacco products was not considered in this analysis, but that the low plasma nicotine levels in the tobacco-using subjects confirmed that subjects had abstained from smoking or taking snuff prior to the examination.

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION, BRIEF DESCRIPTION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Gyllerup et al. 1991 Sweden This study examined whether high mortality in cold regions of Sweden could be explained by smoking, hypertension, or fat consumption.	Descriptive study This study used national acute myocardial infarction mortality data from Swedish males aged 40-64 during the period of 1975-1984. These data were obtained from the Cause of Death Register. Information on the prevalence of snuff use among Swedish men aged 45-64 (n=1,790) came from a national survey of living conditions conducted in 1980 and 1981. "Snuff" is defined in this paper as a moist tobacco, inserted between the lip and gum. The actual number of snuff users was not reported.	No increase in the coefficient of determination for the regional temperature and acute myocardial infarction was detected when both regional temperature and snuff use were considered together. When evaluated independently, the coefficient of determination for the regional prevalence of snuff use and acute myocardial infarctions in middle-aged men was only 0.15.	**The authors concluded that the strong association between cold exposure and coronary mortality was not influenced by the regional variation in snuff use. However, the authors note that a relatively small sample was used to assess snuff use and that results obtained using this data should be interpreted cautiously.**

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION, BRIEF DESCRIPTION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hirsch et al. 1992 Sweden The goal of this study was to investigate the short-term hemodynamic effects of snuff dipping during rest and dynamic exercise in healthy habitual users of oral snuff.	Descriptive study The study population included 9 healthy volunteers (8 males, 1 female) aged 25-31 years who had previous experience with oral snuff. Subjects refrained from snuff use for 9 hrs prior to the experiment. After using snuff, heart rate, blood pressure, and stroke volume were measured. All subjects had "previous experience" with oral snuff; all but one were habitual users. A commercial brand of Swedish snuff was used in this study.	Both systolic and diastolic blood pressure were markedly increased after snuff intake while at rest. Heart rate increased approximately 25% 15-30 minutes after snuff administration. After the dynamic exercise test, heart rate, but not blood pressure, was increased when comparing snuff intake with no snuff. Initially, blood pressure (but not heart rate) was significantly higher after snuff at the start of the isometric exercise. The heart rate response to isometric exercise was slightly more pronounced after snuff, whereas the differences in blood pressure tended to disappear.	**The authors concluded that snuff intake is associated with significant short-term hemodynamic effects during rest, but not exercise.** There was no adjustment for possible confounding factors.

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION, BRIEF DESCRIPTION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Wallenfeldt et al. 2001 Sweden The study examined the association between smokeless tobacco use, smoking, cardiovascular risk factors, inflammation and ultrasound-assessed measures of atherosclerosis in the carotid and femoral arteries. See Appendix O-1 for results on body weight.	Descriptive study (cross-sectional) Subjects were 391 clinically healthy men of Swedish ancestry (all 58 years old), who were randomly selected from the general population. Subjects were excluded if they had cardiovascular or other clinically overt diseases, or if they were taking cardiovascular medications. Cardiovascular risk factors were assessed by biochemical analysis of blood and by ultrasonography of carotid and femoral arteries. Smoking and snuff habits were assessed by questionnaire. Present use of snuff was defined as at least one snuff-dipping per day. 48 men were current snuff users and 33 were previous snuff users. Only 4 of the 81 current or previous snuff users had never smoked. "Snuff" is also referred to as smokeless tobacco, and is described as moist snuff.	Never-users of snuff had lower serum triglyceride concentrations than previous or current snuff users (p=0.001). There were no other statistically significant relationships between snuff use and cardiovascular risk factors (cholesterol, apolipoprotein A1 or B, fasting blood glucose, plasma insulin, or C-reactive protein). There were also no associations between snuff use and ultrasound-assessed measures of atherosclerosis (intima-media thickness, or plaques in the carotid or femoral arteries). Number of snuff-years was related only to serum triglycerides and to waist-hip ratio. There was a close relation between smoking and snuff taking.	**The authors concluded that oral use of moist snuff is not associated with any signs of ultrasound-assessed atherosclerosis in the carotid or femoral arteries, or with elevated levels of C-reactive protein.** They also concluded that smokeless tobacco is associated with much less or no risk for atherosclerotic disease than tobacco smoking. This suggests that inhaled smoke, rather than nicotine itself, may be the most important etiologic factor in atherosclerosis. The authors acknowledge that no conclusions can be drawn regarding causality from this cross-sectional study.

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION, BRIEF DESCRIPTION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Wennmalm et al. 1991 Southwest Sweden The study addressed the effect of tobacco use on the formation of two eicosanoids, thromboxane A_2 and prostacyclin, which have been implicated in both acute and chronic cardiovascular disorders.	Descriptive Subjects were randomly sampled 18-19 year-old men attending a compulsory medical screening for enrollment in the Swedish national defense system. After applying a set of exclusion criteria (recent use of aspirin-like drugs, incomplete data, acute or chronic disease) to 756 initially eligible subjects, the final number of subjects included in the study was 577. Urinary excretion of the metabolites of thromboxane A_2 and prostacyclin (Tx-M and PGI-M, respectively) were analyzed and related to self-reported tobacco use. Systolic and diastolic blood pressure, maximal heart rate, and maximal working capacity were also collected. "Snuff" is defined in this paper as wet (oral) snuff. The study included 127 snuff only users who used an average of 25±1 grams of snuff per day and 377 non-tobacco users.	Snuff only users showed no difference between non-tobacco users with respect to resting systolic blood pressure, resting diastolic blood pressure, maximum heart rate, maximum workload, and excretion of catecholamines. Compared to non-tobacco users, snuff only users, despite having urinary cotinine levels comparable to those in cigarette smokers, had no increase in their urinary excretion of Tx-M. The excretion of PGI-M did not differ between snuff only users and non-tobacco users.	**The authors concluded that cigarette smoking, but not the use of snuff, facilitates the formation of thromboxane A_2.** The authors note that while the unaffected excretion of Tx-M in the snuff-only group seems to disfavor the hypothesis that nicotine can elicit platelet activation, further studies are needed to elucidate whether the differences in pharmacodynamics of tobacco constituents administered via the lungs and via the gastrointestinal tract may explain the discrepancy in Tx-M excretion between smokers and snuff users.

Appendix J2

Case-Control Studies of Cardiovascular Diseases

APPENDIX J-2
CASE-CONTROL STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (N=4)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hergens et al. 2005 Sweden This study assessed whether long-term use of snus increased risk of first-time acute MI in men. See Appendix O-3 for results on body weight and Appendix M-4 for results on diabetes.	Case-control study (population-based) Cases were 1,760 male patients with a first acute MI drawn from two methodologically equivalent case-control studies using identical questionnaires: a study consisting of Swedish men aged 45 to 70 years living in Stockholm County from 1992 to 1993, and a study of men aged 45 to 65 years living in Västernorrland County from 1993 to 1994. 1,432 of these cases provided data on tobacco use (1,173 nonfatal and 259 fatal) Controls consisted of 1,810 men randomly selected after stratification for age and hospital catchment area. "Snuff" was defined as Swedish moist snuff.	<u>Snuff Use</u> **All Cases** Never Former Current **Nonfatal Cases** Never Former Current **Fatal Cases** Never Former Current	<u>Odds Ratio (95% CI)</u> 1.00 (reference) 1.1 (0.78-1.5) 0.98 (0.77-1.3) 1.00 (reference) 1.1 (0.79-1.6) 0.98 (0.76-1.3) 1.00 (reference) 1.1 (0.54-2.1) 1.9 (0.65-1.6)	**The authors concluded that this study does not support the hypothesis that smokeless tobacco increases risk of MI.** Risks of MI among snuff users were also stratified by smoking status (never, former, or current). Risk of MI was not significantly elevated among any group of snuff users who had never smoked. Risk was significantly elevated only among those subjects who were former or current smokers. Odds ratios were adjusted for age, hospital catchment area, and smoking. Adjusting for diabetes, hyperlipidemia, hypertension, overweight, physical inactivity, and job strain had little impact on the risk estimates. The authors speculate that risk of MI is probably not increased by long-term exposure to nicotine, which is present in both smokeless tobacco and cigarettes. Rather, it is probably the various components of cigarette smoke (*e.g.*, carbon monoxide, oxidant gases) that have potential cardiovascular effects. They also suggest another hypothesis: that oral moist snuff contains substances such as fatty acids and flavonoids that could have a protective effect for MI.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX J-2
CASE-CONTROL STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Huhtasaari et al. 1992 Northern Sweden This study examined the risk of myocardial infarction (MI) in snuff users, cigarette smokers, and non-tobacco users in northern Sweden.	Case-control study (population-based) Cases included 585 men aged 35-64 years in the Northern Sweden MONICA Study (Monitoring Trends and Determinants in Cardiovascular Disease) with a first acute MI occurring between April 1989 and April 1991. Controls included 589 men without MI selected from a population survey of cardiovascular risk factors, who were matched by age and location to cases. "Snuff" is not specifically defined, but appears to refer to moist snuff in this paper. Regular snuff dippers were defined as non-smoking men who used snuff at least once daily. There were 146 regular snuff dippers (59 cases, 87 controls) and 104 former snuff dippers (22 cases, 82 controls).	<u>Snuff Use --Cans/Week</u> Non-users of tobacco <2 cans weekly ≥2 cans weekly <u>Snuff Dippers Vs. No Tobacco (by Age Group of Snuff Dippers)</u> Non-users of tobacco 35-54 years 55-64 years All subjects <u>Cigarette Smoking Vs. Snuff Dipping (by Age Group of Tobacco Users)</u> 35-54 years 55-64 years All subjects	<u>Odds Ratios (95% CI)</u> 1.00 (reference) 0.63 (0.41-0.98)** 0.93 (0.61-1.41) 1.00 (reference) 0.96 (0.56-1.67) 1.24 (0.67-2.30) 0.89 (0.62-1.29) 3.22 (1.82-5.70)* 1.09 (0.55-2.16) 2.09 (1.39-3.15)*	**The authors concluded that when snuff dippers were compared with non-tobacco users, the age-adjusted risk for myocardial infarction was not significantly increased in any age group. In men aged 35-54, snuff dipping was associated with a lower risk of myocardial infarction than cigarette smoking.** There was no significantly increased risk of myocardial infarction in snuff users of any age group (35-54 years, 55-64 years, 35-64 years) or consumption level (<2 cans/week or ≤2 cans/week). In comparisons between cigarette smokers and snuff dippers, cigarette smokers had a significantly higher odds ratio for myocardial infarction in the 35-54 age group (but not for the 55-64 year age group) and in all subjects regardless of age. Odds ratios were adjusted for age only.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX J-2
CASE-CONTROL STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Huhtasaari et al. 1999 Northern Sweden This study investigated whether the use of snuff affects the risk of myocardial infarction (MI).	Case-control study (population-based) Cases included 687 men ages 25-64 years in the Northern Sweden MONICA Study (Monitoring Trends and Determinants in Cardiovascular Disease) with acute myocardial infarction (fatal or non-fatal) and sudden death occurring between May 1, 1991 and December 31, 1993. Controls were 687 men with no MI selected from population registries and matched to cases on county of residence and age. "Snuff" is defined in this paper as moist snuff, which the authors state is the only form of smokeless tobacco used in northern Sweden. There were 149 current snuff users with no current smoking (59 cases, 90 controls). There were 31 current snuff users who were also current smokers (20 cases, 11 controls). There were 24 former snuff users who never smoked (11 cases, 13 controls). There were 91 subjects who were former snuff users and as well as former smokers (37 cases, 54 controls).	<u>Tobacco Use</u> **Fatal and nonfatal acute MI** Non-users of tobacco Regular use of snuff Regular smoking **Fatal acute MI only** Non-users of tobacco Regular use of snuff Regular smoking	<u>Odds Ratios (95% CI)</u> 1.00 (reference) 0.58 (0.35-0.94)** 3.53 (2.48-5.03)* 1.00 (reference) 1.50 (0.45-5.03) 8.57 (2.48-30.3)*	**The authors concluded that the risk of MI was not increased in snuff dippers. The observations from this study show that, from a cardiovascular perspective, the deleterious effects of snuff dipping are much less than those of cigarette smoking.** Snuff users had no increased risk of myocardial infarction (fatal and nonfatal cases; either unadjusted or adjusted for multiple cardiovascular risk factors). A possible small or modest detrimental effect of snuff dipping on the risk for sudden death could not be excluded in this study due to a limited number of fatal cases. Odds ratios were adjusted for hypertension, diabetes, high cholesterol, family history of early cardiac death, low education level, and marital status. The authors hypothesize that the great difference in risk for MI between cigarette smoking and snuff dipping observed in this study provides important information on how the effects of smoking on cardiovascular risk are mediated. They speculate that nicotine is probably not an important risk contributor to ischemic heart disease in smokers, and that the moieties specific to tobacco smoke mediate the excess risk.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX J-2
CASE-CONTROL STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Wennberg et al. 2007 Sweden This study investigated the risk of a first myocardial infarction (MI) and sudden cardiac death (SCD) among male snuff users.	Nested case-control study (described by the authors as a "prospective incident case-referent study") The study was nested in 2 population-based surveys conducted in northern Sweden (the Västerbotten Intervention Program and the WHO MONICA study). All cases of MI and SCD that occurred from January 1, 1985 to December 31, 1999 were identified through the MONICA incidence registry. Cases were 525 men who experienced a first MI or SCD between January 1, 1985 and December 31, 1999. Controls were 1,798 men randomly selected from the survey populations who were matched for sex, age (± 2 yrs), date of health survey (± 4 months), and geographical region. Data on tobacco consumption were obtained by self-administered questionnaire. Conditional logistic regression was used to calculate odds ratios and 95% confidence intervals in univariate and multivariate models. Tobacco use was characterized by 8 mutually exclusive categories. "Snuff" is defined in this paper as Swedish snuff.	Tobacco Use **MI** Never used tobacco Never smoked, current snuff Former smoker, current snuff user Current smoker, current snuff user Never smoked, former snuff user Former smoker, former snuff user **Fatal MI within 28 Days** Never used tobacco Never smoked, current snuff Former smoker, current snuff user Current smoker, current snuff user Never smoked, former snuff user Former smoker, former snuff user **SCD with Survival <24 Hr** Never used tobacco Never smoked, current snuff Former smoker, current snuff user Current smoker, current snuff user Never smoked, former snuff user Former smoker, former snuff user **SCD with Survival <1 Hr** Never used tobacco Never smoked, current snuff Former smoker, current snuff user Current smoker, current snuff user Never smoked, former snuff user Former smoker, former snuff user	Odds Ratios (95% CI) 1.00 (reference) 0.82 (0.46-1.43) 1.25 (0.80-1.96) 2.14 (1.28-3.60)* 0.66 (0.32-1.34) 1.34 (0.84-2.12) 1.00 (reference) 1.12 (0.38-3.29) 1.24 (0.44-3.53) 1.11 (0.34-3.69) 0.64 (0.13-3.18) 0.60 (0.18-2.02) 1.00 (reference) 1.18 (0.38-3.70) 1.39 (0.44-4.42) 0.75 (0.17-3.28) 0.70 (0.14-3.64) 0.50 (0.12-2.03) 1.00 (reference) 0.38 (0.08-1.89) 2.67 (0.52-13.80) 0.13 (0.01-2.10) 0.35 (0.03-4.56) ---	**The authors concluded that there was no increased risk of MI or SCD among snuff users who did not have a history of smoking.** ORs were adjusted for BMI, leisure time physical activity, educational level, and cholesterol level. Other variables (diabetes, hypertension, use of nitrates or other heart medicine) were considered, but had little effect and were not included in the multivariate models. This study was prospective in that the data on tobacco use were collected prior to the occurrence of MI or SCD. There were strict and uniform criteria for the diagnosis of the outcomes. Tobacco use at baseline was reassessed among 30% of the subjects in a rescreening (median follow-up of 9 yrs 4 mos); consistency with the baseline screening was fairly good (the authors report consistency of 82% to 96%, depending on the particular tobacco use category). 69 MI cases (including 10 SCD cases) and 130 referents could not be categorized because of missing tobacco data. The authors note that differences among studies of snus and heart disease could be due to differences in study populations. The only study in which snus was

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX J-2
CASE-CONTROL STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
				associated with increased risk (Bolinder et al. 1994) involved a defined socioeconomic group (i.e., construction workers), while other studies were population-based.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix J3

Cohort Studies of Cardiovascular Diseases

APPENDIX J-3
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (N=7)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Bolinder et al. 1994 Sweden This study examined whether long-term exposure to smokeless tobacco is associated with excess risk of dying from cardiovascular disease in users compared with nonusers. [Subjects were selected from the same overall study population as Bolinder et al. 1992. This paper was one of 6 papers that were the basis of Bolinder's 1997 dissertation.] Results on lung cancer, all cancers, and stroke are presented in Appendices H, I, and K-2, respectively. [Updated and extended by Hergens et al. 2008]	Cohort study Subjects were 84,781 Swedish male construction workers identified between 1971 and 1974, and who were alive on January 1, 1974. They were followed for cause-specific mortality (ischemic heart disease, stroke, all cardiovascular disease, and all cancer) from 1974 through 1985 with the aid of the Swedish National Cause of Death Register. The classification of tobacco habits was aimed at isolating subjects in groups with a single type of tobacco exposure. Smokeless tobacco users were subjects who reported only present smokeless tobacco use and no former or present smoking (n=6,297). Smokeless tobacco is not defined in this paper, but is assumed to be Swedish snus as the cohort population is Swedish men.	<u>Cause of Death By Use Or Non-Use of Smokeless Tobacco</u> **All Cardiovascular Disease** Nonusers of smokeless tobacco Smokeless tobacco users <u>Cause-Specific Mortality Ages 35-54</u> **All Cardiovascular Disease** Nonusers Smokeless tobacco users **Ischemic Heart Disease** Nonusers Smokeless tobacco users <u>Cause-Specific Mortality Ages 55-65</u> **All Cardiovascular Disease** Nonusers Smokeless tobacco users **Ischemic Heart Disease** Nonusers Smokeless tobacco users	<u>Relative Risk (95% CI) of death</u> 1.0 (reference) 1.4 (1.2-1.6)* 1.0 (reference) 2.1 (1.5-2.9)* 1.0 (reference) 2.0 (1.4-2.9)* 1.0 (reference) 1.1 (1.0-1.4) 1.0 (reference) 1.2 (1.0-1.5)	**The authors concluded that both smokeless tobacco users and smokers face a higher risk of dying from cardiovascular disease compared to nonusers of tobacco, although the risk is lower for smokeless tobacco users than for smokers.** Increased risk of dying from all cardiovascular disease among snuff users was small but significant (220/6297, or 3.5%). Increased risks of all CVD and IHD were generally observed only among younger men (35-45). Relative risks reported here were adjusted only for age and region of origin. However the authors report that adjustment for area of domicile, BMI, blood pressure, diabetes, and history of heart symptoms and use of blood pressure medication did not affect the estimates.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX J-3
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (N=7) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Johansson et al. 2005 Sweden This purpose of this study was to evaluate the association between smoking and snuffing habits and the incidence rate of coronary heart disease (CHD). [Updated and extended by Haglund et al. 2007]	Cohort study Subjects were participants in the Swedish Annual Level-of-Living Survey (a random sample of the adult, non-institutionalized Swedish population). The sample included all healthy men (n=3,120; ages 30 to 74) surveyed in 1988-1989. Subjects were followed until hospitalization for a first fatal or nonfatal CHD event, death, or the end of the study on December 31, 2000. Mean follow-up was 11.2 years. There were 277 CHD events during the study period. Subjects were divided into six mutually exclusive categories based on their smoking and snuffing habits: never-smokers, former smokers, daily smokers, daily snuffing never-smokers, daily snuffing former smokers, and those who used snuff daily and smoke daily. Hazard ratios were calculated using 3 different statistical models. Smokeless tobacco is not defined in this paper, but is assumed to be Swedish snus as the cohort population is Swedish men.	<u>Tobacco Use</u> **Model 1** Never smokers Former smokers Daily smokers Daily snuffer/never smokers Daily snuffer/former smokers Daily snuffer/daily smokers **Model 2** Never smokers Former smokers Daily smokers Daily snuffer/never smokers Daily snuffer/former smokers Daily snuffer/daily smokers **Model 3** Never smokers Former smokers Daily smokers Daily snuffer/never smokers Daily snuffer/former smokers Daily snuffer/daily smokers	<u>Hazard Ratio (95% CI)</u> 1.00 (reference) 1.45 (1.05-1.99)* 2.19 (1.59-3.03)* 1.62 (0.70-3.75) 1.38 (0.80-2.39) 2.66 (1.32-5.36)* 1.00 (reference) 1.46 (1.06-2.02)* 2.27 (1.64-3.14)* 1.52 (0.66-3.53) 1.31 (0.76-2.38) 2.53 (1.25-5.10)* 1.00 (reference) 1.47 (1.07-2.03)* 2.30 (1.66-3.19)* 1.41 (0.61-3.28) 1.18 (0.67-2.06) 2.73 (1.35-5.53)*	**The authors concluded that the association between daily snuffing and CHD was non-significant.** The authors presented data from three different proportional hazard models that were based on stepwise inclusion of explanatory variable. Model 1 was adjusted only for age. Model 2 was adjusted also for physical activity and body mass index. Model 3 was adjusted also for diabetes and hypertension. In this study, daily smokers, former smokers, and those who combined smoking and snuffing all had significantly higher hazard ratios than never-smokers. The authors noted that, although the association between daily snuffing and CHD was not significant, the hazard ratio was "markedly increased," and that smokers should not use snuff to quit smoking. A major weakness of this study is that tobacco habits were assessed only at baseline and not again during the follow-up period. The authors note that they had data on former smoking, but not former snuff use. In addition, only 3.4% of the subjects (n=107) were never-smoking daily snuffers.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX J-3
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (N=7) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Hansson et al. 2009 Sweden This study examined the association between snus use and risk of cardiovascular disease (stroke and ischemic heart disease). Results on stroke are presented in Appendix K-2.	Cohort study Participants were 16,642 males, free of cardiovascular disease, who were identified from the Swedish Twin Registry in 1998-2002. The Swedish Twin Registry, established in 1950s attempted to include all Swedish twins born in 1958 or earlier; the study included twins born 1926-1958 (40 years or older at the time of the study). Using a telephone interview, participants in the registry were asked about tobacco use, including smoking and snus use. Never tobacco users were compared to current snus users. Incident cases of and death due to ischemic heart disease (IHD, myocardial infarction or coronary revascularization; ICD-10:I20-21, I24-25, excluding I25.2]) were identified from inpatient and national death registers. Participants were followed through 2003 for mortality and 2005 for hospitalization.	<u>IHD risk by tobacco exposure</u> Never tobacco users Current snus use Former snus use Never snus users Snus use ≤4 cans/week Snus use >4 cans/week Never snus users Snus use <20 years Snus use ≥ 20 years [see Hansson et al. 2009 for additional analyses]	<u>Hazard ratio (95% CI) for IHD</u> 1.0 (reference) 0.85 (0.51-1.41) 1.07 (0.56-2.03) 1.0 (reference) 0.84 (0.62-1.13) 0.92 (0.52-1.63) 1.0 (reference) 0.87 (0.55-1.38) 0.85 (0.62-1.18)	**The authors concluded that no evidence of an association between snus use and risk for cardiovascular disease (stroke and ischemic heart disease risk) was observed, and there was no indication of an increased IHD risk by weekly use or by increasing duration of snus use.** The authors presented relative risks adjusted for three sets of variables: (1) age; (2) age and smoking status (former or current); and (3) age, smoking status, diabetes mellitus, high blood pressure, and high cholesterol. These latter, multivariate risk estimates are presented in this table.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX J-3
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (N=7) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Haglund et al. 2007 Sweden This study examined the association between snus use and risk of stroke and ischemic heart disease. It extends the results of Johannson et al. (2005) by including a larger sample, an additional three years of follow-up, and examines stroke as well as ischemic heart disease. Results on stroke are presented in Appendix K-2.	Cohort Participants were 5,002 males ages 16 to 74 years old who responded to questions about tobacco use on the 1988-1989 Swedish Survey of Living Conditions, a population-based, representative, random sample of the Swedish population. Incident cases of and death due to ischemic heart disease (IHD) were identified through 2003 from inpatient and national death registers [ICD-9:410-414; ICD-10:I20-I25]. Participants were followed through 2003 for mortality and 2005 for hospitalization. Current Swedish moist snuff (snus) and other tobacco use assessed. Information on prior tobacco use not assessed.	<u>IHD risk by tobacco habits</u> No tobacco Snuff Smoke and snuff No tobacco Snuff Smoke and snuff	<u>Incidence Rate Ratios</u> 1.0 (reference) 0.77 (0.51-1.15) 1.64 (0.96-2.79) <u>Mortality Risk Ratio</u> 1.0 (reference) 1.15 (0.54-2.41) 1.69 (0.52-5.46)	**The authors concluded that no significant excess IHD risks for snuff users compared with non-tobacco users were observed. They noted, however, that a nonsignificant increased risk of fatal IHD was observed among snuff users.** No information was available on past tobacco use. The authors note that available scientific literature reports an increased risk of IHD from smoking observed up to five years after smoking cessation. Adjusted for age at event, SES, residential area, self-reported health, number of longstanding illnesses, and physical activity. The number of fatal events to determine mortality risks was small (8 fatal IHD cases among snuff users).

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX J-3
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (N=7) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Hergens et al. 2007 Sweden This study examined the long-term use of snus in males on morbidity and mortality from myocardial infarction compared to nonsmoking males. [Updates and extends Bolinder et al. 1994]	Cohort study Participants were 118,395 male construction workers who had never smoked regularly. Incident cases of and death due to myocardial infarction (MI) [ICD-7:420.10-420.17; ICD-8: 410; ICD-9: 410; ICD-10: I21-I22] were identified from inpatient and national death registers. Participants were followed through 2004. The association between snus use and the risk of MI (fatal, nonfatal, total) was compared to the rates of these events among nontobacco users in the construction workers cohort. Subjects were originally construction workers identified between 1971 and 1974, and who were alive on January 1, 1974. Follow-up visits occurred between 1971 and 1993, and tobacco exposure information was obtained from follow-up visits starting in 1978 as snuff use data prior to 1978 was deemed incomplete. Regular snuff use was defined as 1 gram/day for at least 1 year. Former snuff users were those who had stopped using snuff for at least 1 year.	<u>MI risk among never smokers</u> <u>Total MI</u> Never tobacco users Current snuff users Former snuff users <u>MI - Nonfatal</u> Never tobacco users Current snuff users Former snuff users <u>MI - Fatal</u> Never tobacco users Current snuff users Former snuff users <u>Total MI – by snuff use</u> Never tobacco users ≤ 12.5 g/day 12.5-24.9 g/day 25-49.9 g/day ≥ 50 g/day <u>MI – Nonfatal – by snuff use</u> Never tobacco users ≤ 12.5 g/day 12.5-24.9 g/day 25-49.9 g/day ≥ 50 g/day <u>MI – Fatal – by snuff use</u> Never tobacco users ≤ 12.5 g/day 12.5-24.9 g/day 25-49.9 g/day ≥ 50 g/day	<u>Hazard Ratio for MI</u> 1.0 (reference) 1.02 (0.92-1.14) 0.76 (0.55-1.05) 1.0 (reference) 0.94 (0.83 -1.06) 0.70 (0.48-1.02) 1.0 (reference) 1.32 (1.08-1.61)* 1.00 (0.54-1.88) 1.0 (reference) 1.12 (0.95-1.30) 0.93 (0.79-1.09) 0.95 (0.73-1.24) 1.24 (0.89-1.73) 1.0 (reference) 1.02 (0.84-1.22) 0.85 (0.70-1.03) 0.95 (0.71-1.29) 1.06 (0.71-1.58) 1.0 (reference) 1.45 (1.09-1.93)* 1.22 (0.90-1.65) 0.95 (0.54-1.69) 1.96 (1.08-3.58)*	**The authors concluded that they found no evidence for an overall elevated risk of myocardial infarction among snuff users compared to tobacco nonusers. They did observe, however, a significant increase in fatal MI among snuff users. The authors noted that the risk of fatal MI was most evident among heavy users (50 grams or more per day).** Relative risks were adjusted for age, BMI, and region of residence in Sweden. . The authors noted that when fatal MI was further adjusted for high blood pressure, relative risk estimates were reduced, suggesting "elevated blood pressure might be in the causal pathway between snuff use and myocardial infarction." The authors suggested that potential confounding from socioeconomic status or education are minimized in this cohort of relatively homogenous construction workers. No information was available on alcohol consumption, and tobacco use was obtained only through 1993. This analysis differed from that of Bolinder et al. (1994) in that Hergens et al. used updated tobacco use information collected during participants' follow-up visits after the initial visit in the 1970s. The data collection form from the initial interviews has been criticized as not adequate for collecting information on snus use.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX J-3
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (N=7) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Janzon and Hedblad 2009 Sweden The purpose of this population-based study was to explore whether snuff users have an increased incidence of myocardial infarction or stroke. [Results for stroke presented in Table K-2]	Cohort study The study population included 27,227 male and female residents of Malmö, Sweden, ages 45-73 years old at time of study entry, 1991-1996 (approximately 40% of eligible participants) who had no history of MI or stroke, and had available information on BMI, blood pressure, diabetes, and tobacco use. First incident MI or fatal ischemic heart disease [ICD-9: 410-414] was obtained from hospital discharge registries through December 2004. Participants completed a self-administered questionnaire on tobacco use. Smokers were categorized as never, ex-, or current smokers, and current snuff use (categorized as yes/no) was quantified into low (1-2), medium (3-5), and high (≥ 6) packages per week.	<u>First ever MI</u> **Males – risk factor adjusted:** Nontobacco users Snuff user, never smoker **Females** Nontobacco users Snuff user	<u>Relative Risk (95% CI)</u> 1.0 (reference) 0.75 (0.3-1.8) 1.0 (reference) 0 cases	**The authors concluded that the present study does not support the hypothesis that snuff is a risk factor for incident myocardial infarction for men.** There were too few cardiovascular events (MI or stroke) among female snuff users to examine this outcome in this cohort. In this cohort, 7 % of males and 0.4% of females were snuff users; of these, 34% of males and 28% of females were dual users (also current smokers). Relative risks were adjusted for age, BMI, smoking habits, diabetes mellitus, hypertension, physical activity, marital status, and occupation. Male snuff users compared to snuff nonusers were younger, less likely to use blood pressure medication, be ex- or current smokers, have low- or medium-level occupations, and be unmarried (single). The authors report that even after adjusting for age and BMI, mean blood pressure showed no statistically significant difference between male and female snuff users and non-users (which may include smokers). No dose-response analysis was presented though information on the amount of snuff used weekly was collected.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX J-3
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (N=7) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Roosaar et al. 2008 Sweden This study evaluated and compared the effects of snus and smoking on cancer incidence and cardiovascular deaths [ICD8,9: 390-458; ICD10: I00-I99]. Results on smoke-related cancers and any cancer are presented in Appendix H and head and neck cancers in Appendix C.	Retrospective cohort study Subjects were identified from a cohort established in 1973-74 and followed up for mortality and cancer incidence between 1973 and 2002 using national registers. Subjects were 9,976 males from Uppsala County, central Sweden, who completed a questionnaire about tobacco and alcohol consumption, and all underwent a clinical examination of the oral cavity. 867 men (9%) were ever daily snus users (but never daily smokers), 5,309 (53%) were ever daily smokers (but never ever daily snus users) and 692 (7%) were both ever daily snus users and ever daily smokers	<u>Oral Snuff and Smoking Usage</u> Cardiovascular death Snus use Never daily use Ever daily use Restricted to never smokers Snus use Never daily use Ever daily use	<u>Hazard Ratio (95% CI)</u> Reference 1.11 (0.98-1.25) Reference 1.15 (0.97-1.37)	**The authors conclude that their results are inconsistent with claims that the use of snus is without demonstrable risk. Relative risks are consistently lower than those associated with smoking.** Models were adjusted for alcohol consumption, area of residence, calendar period, smoking or snus use, and several interaction terms (with age). The follow up time of the cohort was long (up to 29 years). The authors stated that the residual negative confounding from smoking is an important concern for those who both smoke and use snus. To examine the potential for change in tobacco habits from time of study entry (1973), the authors conducted a sensitivity analysis for all cancer, all mortality, and oral/pharyngeal cancer that included only males aged 25 and older at time of entry. They reported that results were essentially unchanged, and concluded that "since smoking is rarely taken up after age 25, the analyses that were restricted to never-smokers should not have been seriously affected by changes in smoking habits." No information on the amount or duration of snus use was available for dose-response analyses.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix J4

Experimental Studies of Cardiovascular Effects

APPENDIX J-4
EXPERIMENTAL STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (N=1)

CITATION, LOCATION	STUDY TYPE, POPULATION, BRIEF DESCRIPTION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Rohani and Agewall 2004 Sweden This study examined the effect of snuff use on the response of the vasculature to increases in blood flow.	Experimental human study Subjects were 20 healthy middle-aged snuff users (18 men and 2 women), mean age of 34 years. They took no drugs and had no history of cardiovascular disease or diabetes. 10 subjects were examined twice in a randomized cross-over design, once with snuff and once with placebo. 10 additional subjects were evaluated only after using snuff. Using ultrasonography and pulsed Doppler imaging, investigators measured the diameter and blood flow in the brachial artery under resting conditions and after an increase in blood flow caused by the release of a blood pressure cuff to assess flow-mediated dilatation. The degree of dilatation and blood flow was measured prior to and at 20 and 35 min. after beginning using snuff or use of an unidentified placebo. Heart rate and blood pressure were also measured.	35 min. after beginning snuff use, there was a statistically significantly decline in dilatation of the brachial artery in response to increased blood flow compared to that seen under resting conditions ($p=0.004$). No significant difference in dilatation was seen at 20 min. after starting snuff use. Heart rate and blood pressure were significantly increased at 20 min. and heart rate was significantly increased at 35 min. after beginning snuff use. No significant changes were reported in flow-mediated dilatation, heart rate, or blood pressure under the placebo conditions. (These data are not presented).	**The investigators concluded that use of oral moist snuff significantly impairs endothelial function, which is a predictor of cardiovascular morbidity. Consequently, snuff use should be discouraged.** This study compared dilatation readings obtained after snuff use to baseline readings, rather than to readings obtained under placebo conditions. The conclusions that can be drawn, therefore, are limited to effects before and during snuff use rather than a comparison of snuff use versus no snuff use. Thus, the results may just reflect a change over time rather than a change inherent to product use. Further, the statistical test was inappropriate. A repeated measures analysis of variance rather than a t-test should have been used. The t-test overestimates statistical significance in this situation. The investigators over-extrapolate the study findings to conclude that snuff use increases cardiovascular morbidity. Although impaired flow-mediated dilatation has been seen in populations at greater risk for cardiovascular events, this study was not designed to assess any difference between snuff users and nonusers.

Appendix K1

Case-Control Studies of Stroke

APPENDIX K-1
CASE-CONTROL STUDIES OF STROKE AMONG SWEDISH SNUS USERS (N=2)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Asplund et al. 2003 Sweden This study investigated whether use of snuff increased the risk of stroke in men. Risk among snuff users was compared to that among cigarette smokers and nonusers of tobacco.	Nested case-control study (population-based) Cases and controls were identified from 2 cohort studies, the Northern Sweden MONICA Project and the Västerbotten Intervention Project (VIP). Cases were 276 male patients with a first-ever confirmed stroke (brain infarction or intracerebral hemorrhage), either fatal or nonfatal, that occurred from 1985 to 2000. For each stroke case, 2 matched control subjects with no history of cardiovascular disease were selected from the MONICA and VIP cohorts. Controls were matched by sex, age, geographical region, year of baseline examination, and cohort. Data presented here are for exclusive, life-long users of the specified product. Snuff is not defined in this paper, but is assumed to be Swedish snus as the cohort populations are from Sweden.	<u>First-Ever Fatal or Nonfatal Stroke</u> **Univariate Analyses** Never users of tobacco All snuff users (including smokers) Exclusive snuff users All cigarette smokers (including snuffers) Exclusive cigarette smokers **Conditional Logistic Regression** Regular snuff users Regular cigarette smokers	<u>Odds Ratio (95% CI)</u> 1.00 (reference) 1.16 (0.60-2.22) 1.05 (0.37-2.04) 1.86 (1.13-3.05)* 2.21 (1.29-3.79)* 0.87 (0.41-1.83) 1.74 (0.85-3.54)	**The authors concluded that snuff was not associated with any excess risk of stroke.** They note that "the deleterious effects of snuff dipping are considerably less than those of cigarette smoking." Odds ratios from the conditional logistic regression analysis were adjusted for elevated blood pressure, low level of education, not married or cohabitant, diabetes, and serum cholesterol. The reference group for these analyses was not specifically defined. A key strength of the nested case-control design is that information on risk factors is collected prior to the health event of interest, eliminating recall bias. In attempting to explain the difference in risk of stroke associated with cigarette smoking and snuff dipping, the authors speculate that nicotine is a minor contributor and that moieties specific to tobacco smoke are more important in conferring excess risk.

* denotes statistically significant increase in risk
** demotes statistically significant decrease in risk

APPENDIX K-1
CASE-CONTROL STUDIES OF STROKE AMONG SWEDISH SNUS USERS (N=2) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Koskinen and Blomstedt 2006 Sweden This study investigated whether smoking or use of snuff increased the risk of subarachnoid hemorrhage (SAH).	Case-control study (population-based) Cases were 120 consecutive patients with spontaneous SAH admitted to the Department of Neurosurgery at the Umeå University Hospital (serving the northern part of Sweden) from January 1, 1997 to December 31, 1998. The reference population is not described in detail in this paper; it was chosen randomly from all areas in the country in proportion to the inhabitants and apparently was matched to the distribution of smokers in 2001 and snuffers in 1996-1997. Information concerning tobacco use and other possible risk factors was obtained using a standardized questionnaire. Snuff is defined in this paper as Swedish snuff.	Tobacco Use **Among Men** Reference not defined Smokers Snuffers **Among Women** Reference not defined Smokers Snuffers	Relative Risk (95% CI) 1.00 (reference) 2.63 (1.20-5.72)* 0.48 (0.17-1.30) 1.00 (reference) 2.26 (1.69-3.01)* 1.30 (0.33-5.18)	**The authors concluded that consumption of snuff was not associated with increased risk of subarachnoid hemorrhage.** The exact design of this study is unclear. It appears to most closely resemble a case-control study, although controls were not matched individually to cases, and the authors refer to it as a cohort. The data on "snuffers" likely includes people who also smoked or smoked previously. In this study 77.1% of the subjects were current or former smokers. The authors noted that it is unlikely that nicotine is solely responsible for the increase in risk of SAH. It does not appear that the relative risks were adjusted for potential confounders. The reference group for these analyses was not specifically defined. The mean duration of consumption of non-smoking tobacco was 20.4 years (range 4-55 years) with a mean consumption of 3 days/packet (range 1-14).

* denotes statistically significant increase in risk
** demotes statistically significant decrease in risk

Appendix K2

Cohort Studies of Stroke

APPENDIX K-2
COHORT STUDIES OF STROKE AMONG SWEDISH SNUS USERS (N=5)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Bolinder et al. 1994 Sweden This study examined whether long-term exposure to smokeless tobacco is associated with excess risk of dying from stroke in users compared with nonusers. [Subjects were selected from the same overall study population as Bolinder et al. 1992. This paper was one of 6 papers that were the basis of Bolinder's 1997 dissertation.] Results on lung cancer, all cancers, and cardiovascular disease are presented in Appendices H, I, and J-3, respectively. [Construction workers cohort extended and updated by Hergens et al. 2008]	Cohort study Subjects were 84,781 Swedish male construction workers identified between 1971 and 1974, and who were alive on January 1, 1974. They were followed for cause-specific mortality (ischemic heart disease, stroke, all cardiovascular disease, and all cancer) from 1974 through 1985 with the aid of the Swedish National Cause of Death Register. The classification of tobacco habits was aimed at isolating subjects in groups with a single type of tobacco exposure. Smokeless tobacco users were subjects who reported only present smokeless tobacco use and no former or present smoking (n=6,297). Smokeless tobacco is not defined in this paper, but is assumed to be Swedish snus as the cohort population is Swedish men.	Cause-Specific Mortality Ages 35-54 **Stroke** Nonusers Smokeless tobacco users Cause-Specific Mortality Ages 55-65 **Stroke** Nonusers Smokeless tobacco users	Relative Risk (95% CI) of death 1.0 (reference) 1.9 (0.6-5.7) 1.0 (reference) 1.2 (0.7-1.8)	The authors concluded that there was an apparent excess risk of death from cardiovascular and cerebrovascular diseases of from 40% to 100% among smokeless tobacco users, compared to nonusers, when possible confounding factors are taken into account. Smokers face even higher risks of both cardiovascular and cerebrovascular causes of death. Relative risks reported were adjusted only for age. However the authors report that adjustment for area of domicile, BMI, blood pressure, diabetes, and history of heart symptoms and use of blood pressure medication did not affect the estimates. The baseline information on tobacco use was collected in the 1970s, and was not updated for the analysis. Tobacco use may have changed after collection of these data.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX K-2
COHORT STUDIES OF STROKE AMONG SWEDISH SNUS USERS (N=5) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Hergens et al. 2008 Sweden This study examined the long-term use of snus in males on morbidity and mortality from stroke and stroke subtypes compared to nonsmoking males. [Updates the study reported by Bolinder et al. 1994]	Cohort study Participants were 118,465 male construction workers who had never smoked regularly. Incident cases of and death due to stroke were identified from inpatient and national death registers. Participants were followed through 2003. The association between snus use and the risk of stoke, including stroke subtypes, was compared to the incidence of these events among nontobacco users in the construction workers cohort. Subjects were originally construction workers identified between 1971 and 1974, and who were alive on January 1, 1974. Follow-up visits occurred between 1971 and 1993, and tobacco exposure information was obtained from follow-up visits starting in 1978 as snuff use data prior to 1978 was deemed incomplete. Of the 118,465 participants who had never smoked regularly, 71% had never used snuff, 2% were former users, and 27% were current snuff users.	<u>Stroke risk among never smokers</u> <u>All stroke types</u> Never tobacco users (n=2805) Current snuff users (n=412) Former snuff users (n=31) <u>All stroke types - Nonfatal</u> Never tobacco users Current snuff users Former snuff users <u>All stroke types - Fatal</u> Never tobacco users Current snuff users Former snuff users <u>Ischemic stroke - All</u> Never tobacco users Current snuff Former snuff users <u>Ischemic stroke - Nonfatal</u> Never tobacco users Current snuff users Former snuff users <u>Ischemic stroke - Fatal</u> Never tobacco users Current snuff users Former snuff users <u>Hemorrhagic stroke - All</u> Never tobacco users Current snuff users Former snuff users	<u>Hazard Ratio for stroke</u> 1.0 (reference) 1.05 (0.95-1.17) 0.72 (0.50-1.02) 1.0 (reference) 1.02 (0.91-1.14) 0.75 (0.53-1.08) 1.0 (reference) 1.38 (0.99-1.91) 0.30 (0.04-2.11) 1.0 (reference) 1.07 (0.94-1.22) 0.68 (0.44-1.06) 1.0 (reference) 1.04 (0.91-1.18) 0.67 (0.43-1.06) 1.0 (reference) 1.72 (1.06-2.78)* 0.82 (0.12-5.93) 1.0 (reference) 0.85 (0.65-1.10) 0.90 (0.45-1.82)	**The authors concluded that they found no evidence for an overall elevated risk of stroke or nonfatal stroke among snuff users compared to tobacco nonusers. The authors noted, however, an increased risk of fatal ischemic and unspecific stroke among snuff users compared to tobacco nonusers.** No evidence of a dose-response relationship was observed for any stroke type or by stoke survival or mortality. In the dose-response analysis, the only statistically significant increase in risk of any ischemic stroke was observed in the lowest daily dose group (<12.5 grams/day). Relative risks were adjusted for age, BMI, and region of residence in Sweden. The authors suggested that potential confounding from socioeconomic status or education are minimized in this cohort of relatively homogenous construction workers. No information was available on alcohol consumption, and tobacco use was obtained only through 1993. This analysis differed from that of Bolinder et al. 1994 in that Hergens et al. used updated tobacco use information collected during participants' follow-up visits after the initial visit in the 1970s. The data collection form from the initial interviews has been criticized as not adequate for collecting information on snus use.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX K-2
COHORT STUDIES OF STROKE AMONG SWEDISH SNUS USERS (N=5) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
		Hemorrhagic stroke - Nonfatal		
		Never tobacco users	1.0 (reference)	
		Current snuff users	0.77 (0.57-1.04)	
		Former snuff users	1.10 (0.54-2.21)	
		Hemorrhagic stroke - Fatal		
		Never tobacco users	1.0 (reference)	
		Current snuff users	1.17 (0.68-2.01)	
		Former snuff users	0	
		Unspecified stroke - All		
		Never tobacco users	1.0 (reference)	
		Current snuff users	1.35 (1.02-1.80)*	
		Former snuff users	0.66 (0.21-2.06)	
		Unspecified stroke - Nonfatal		
		Never tobacco users	1.0 (reference)	
		Current snuff users	1.31 (0.98-1.77)	
		Former snuff users	0.69 (0.22-2.14)	
		Unspecified stroke - Fatal		
		Never tobacco users	1.0 (reference)	
		Current snuff users	1.14 (0.51-5.54)	
		Former snuff users	0	

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX K-2
COHORT STUDIES OF STROKE AMONG SWEDISH SNUS USERS (N=5) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Hansson et al. 2009 Sweden This study examined the association between snus use and risk of stroke and ischemic heart disease. Results on ischemic heart disease are presented in Appendix J-2.	Cohort study Participants were 16,642 males, free of cardiovascular disease, who were identified from the Swedish Twin Registry in 1998-2002. The Swedish Twin Registry, established in 1950s attempted to include all Swedish twins born in 1958 or earlier; the study included twins born 1926-1958 (40 years or older at the time of the study). Using a telephone interview, participants in the registry were asked about tobacco use, including smoking and snus use. Never tobacco users (n=12,525 of whom 20% are current smokers and 30% are former smokers) were compared to current snus users (n=2661). Incident cases of and death due to stroke were identified from inpatient and national death registers. Participants were followed through 2003 for mortality and 2005 for hospitalization.	<u>Stroke risk by tobacco exposure, never smokers</u> Never tobacco users (n=155) Current snus use (n=14) Former snus use (n=8) Never snus users Snus use ≤ 4 cans/week Snus use >4 cans/week Never snus users Snus use <20 years Snus use ≥ 20 years	<u>Hazard ratio (95% CI) for stroke</u> 1.0 (reference) 1.18 (0.67-2.08) 1.35 (0.65-2.82) 1.0 (reference) 0.75 (0.49-1.15) 1.75 (0.95-3.21) 1.0 (reference) 1.13 (0.63-2.01) 0.80 (0.51-1.25)	**The authors concluded that they found no clear evidence of an association between snus use and risk for cardiovascular disease (stroke and ischemic heart disease risk). They noted an indication of an increased risk of stroke among snus users of four or more cans per week, but cautioned that no increased risk was observed in the group with moderate use of snus and no increased risk was observed with increasing duration of use.** The authors presented relative risks adjusted for three sets of variables: (1) age; (2) age and smoking status (former or current); and (3) age, smoking status, diabetes mellitus, high blood pressure, and high cholesterol. These latter, multivariate risk estimates are presented in this table; relative risk estimates adjusted for age only, and for age and smoking status, are presented in the publication.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX K-2
COHORT STUDIES OF STROKE AMONG SWEDISH SNUS USERS (N=5) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Haglund et al. 2007 Sweden This study examined the association between snus use and risk of stroke and ischemic heart disease. It extends the results of Johannson et al. (2005) by including a larger sample, an additional three years of follow-up, and examines stroke as well as ischemic heart disease. Results on ischemic heart disease are presented in Appendix J-2.	Cohort Participants were 5,002 males ages 16 to 74 years old who responded to questions about tobacco use on the 1988-1989 Swedish Survey of Living Conditions, a population-based, representative, random sample of the Swedish population. Incident cases of and death due to stroke were identified through 2003 from inpatient and national death registers. Participants were followed through 2003 for mortality and 2005 for hospitalization. Current Swedish moist snuff (snus) and other tobacco use assessed. Information on prior tobacco use not assessed.	<u>Stroke risk by tobacco habits</u> No tobacco Snuff Smoke and snuff No tobacco Snuff Smoke and snuff	<u>Incidence Rate Ratios</u> 1.0 (reference) 1.07 (0.65-1.77) 1.98 (1.00-3.95) <u>Mortality Risk Ratio</u> 1.0 (reference) 1.01 (0.35-2.92) 4.30 (1.22-15.1)	**The authors concluded that no excess stroke risks for snuff users compared with non-tobacco users were observed. They noted that the highest risk of stroke incidence and mortality was observed for those who smoke and use snuff simultaneously (dual users).** No information was available on past tobacco use. The authors note that available scientific literature reports an increased risk of IHD from smoking observed up to five years after smoking cessation. Adjusted for age at event, SES, residential area, self-reported health, number of longstanding illnesses, and physical activity, but not for other cardiovascular risk factors. The number of fatal events to determine mortality risks was small (4 fatal stokes among snuff users, 3 among dual users).

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX K-2
COHORT STUDIES OF STROKE AMONG SWEDISH SNUS USERS (N=5) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Janzon and Hedblad 2009 Sweden The purpose of this population-based study was to explore whether snuff users have an increased incidence of stroke or myocardial infarction (MI). [Results for MI presented in Table J-2]	Cohort study The study population included 27,227 male and female residents of Malmö, Sweden, ages 45-73 years old at time of study entry, 1991-1996 (approximately 40% of eligible participants) who had no history of MI or stroke, and had available information on BMI, blood pressure, diabetes, and tobacco use. First incident MI or stroke was obtained from hospital discharge registries through December 2004. Participants completed a self-administered questionnaire on tobacco use. Smokers were categorized as never, ex-, or current smokers, and current snuff use (categorized as yes/no) was quantified into low (1-2), medium (3-5), and high (≥ 6) packages per week.	<u>First ever stroke</u> **Males – risk factor adjusted:** Nontobacco users Snuff user, never smoker Smokers **Females** Nontobacco users Snuff user	<u>Relative Risk (95% CI)</u> 1.0 (reference) 0.59 (0.2-1.5) 1.13 (0.6-2.0) 1.0 (reference) 1 case (relative risk not presented)	**The authors concluded that the present study does not support the hypothesis that snuff is a risk factor for incident stroke for men.** There were too few cardiovascular events (MI or stroke) among female snuff users to examine this outcome in this subcohort. In this cohort, 7 % of males and 0.4% of females were snuff users; of these, 34% of males and 28% of females were dual users (also current smokers). Both male and female dual users were significantly more likely to have lower daily consumption of cigarettes and male snuff users were significantly more likely to be former smokers. Only 9% of male snuff users had never been smokers. Relative risks were adjusted for age, BMI, smoking habits, diabetes mellitus, hypertension, physical activity, marital status, and occupation. Male snuff users compared to snuff nonusers were younger, less likely to use blood pressure medication, be ex- or current smokers, have low- or medium-level occupations, and be unmarried (single). The authors report that even after adjusting for age and BMI, mean blood pressure showed no statistically significant difference between male and female snuff users and non-users (which may include smokers). No dose-response analysis was presented though information on the amount of snuff used weekly was collected.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix L1

Descriptive Studies of Gastrointestinal Effects

APPENDIX L-1
DESCRIPTIVE STUDIES OF GASTROINTESTINAL EFFECTS AMONG SWEDISH SNUS USERS (N=1)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Bolinder et al. 1992 Sweden The aim of this study was to investigate the relationship between tobacco consumption habits and general health status. [This study includes individuals from the same study population as Bolinder et al. 1994.] Data on cardiovascular outcomes and body weight effects in this study are also summarized in Appendices J-1 and O-1 respectively.	Descriptive study (cross-sectional) Subjects in this population survey were 97,586 male construction workers (16-65 years of age) who received health examinations from 1971 to 1974. Physical examinations included blood pressure and heart rate measurements and included a questionnaire about tobacco use and health status. Information was also acquired on sick leave and the allocation of disability pensions. Of the 97,586 subjects examined, 59,864 were excluded because of use of more than one type of tobacco product or because they were ex-smokers. The remaining subjects (n=37,722; 1,370 of whom were disability pensioners) were grouped for analysis by tobacco habit: non-users who had never used tobacco products (n=23,885), smokeless tobacco users who had never been regular smokers (n=5,014), and smokers of $\geq$ 15 cigarettes per day who had never been regular users of smokeless tobacco (n=8,823). The authors define smokeless tobacco as "mainly moist snuff."	Tobacco Use **Heartburn** Non-users of tobacco Smokeless tobacco users **Peptic Ulcer** Non-users of tobacco Smokeless tobacco users	Odds Ratio (95% CI) 1.0 (reference) 0.9 (0.8-0.9) 1.0 (reference) 1.1 (0.9-1.2)	**The authors concluded that users of smokeless tobacco did not have any excess risk of peptic ulcer and that they had a significantly lower tendency to suffer from heartburn than non-users.** Odds ratios appear to be unadjusted for potential confounding factors. Smokers of $\geq$15 cigarettes per day had significantly higher risks of heartburn and peptic ulcer than non-users of tobacco products. The reason for a lower risk of heartburn in smokeless tobacco users was not clear, but the authors speculate that the high pH of moist Swedish snuff (8.5) could be important when saliva is swallowed. The authors also stated that smokeless tobacco users appear to have a better general health profile than those who use smoked tobacco, although their profile is worse than that of the non-users.

* denotes statistically significant increase in risk
** demotes statistically significant decrease in risk

Appendix L2

Case-Control Studies of Gastrointestinal Effects

APPENDIX L-2
CASE-CONTROL STUDIES OF GASTROINTESTINAL EFFECTS AMONG SWEDISH SNUS USERS (N=1)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Persson et al. 1993 Stockholm, Sweden This study examined the association between oral moist snuff use and inflammatory bowel disease (Crohn's disease and ulcerative colitis).	Case-control study (hospital-based cases, population-based controls) Cases included 365 subjects aged 15-79 years, with confirmed diagnoses of Crohn's disease (n=184) or ulcerative colitis (n=181). Cases were residents of Stockholm County from 1980 to 1984, and were selected from a central register of all hospital admissions in that county. After narrowing the analysis to males with completed questionnaires (and excluding subjects who smoked only a pipe or cigars), 60 cases of Crohn's disease and 82 cases of ulcerative colitis remained. Controls were 390 subjects obtained by random sample of a register of the inhabitants of Stockholm county. Controls were stratified by age (5-year age groups) and gender. After narrowing the analysis to males with completed questionnaires, 145 controls remained. "Snuff" was defined as oral moist snuff. Snuff use was reported by 16 Crohn's disease cases, 24 ulcerative colitis cases, and 21 controls.	<u>Moist Snuff Use Among Never-Smokers:</u> **Crohn's disease** Never Ever **Ulcerative colitis** Never Ever <u>Moist Snuff Use Among All Subjects (Never, Former, Current Smokers):</u> **Crohn's disease** Never Ever **Ulcerative colitis** Never Ever	<u>Relative Risks (95% CI)</u> **Crohn's disease** 1.0 (reference) 0.9 (0.3-3.1) **Ulcerative colitis** 1.0 (reference) 1.1 (0.4-3.1) **Crohn's disease** 1.0 (reference) 2.1 (1.0-4.6) **Ulcerative colitis** 1.0 (reference) 2.2 (1.1-4.4)*	**The authors found that use of oral moist snuff alone was not associated with increased risk of Crohn's disease or ulcerative colitis.** Relative risk estimates for Crohn's disease and ulcerative colitis in snuff-using never-smokers were adjusted for age only. Relative risk estimates for Crohn's disease and ulcerative colitis in snuff users (including smokers and never-smokers) were adjusted for age and smoking status. The authors found a synergistic interaction between oral moist snuff and cigarette smoking; users of both products had a more than 3-fold increased risk of both diseases. However, it is not clear whether this was tested statistically through an interaction term in the logistic regression model.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix M1

Descriptive Studies of Insulin Resistance and Diabetes

APPENDIX M-1
DESCRIPTIVE STUDIES OF INSULIN RESISTANCE AND DIABETES AMONG SWEDISH SNUS USERS (N=2)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Persson et al. 2000 Sweden This study investigated the association between cigarette smoking and/or oral snuff use, and three endpoints of impaired glucose tolerance and type 2 diabetes.	Cross-sectional (population-based) Subjects included 3,162 males aged 35-56 years who resided in Stockholm. Half of the participants had a strong family history of diabetes. All subjects were given an oral glucose tolerance test and then classified as having normal or impaired glucose tolerance, or diabetes, according to WHO 1985 criteria. All subjects were asked if they currently used snuff, and if so were classified into never, former or current users. Additionally, information regarding the weekly number of boxes (50 g each) consumed was collected.	<u>Oral Snuff Usage Among Exclusive Users of Snuff</u> **Impaired glucose tolerance** Never users of tobacco Moist snuff only **Type 2 diabetes** Never users of tobacco Moist snuff only	<u>Prevalence Odds Ratios (95% CI)</u> 1.0 (reference) 0.9 (0.4-2.1) 1.0 (reference) 3.9 (1.1-14.3)*	**The authors concluded that heavy users of moist snuff have an increased risk of type 2 diabetes. According to the authors, this study is the first to illustrate an association between oral snuff use and diabetes.** The data presented here are for exclusive users of moist snuff (*i.e.*, those without cigarette use). The authors also present prevalence odds ratios for impaired glucose tolerance and type 2 diabetes among snuff users who apparently may also have smoked. Among this latter group of snuff users, the prevalence of type 2 diabetes was significantly higher only among current snuffers who used 3+ boxes per week. Although current moist snuffers had almost a 4-fold increased prevalence of diabetes, the authors note that the confidence interval for this result is wide. A wide confidence interval indicates that that the risk estimate is based on small numbers (in this case, only 4 subjects with diabetes). Odds ratios were adjusted for age, body mass index, family history of diabetes, physical activity, and alcohol consumption using multiple logistic regression. This study, like all cross-sectional studies, has inherent weaknesses. It examines prevalence of disease, not incidence, and thus cannot comment on factors that affect the development of disease. Furthermore, cross-sectional studies cannot address temporal sequence (*i.e.*, whether the snuff use preceded the diabetes or not).

* Denotes statistically significant increase in risk
** Denotes statistically significant decrease in risk

APPENDIX M-1
DESCRIPTIVE STUDIES OF INSULIN RESISTANCE AND DIABETES AMONG SWEDISH SNUS USERS (N=2) CONTINUED

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Wandell et al. 2008 Sweden This study examined the potential association between use of tobacco, including smokeless tobacco, and metabolic syndrome and diabetes. Results on metabolic syndrome presented in Appendix N-2.	Population based cross-sectional study Subjects were 1,859 men, aged 60 years old living in Stockholm County from August 1997-March 1999. The men underwent a physical exam, lab tests, and a questionnaire, including medical data, and questions on demographic, socio-economic and life style factors. Use of tobacco was coded as never users of tobacco (n = 594), former smokers (n = 737), former smokers but current daily users of snuff (n = 113), current daily smokers (n = 360), former snuffers (n = 12), current snuffers (n = 16) and current daily smokers and snuffers (n = 27).	<u>Oral Snuff and Smoking Usage</u> **Diabetes** Ex-smokers Ex-smokers, current snuffers Current smokers Ex-snuffers Current snuffers Current smokers and snuffers Smoking duration, short (<20 years) Smoking duration, long (≥20 years) Snuff, low consumers (<3 cans/w) Snuff, high consumers (≥3 cans/w)	<u>Odds Ratio (95% CI)</u> 1.41 (0.76-2.60) 1.71 (0.67-4.35) 1.40 (0.68-2.89) 3.10 (0.36-26.84) 2.12 (0.25-17.71) 2.48 (0.52-11.82) 1.3 (0.64-2.66) 1.46 (0.79-2.68) 1.30 (0.49-3.40) 1.80 (0.67-4.85)	**The authors conclude that an association between use of snuff and risk of diabetes was not found.** Although not statistically significant, ORs for former and current snuff users were the highest among tobacco users. The prevalence of smokers and snus users in this cohort was comparable to the general Swedish population of the same age. The authors collected information on smoking duration and snus consumption so a potential tendency for a dose-response relationship could be assessed. Due to the nature of their design, causality cannot be determined from cross-sectional studies since disease and exposure are measured simultaneously. The power to detect a potential association in this study was low.

* Denotes statistically significant increase in risk
** Denotes statistically significant decease in risk

Appendix M2

Cohort Studies of Insulin Resistance and Diabetes

APPENDIX M-2
COHORT STUDIES OF INSULIN RESISTANCE AND DIABETES AMONG SWEDISH SNUS USERS (N=1)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Eliasson et al. 2004 Sweden This study investigated the effect of smoking and snus use on the risk of type 2 diabetes and pathological glucose tolerance (PGT; defined as impaired glucose tolerance or undiagnosed diabetes).	Cross-sectional and prospective follow-up (population-based) Subjects were 3,384 men (aged 25-74 years at study entry) who participated in one of 4 MONICA project surveys (1986, 1990, 1994, or 1999). The prevalence of self-reported clinically diagnosed, known diabetes was assessed at study entry and at follow-up 5-13 years later. An oral glucose tolerance test was administered to 1,158 men without diabetes at entry to identify those with PGT (n=98). 1,757 men returned in 1999 for re-examination. Subjects were classified as ex, current, or never users of cigarettes or snus. Current snus users were categorized by amount used weekly (<2 boxes, 2-3 boxes, >3 boxes).	<u>Prevalence Results</u> **Known Type 2 diabetes** Never user of tobacco Ever smoker (exclusive) Ever snus use (exclusive) Current smoker Current snus user Ex-smoker Ex-snus user **PGT** Never user of tobacco Ever smoker (exclusive) Ever snus use (exclusive) Current smoker Current snus user Ex-smoker Ex-snus user <u>Incidence Results</u> **Known Type 2 diabetes** Consistent no tobacco Consistent exclusive snus Consistent exclusive smoking Ex-smokers Ex-snus users Smokers switched to snus	<u>Prevalence Odds Ratios (95% CI)</u> 1.00 (reference) 1.77 (1.10-2.87)* 1.21 (0.59-2.49) 1.62 (0.86-3.05) 1.06 (0.43-2.64) 1.87 (1.10-3.20)* 1.45 (0.54-3.87) 1.00 (reference) 1.23 (0.74-2.04) 1.05 (0.51-2.17) 0.94 (0.46-1.92) 0.78 (0.29-2.09) 1.45 (0.82-2.56) 1.48 (0.57-3.80) <u>Odds Ratios (95% CI)</u> 1.00 (reference) 0 cases 4.61 (1.37-15.5)* 3.13 (1.13-8.67)* 1.72 (0.20-14.8) 3.25 (0.78-13.6)	**The authors concluded that risk of diabetes was not significantly increased among snus users. Smoking was associated with both prevalent and incident cases of diabetes.** Prevalence odds ratios were adjusted for age and waist circumference. Incidence odds ratios were adjusted for age, follow up, and annual percentage weight gain between baseline and follow-up. At study entry, the prevalence of diabetes was significantly higher among ever- and ex-smokers compared to never-tobacco users, but the prevalence was not significantly elevated among any category of snus users (ever, current, ex). The authors also analyzed the prevalence of diabetes in exclusive snus users according to the amount of snus used per week, but found no dose-response relationship. The prevalence of PGT was not significantly elevated among snus users or smokers. No cases of diabetes developed among consistent exclusive snus users. Odds ratios for incident known diabetes associated with exclusive smoking or ex-smoking were significantly elevated compared to non-tobacco users, regardless of adjustment for various confounders. Smokers who switched to snus were not at significantly elevated risk of diabetes.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX M-2
COHORT STUDIES OF INSULIN RESISTANCE AND DIABETES AMONG SWEDISH SNUFF USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Eliasson et al. 2004 (continued)		Among 513 men with normal OGT at baseline	Odds Ratios (95% CI)	An oral glucose tolerance test was administered to 513 men who had normal glucose tolerance at baseline; these men formed the population at risk for impaired glucose tolerance (IGT) or diabetes. Risk of impaired glucose tolerance, diabetes, or PGT was not significantly increased among any category of tobacco user. The authors note that nonsignificantly elevated odds ratios among ex-snus users may be a chance finding, but deserve further examination. The authors appropriately note that a causal link between tobacco use and disease cannot be claimed on the basis of cross-sectional prevalence data. Cross-sectional studies only examine the relationship between exposure and disease at a single point in time, and thus can only address prevalence. In addition, the authors note that a limitation of this study is the small number of cases of diabetes. However, this study also provides strong data on incidence (*i.e.*, development of disease over time among individuals who were not diseased at study entry); causal conclusions *can* be drawn from such data. This is the first prospective study that demonstrates that use of snus does not carry the same increased risk for diabetes as smoking. Other study strengths include: a large number of subjects; about half of the incident cases of diabetes were confirmed by oral glucose tolerance test; and tobacco use was validated biochemically in a subgroup of subjects.
		Impaired GT		
		Consistent no tobacco	1.00 (reference)	
		Consistent exclusive smoking	0.68 (0.19-2.44)	
		Consistent exclusive snus	0.23 (0.03-1.80)	
		Ex-smokers	0.48 (0.21-1.08)	
		Ex-snus users	0.75 (0.16-3.57)	
		Smokers who switched to snus	1.18 (0.51-2.74)	
		Type 2 diabetes		
		Consistent no tobacco	1.00 (reference)	
		Consistent exclusive smoking	0.66 (0.08-5.58)	
		Consistent exclusive snus	0.91 (0.10-8.01)	
		Ex-smokers	1.27 (0.48-3.34)	
		Ex-snus users	3.97 (0.86-18.33)	
		Smokers who switched to snus	0 cases	
		PGT		
		Consistent no tobacco	1.00 (reference)	
		Consistent exclusive smoking	0.77 (0.25-2.41)	
		Consistent exclusive snus	0.45 (0.10-2.04)	
		Ex-smokers	0.73 (0.38-1.43)	
		Ex-snus users	1.85 (0.60-5.70)	
		Smokers who switched to snus	1.05 (0.46-2.44)	

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix M3

Experimental Studies of Insulin Resistance and Diabetes

APPENDIX M-3
EXPERIMENTAL STUDIES OF INSULIN RESISTANCE AND DIABETES AMONG SWEDISH SNUS USERS (N=1)

CITATION, LOCATION	STUDY TYPE, POPULATION, BRIEF DESCRIPTION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Attvall et al. 1993 Sweden This study examined the acute effect of smoking and snuffing on insulin sensitivity in a group of healthy habitual smokers.	Experimental human study Subjects were 7 healthy smokers (4 females and 3 males) aged 31 ± 2 years. All normal participants smoked at least 20 cigarettes per day for at least five years, and were of normal weight, took no regular medications, consumed moderate amounts of alcohol, and had no family history of diabetes or hypertension. Tests used to measure the acute effect of tobacco on insulin sensitivity included the euglycemic clamp technique, combined with the subcutaneous injection of a bolus of fast-acting insulin. Each subject underwent the following three studies (in random order) during a 4 week interval: 1) Smoking one filtered cigarette per hour during the clamp; 2) Consuming one bag of snuff every hour during the clamp following a 2 day abstinence from cigarettes; 3) Total tobacco abstinence for 2 days before, as well as during the clamp.	There was no difference observed in the insulin effect (the amount of glucose needed to maintain normoglycemia during the 6-hour clamp) between abstainers and snuffers. Abstainers and snuffers also experienced a significant increase ($p<0.05$) in basal glucose utilization when compared to smokers during the last 3-hours of the clamp. When examining insulin-antagonistic hormones, results indicate that growth hormone levels more than doubled ($p<0.01$) during both smoking and snuffing when compared to abstaining.	**The authors did not draw any specific conclusions about the effect of snuffing on insulin sensitivity. However, it can be noted that there was no difference in insulin action between snuffers and abstainers.** Due to the nature of their design, experimental studies are able to control exposure dose and duration. Experimental studies in theory should generate results with less variability than case-control or cohort studies, because outside factors influencing exposure data are eliminated. However, results cannot be generalized beyond the population studied (*i.e.*, young, healthy smokers).

Appendix M4

Case Control Studies of Insulin Resistance and Diabetes

APPENDIX M-4
CASE-CONTROL STUDIES OF INSULIN RESISTANCE AND DIABETES AMONG SWEDISH SNUS USERS (N=1)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hergens et al. 2005 Sweden This study assessed whether long-term use of snus increased risk of diabetes See Appendix J-2 for results on MI and Appendix O-3 for results on body weight.	Case-control study (population-based) Cases were 1,760 male patients with a first acute MI drawn from two methodologically equivalent case-control studies using identical questionnaires: a study consisting of Swedish men aged 45 to 70 years living in Stockholm County from 1992 to 1993, and a study of men aged 45 to 65 years living in Västernorrland County from 1993 to 1994. 1,432 of these cases provided data on tobacco use (1,173 nonfatal and 259 fatal). Controls consisted of 1,810 men randomly selected after stratification for age and hospital catchment area. Risk factors of MI were also investigated among the controls (including diabetes). "Snuff" was defined as Swedish moist snuff.	<u>Snuff Use</u> **Diabetes** Never Former Current	<u>Odds Ratio (95% CI)</u> 1.00 (reference) 1.1 (0.40–3.3) 1.5 (0.76–2.9)	**The authors state that "it is unclear to what extent snuff use could influence some of these risk factors [including diabetes]." The authors concluded that this study does not support the hypothesis that smokeless tobacco increases risk of MI.** The risk of diabetes among former or current snus users was not significantly elevated. Adjusting for diabetes, hyperlipidemia, hypertension, overweight, physical inactivity, and job strain had little impact on the risk estimates for MI. Odds ratios for diabetes were adjusted for age, hospital catchment area, and smoking.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix N1

Cohort Studies of Metabolic Syndrome

APPENDIX N-1
COHORT STUDIES OF METABOLIC SYNDROME (N=1)

CITATION, LOCATION,	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Norberg et al. 2006 Sweden This study was done to investigate associations between lifestyle factors and metabolic syndrome (MetSy), with a focus on the role of snus. Results on obesidy are presented in Appendix O-2.	Cohort study Subjects were a subset of the Västerbotten Intervention Programme, a community-based program to prevent CVD and diabetes. All inhabitants of Västerbotten are invited to participate in a health survey at the ages of 30, 40, 50, and 60 years. As part of the health survey, information on lifestyle is obtained by questionnaire and information on BMI, blood pressure, blood lipids, and glucose tolerance is obtained by physical exam. Subjects in this analysis were 16,492 men and women aged 30, 40, or 50 who were first examined in 1990-94 and who returned for follow-up 10 years later. Univariate and multivariate logistic regression analyses were performed, with lifestyle variables at baseline as predictors and the presence of MetSy at follow-up as the outcome. At study initiation, 2.7% of women and 18.9% of men used ≤4 cans of snus/week; 0.4% of women and 5.7% of men used >4 cans of snus/week. In this paper, snuff was defined as Swedish moist snuff.	Snus Use **Metabolic Syndrome** No use ≤4 cans/week >4 cans/week **Components of MetSy** Glucose >5.6 or Diabetes No use ≤4 cans/week >4 cans/week Triglycerides >1.7 No use ≤4 cans/week >4 cans/week Low HDL Cholesterol No use ≤4 cans/week >4 cans/week Hypertension No use ≤4 cans/week >4 cans/week Body Mass Index >30 No use ≤4 cans/week >4 cans/week	Odds Ratio (95% CI) 1.00 (reference) 1.0 (0.85-1.22) 1.6 (1.26-2.15)* 1.00 (reference) 1.0 (0.86-1.08) 1.8 (0.69-1.02) 1.00 (reference) 1.2 (1.05-1.35)* 1.6 (1.30-1.95)* 1.00 (reference) 1.0 (0.86-1.18) 1.1 (0.82-1.42) 1.00 (reference) 0.9 (0.84-1.05) 1.2 (0.99-1.46) 1.00 (reference) 1.0 (0.88-1.20) 1.7 (1.36-2.18)*	**The authors concluded that heavy use of snus is independently associated with the metabolic syndrome, even after adjustment for smoking. Snus has the greatest effect on hypertriglyceridemia and obesity.** The odds ratios for MetSy were adjusted for age, sex, and family history of CVD or diabetes. Those for the components of MetSy were adjusted for those factors and education, exercise, and alcohol use. It is unclear whether they were adjusted for smoking. The study had several strengths: it was large and population-based. The authors considered several definitions of MetSy, apparently with consistent results. However, it appears that people who had the disease of interest were not eliminated at baseline, as is necessary in a cohort study. Consequently, this study cannot demonstrate a temporal relationship. Furthermore, those who had MetSy at baseline may have been more likely to die and not return for follow-up; the authors do not address how this was handled. Although the investigators had data on tobacco use at baseline and 10 years later, this analysis only considered tobacco use at baseline. Subjects may have changed their tobacco habits during the long follow-up period, especially since this was an intervention program, in which subjects were advised how to reduce risk of CVD. The authors acknowledge that this study cannot explain the mechanism by which snus use could increase risk of MetSy.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix N2

Descriptive Studies of Metabolic Syndrome

APPENDIX N-2
DESCRIPTIVE STUDIES OF METABOLIC SYNDROME (N=1)

CITATION, LOCATION,	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Wandell et al. 2008 Sweden This study examined the potential association between use of tobacco, including smokeless tobacco, and metabolic syndrome and diabetes. Results on diabetes presented in Appendix M-1.	Population based cross-sectional study Subjects were 1,859 men, aged 60 years old living in Stockholm County from August 1997-March 1999. The men underwent a physical exam, lab tests, and a questionnaire, including medical data, and questions on demographic, socio-economic and life style factors, was completed. Metabolic syndrome was defined by the criteria from the National Cholesterol Education Program Adult Treatment Panel III (ATP III), from the European Group for the Study of Insulin Resistance (EGIR), and from the International Diabetes Federation (IDF). Use of tobacco was coded as never users of tobacco (n = 594), former smokers (n = 737), former smokers but current daily users of snuff (n = 113), current daily smokers (n = 360), former snuffers (n = 12), current snuffers (n = 16) and current daily smokers and snuffers (n = 27).	<u>Oral Snuff and Smoking Usage</u> **Metabolic Syndrome** Ex-smokers ATP III EGIR IDF Ex-smokers, current snuffers ATP III EGIR IDF Current smokers ATP III EGIR IDF Ex-snuffers ATP III EGIR IDF Current snuffers ATP III EGIR IDF Current smokers and snuffers ATP III EGIR IDF	<u>Odds Ratio (95% CI)</u> 1.49 (1.15-1.92)* 1.55 (1.17-2.06)* 1.44 (1.14-1.83)* 1.14 (0.71-1.82) 1.29 (0.78-2.14) 1.18 (0.76-1.83) 1.18 (0.86-1.62) 0.95 (0.66-1.37) 1.00 (0.74-1.35) 0.69 (0.14-3.28) 0.97 (0.20-4.67) 0.48 (0.10-2.26) 1.55 (0.52-4.62) 0.71 (0.16-3.24) 1.81 (0.65-5.02) 1.46 (0.63-3.41) 0.47 (0.14-1.63) 0.85 (0.36-2.02)	**The authors conclude that an association between use of snuff and risk of metabolic syndrome was not found.** The prevalence of smokers and snus users in this cohort was comparable to the general Swedish population at the same age. The authors collected information on smoking duration and snus consumption so a potential tendency for a dose-response relationship could be assessed. Due to the nature of their design, causality cannot be determined from cross-sectional studies since disease and exposure are measured simultaneously. The power to detect a potential association in this study was low.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix O1

Descriptive Studies of Body Weight

APPENDIX O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (N=8)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Saarni et al. 2004 Finland This study investigated whether cigarette smoking and lifetime snuff use were associated with intentional weight loss in young adults.	Cross-sectional (population-based) Subjects included 4,521 young adult Finnish twins aged 23-27 years. Subjects responded to a questionnaire about how many times they had intentionally lost at least 5 kg; those who reported having done so at least 2 times were classified as having intentional recurrent weight loss episodes. Data were also gathered on BMI, cigarette smoking, snuff use, educational level, and number of children. Snuff use was classified in 3 categories according to the number of times ever used (0-1; 2-50; or > 50 times). The association between tobacco use and weight loss was analyzed by logistic regression.	<u>Lifetime Frequency of Snuff Use</u> **Men** 0-1 time 2-50 times >50 times **Women** 0-2 time 2-50 times >50 times	<u>Odds Ratios (95% CI)</u> 1.00 (reference) 1.51 (1.08-2.13)* 1.41 (0.91-2.19) 1.00 (reference) 1.63 (0.98-2.70) -----	**The authors concluded that frequent lifetime snuff use was statistically significantly associated with recurrent intentional weight loss episodes in men.** Odds ratios were adjusted for BMI, age, educational level, and number of children. Snuff use was quite uncommon among women; only 4 women reported using snuff at least 2 times. This study, like all cross-sectional studies, has inherent weaknesses. It examines prevalence of the outcome, not incidence, and thus cannot comment on factors that affect the development of disease. Furthermore, cross-sectional studies cannot address temporal sequence (*i.e.*, whether the snuff use preceded the weight loss or not).

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (N=8) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Sundbeck et al. 2009 Sweden This study investigated whether snuff consumption was associated with obesity.	Cross-sectional (population-based) Subjects included 834 men aged 30–75 years with a mean age of 48.2 years old who's habits of smoking and snuff use were assessed by self-reported questionnaires. Of these men 21% (n=179) were snuff users, 13% (n=109) were current smokers, and 65% (n=546) were non-users. Of all snuff users 65% (n=116) were former smokers, and 35% (n=63) were exclusive snuff users. Obesity was measured by Body mass index (BMI), and also waist circumference (WC) and waist-hip ratio (WHR) which define abdominal obesity. The association between snuff use and obesity was analyzed by logistic regression.	<u>Oral Snuff and Smoking Usage</u> **BMI ≥30 kg/m^2** All All snuff users Current exclusive snuff users Current snuff users who quit smoking Quit smoking without any nicotine substitute Current exclusive smokers **WHR ≥1.0** All All snuff users Current exclusive snuff users Current snuff users who quit smoking Quit smoking without any nicotine substitute Current exclusive smokers **WC >102 cm** All All snuff users Current exclusive snuff users Current snuff users who quit smoking Quit smoking without any nicotine substitute Current exclusive smokers See Sundbeck et al. 2009 for further analyses.	<u>Odds Ratio (95% CI)</u> 1.24 (0.75-2.06) 0.83 (0.36-1.90) 1.51 (0.87-2.63) 2.10 (1.32-3.35)* 1.11 (0.65-2.04) 1.04 (0.55-1.95) 0.60 (0.20-1.82) 1.31 (0.66-2.61) 1.84 (1.08-3.12)* 1.16 (0.59-2.27) 1.27 (0.78-2.06) 1.01 (0.47-2.17) 1.45 (0.84-2.50) 1.71 (1.08-2.72)* 1.18 (0.67-2.10)	**The authors conclude that the study showed that abdominal obesity was greater the higher the snuff consumption. This association was limited to former smokers, however, and was not seen among exclusive snuff users. The authors note: "Thus, the weight increase commonly seen among former smokers should be considered as the possible causal factor."** Odds ratios were adjusted for differences in age, physical activity and education. The authors collected information on individual snuff consumption so a potential tendency for a dose-response relationship could be assessed. Since exclusive snuff users were specifically examined, the remaining effects of smoking could be excluded. This study is limited in that alcohol consumption and energy-intake could not be accounted for in addition to the low sample size. Former smokers who quit smoking without use of any nicotine replacement were the only group with a significant association with overall obesity, and no associations were found between any category of snuff use and overall obesity compared to non-users.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (N=8) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Bolinder et al. 1997a Sweden This study investigated the possible influence of long-term exposure to smokeless tobacco on the atherosclerotic process and risk factors including waist/hip-ratio and BMI in middle-aged men in Sweden. [This study includes individuals from the same study population as Bolinder et al. 1997b, and Bolinder and de Faire 1998. This paper was one of 6 papers that were the basis of Bolinder's 1997 dissertation.] See Appendix J-1 for results on CV Effects.	Descriptive study The study population included 143 healthy male firefighters aged 35-60 years old. Atherosclerotic development was determined using carotid ultrasonography of the right carotid artery. In addition, blood levels of biochemical risk factors for cardiovascular disease (serum lipids, serum lipoproteins, and plasma fibrinogen) were determined. Study subjects were classified into major tobacco habit groups of smokeless tobacco users who had never smoked (n=28), smokers (n=29), and never users of tobacco (n=40). Inter-group comparisons used only these three groups. The remaining subjects (n=46) included ex-tobacco users or those who had switched from one tobacco habit to the other. "Snuff" is also referred to as smokeless tobacco, and is defined in this paper as ground and moistened dark tobacco, buffered to a pH of about 8.5 with sodium carbonate.	<u>Oral Snuff and Smoking Usage</u> **BMI** Never-users of tobacco Smokeless tobacco users Smokers **Waist/hip-Ratio** Never-users of tobacco Smokeless tobacco users Smokers	<u>Level of significance for differences between smokers or smokeless tobacco users and never-users of tobacco</u> Nonsignificant Nonsignificant p<0.001* Nonsignificant Nonsignificant p<0.001*	**The authors concluded that the group of smokeless tobacco users did not differ significantly from the never-users regarding body mass index or waist hip ratio.**

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (N=8) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Eliasson et al. 1995 Northern Sweden This study examined the influence of cigarette smoking and use of smokeless tobacco on BMI and Waist/hip-ratio (WHR). See Appendix J-1 for results on CV Effects.	Descriptive study Subjects included 1,583 participants of the MONICA study (Monitoring Trends and Determinants in Cardiovascular Disease), who were selected from a group of 2000 (1000 men and 1000 women) aged 25-64 years. Between January 1990 and April 1990 subjects underwent blood sampling for plasma fibrinogen levels and fibrinolytic activity (tissue plasminogen activator [tPA] activity and plasminogen activator inhibitor type 1 [PAI-1] activity). A subset of these subjects (n=754) underwent oral glucose tolerance testing. Subjects were classified into five categories of tobacco use. Snuff dippers were defined as regular users of moist snuff who did not use other types of tobacco (n=92 men and 12 women). The women snuff dippers were excluded from this analysis. "Snuff" is also referred to as smokeless tobacco, and is defined in this paper as a form of moist oral snuff.	<u>Oral Snuff and Smoking Usage</u> **BMI** Non-tobacco users Ex-smokers Smokers Snuff dippers Snuff and cigarette users **Waist/hip-Ratio** Non-tobacco users Ex-smokers Smokers Snuff dippers Snuff and cigarette users	<u>Level of significance for differences across groups</u> Nonsignificant p<0.001* (WHR not significantly greater among snuff users compared to non-tobacco users)	**The authors concluded that BMI did not differ significantly between groups, and that men who were current or previous smokers had greater WHR than non-tobacco users and snuff users.** The WHR for snuff users was not significantly greater than the WHR among non-tobacco users.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (N=8) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Wallenfeldt et al. 2001 Sweden The study examined the association between smokeless tobacco use, smoking, BMI and waist-hip ratio(WHR). See Appendix J-1 for results on CV Effects.	Descriptive study (cross-sectional) Subjects were 391 clinically healthy men of Swedish ancestry (all 58 years old), who were randomly selected from the general population. Subjects were excluded if they had cardiovascular or other clinically overt diseases, or if they were taking cardiovascular medications. Cardiovascular risk factors were assessed by biochemical analysis of blood and by ultrasonography of carotid and femoral arteries. Smoking and snuff habits were assessed by questionnaire. Present use of snuff was defined as at least one snuff-dipping per day. 48 men were current snuff users and 33 were previous snuff users. Only 4 of the 81 current or previous snuff users had never smoked. "Snuff" is also referred to as smokeless tobacco, and is described as moist snuff.	<u>Oral Snuff and Smoking Usage (Snuff-years)</u> **BMI** **WHR** **BMI** **WHR**	<u>Spearman's r-value</u> 0.09 0.11* (p-value<0.01) <u>Level of significance for differences across groups</u> Nonsignificant Nonsignificant	**The authors concluded that oral use of moist snuff (in snuff years) is associated with waist-hip ratio, but not BMI.** However, no significant differences in BMI or WHR were observed among never, ex- and current snuff users. The authors acknowledge that no conclusions can be drawn regarding causality from this cross-sectional study. There was a close relation between smoking and snuff taking.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (N=8) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Bolinder et al. 1992 Sweden The aim of this study was to investigate the relationship between tobacco consumption habits and general health status. [This study includes individuals from the same study population as Bolinder et al. 1994**.** This paper was one of 6 papers that were the basis of Bolinder's 1997 dissertation.] Data on gastrointestinal and CV effects observed in this study are summarized in Appendices L-1 and J-1 respectively.	Descriptive study (cross-sectional study) Subjects in this population survey were 97,586 male construction workers (16-65 years of age) who received health examinations during 1971 through 1974. Physical examinations included blood pressure and heart rate measurements and included a questionnaire about tobacco use and health status. Information was also acquired on sick leave and the allocation of disability pensions. Of the 97,586 subjects examined, 59,864 were excluded because of use of more than 1 type of tobacco product or because they were ex-smokers. The remaining subjects (n=37,722; 1,370 of whom were disability pensioners) were grouped for analysis by tobacco habit: non-users (n=23,885), smokeless tobacco users who had never been regular smokers (n=5,014), and smokers of = 15 cigarettes per day who had never been	<u>Snus and Smoking Usage</u> **BMI<22** Snus Users Age (years) ≤35 36-45 46-55 ≥56 Smokers Age (years) ≤35 36-45 46-55 ≥56 **BMI>26** Snus Users Age (years) ≤35 36-45 46-55 ≥56 Smokers Age (years) ≤35 36-45 46-55 ≥56	<u>Odds Ratios (95% CI):</u> 1.0 (0.9-1.1) 1.0 (0.7-1.2) 1.0 (0.8-1.3) 1.1 (0.9-1.3) 1.3 (1.2-1.4)* 1.5 (1.3-1.7)* 2.2 (1.9-2.6)* 2.9 (2.4-3.6)* 1.1 (0.9-1.2) 1.3 (1.1-1.5)* 1.5 (1.3-1.7)* 1.2 (1.1-1.4)* 1.0 (0.9-1.1) 0.9 (0.7-1.0) 0.7 (0.6-0.8) 0.5 (0.4-0.6)	**The authors concluded that snus users did not differ from non-users in the prevalence of underweight (BMI<22) though prevalence of overweight (BMI>26) was significantly elevated among some age groups (36-45, 46-55 and ≥56 years) but not among those 35 or younger. The prevalence of underweight among smokers was significantly higher whereas the prevalence of overweight did not differ from non-users of tobacco.** The authors note that the reasons for lower BMI among smokers and higher obesity among snus users could be related to behavior.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (N=8) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Bolinder et al. 1992 (continued)	regular users of smokeless tobacco (n=8,823). "Snuff" is referred to as smokeless tobacco, and is defined as mainly moist snuff in this paper.			

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (N=8) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Eliasson et al. 1991 Sweden This study evaluated cardiovascular risk factors among healthy young males who were habitual snuffusers, and compared them with the same risk factors in nontobacco users and cigarette smokers.	Descriptive study This study used young male volunteers recruited from university students, teachers, and blue-collar workers. All subjects were ≤ 31 years old and weighed ≤ 28 kg. All subjects underwent a physical exam (including blood pressure, blood chemistry, and hematology) completed a questionnaire about habits. All testing was completed after an overnight fast and abstention from tobacco and abstention from alcohol for 24 hours. Subjects included never-users of tobacco (n=18), users of at least 50 g of moist snuff per week for 2 years (n=21; 5 of whom were ex-smokers), and smokers of at least 10 cigarettes per day for 2 years (n=19; 1 of whom had used snuff previously). "Snuff" is also referred to as smokeless tobacco and is defined as moist oral snuff in this paper.	<u>Oral Snuff and Smoking Usage</u> **BMI** Snuff users Smokers	<u>Level of significance for differences across groups</u> Nonsignificant Nonsignificant	**The authors found that BMI did not differ significantly between non-tobacco users and snuff-users or for smokers.** The authors noted that considerable differences in life style were observed across the groups, with lower levels of physical activity and higher levels of alcohol and coffee consumption among tobacco users. The authors also noted that the timing of use of tobacco products was not considered in this analysis, but that the low plasma nicotine levels in the tobacco-using subjects confirmed that subjects had abstained from smoking or taking snuff prior to the examination.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (N=8) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Bolinder and de Faire 1998 Sweden The goal of this study was to investigate whether the use of smokeless tobacco among healthy middle-aged men is associated with any alteration in blood pressure and heart rate during daytime and nighttime, compared with smokers and nonusers of tobacco. [This study includes individuals from the same study population as Bolinder et al. 1997a, and Bolinder et al. 1997b. This paper was one of 6 papers that were the basis of Bolinder's 1997a dissertation.]	Descriptive study The study population included 135 healthy male firefighters aged 35-60 years. Subjects received both a clinical blood pressure measurement and 24-hour ambulatory blood pressure recordings. Study subjects were classified into three major tobacco habit groups of smokeless tobacco users (n=47), smokers (n=29), and non-users of tobacco (n=59). Smokeless tobacco users in this analysis included both subjects who had never smoked but used smokeless tobacco (n=27) and ex-smokers who currently used smokeless tobacco (n=20). "Snuff" is also referred to as smokeless tobacco, and is not defined in this paper.	<u>Oral Snuff and Smoking Usage</u> **BMI** Snuff users Smokers **Waist-hip ratio** Snuff users Smokers	<u>Level of significance for differences across groups</u> Nonsignificant Nonsignificant Nonsignificant p-value<0.001	**The authors found that BMI did not differ significantly between non-tobacco users and snuff-users or for smokers. Smokers, however, had a significantly higher waist-hip ratio compared with non-tobacco users.**

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix O2

Cohort Studies of Body Weight

APPENDIX O-2
COHORT STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (N=3)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Nafziger et al. 2007 Sweden The goal of this study was to characterize who did not gain weight during a 10-year period in Sweden.	Cross-sectional and prospective follow-up Subjects were participants in the Västerbotten Intervention Program (aged 30, 40, 50, and 60 years). The cross-sectional study included 82,927 adults; the longitudinal study included 14,867 adults. The prevalence of obesity was calculated for the 40, 50, and 60-year-olds from the annual cross-sectional studies between 1990 and 2004. In the longitudinal study, 10-year non-gain (lost weight or maintained body weight within 3% of baseline weight) or weight gain (≥3%) was calculated for individual aged 30, 40, or 50 years at baseline (1990-1994) and at 10-year follow-up (2000-2004). Multivariate logistic regression identified factors associated with weight non-gain. Snus use was assessed only as "yes" or "no."	Weight Non-Gain in the Longitudinal Study **Snuff Use** No Yes	Odds Ratios (95% CI) 1.00 (reference) 0.83 (0.74-0.92)**	**The authors concluded that lack of snuff use increased the chances of not gaining weight.** The longitudinal analysis was restricted to subjects with a baseline BMI of 18.5-29.9. In the cross-sectional studies, the prevalence of obesity (BMI ≥30 kg/m^2) increased from 9.4% in 1990 to 17.5% in 2004. In the longitudinal study, 35.3% of adults were categorized as non-gainers. Other variables associated with weight non-gain were older age, being female, being classified as overweight by baseline BMI, later survey year, and baseline diagnosis of diabetes. It is unclear whether the odds ratios presented here were adjusted for these variables. "Weight gain" is defined very stringently in this study as 3% of baseline body weight. The authors do not discuss the significance of this reported finding, nor do they speculate on a mechanism to explain this reported association. The authors noted that there were differences between participants and nonparticipants in the longitudinal study that should have resulted in more conservative odds ratios.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX O-2
COHORT STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (N=3) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Rodu et al. 2004 Sweden This study investigated the effect of tobacco use (cigarettes and smokeless tobacco) and cessation on body weight.	Cross-sectional and prospective follow-up Subjects included 2,993 men aged 25-64 years who participated in the northern Sweden MONICA study in 1986, 1990, or 1994; 1,650 of whom were followed up in 1999. The prevalence of overweight (BMI $\geq$ 27) was determined among cigarette smokers, snus users and nonusers of tobacco at study entry. Average annual weight gain was reported according to tobacco use at entry and at follow-up, and the development of overweight among various tobacco use groups was calculated using standardized incidence ratios. There were 3 mutually exclusive categories of snus users: ex, current, and never.	Prevalence of Overweight at Study Entry **Tobacco Use** Never use Current exclusive smoking Current exclusive snus use Current combined use Development of Overweight During Follow-up Among Men Not Overweight at Entry **Tobacco Use At Entry/At Follow-Up** Never/no tobacco Smoking/smoking Smoking/snus Smoking/no tobacco Snus/snus Snus/no tobacco	Prevalence Ratios (95% CI) 1.00 (reference) 0.87 (0.73-1.03) 1.20 (1.01-1.42)* 1.25 (1.03-1.63)* Standardized Incidence Ratios (95% CI) ----- 88 (49-145) 80 (22-205) 198 (124-299)* 120 (84-167) 142 (78-264)	**The authors concluded that primary snus use does not have major implications for weight gain, and that smokers who switch to snus may avoid the weight gain that typically occurs after quitting smoking.** Prevalence ratios were adjusted for age and entry year. Standardized incidence ratios were adjusted for age and years of follow-up. At study entry, the prevalence of overweight varied by group, ranging from 28.7% among smokers to 32.5% among snus users to 42.1% among ex-smokers. Smokers who quit all tobacco during follow-up gained significantly more weight (average annual gain of 0.96%) than those who switched to snus (0.51%) ($p<0.05$). Snus users who quit gained more weight than nonusers (0.70% vs. 0.44%, $p<0.05$) or those who continued to use snus (0.42%).

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX O-2
COHORT STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (N=3) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Norberg et al. 2006 Sweden This study was done to investigate associations between lifestyle factors and metabolic syndrome (MetSy), with a focus on the role of snus. Analyses were carried out to investigate associations with separate components of metabolic syndrome, including obesity. Results on metabolic syndrome are presented in Appendix N-1.	Cohort study Subjects were a subset of the Västerbotten Intervention Programme, a community-based program to prevent CVD and diabetes. All inhabitants of Västerbotten are invited to participate in a health survey at the ages of 30, 40, 50, and 60 years. As part of the health survey, information on lifestyle is obtained by questionnaire and information on BMI, blood pressure, blood lipids, and glucose tolerance is obtained by physical exam. Subjects in this analysis were 16,492 men and women aged 30, 40, or 50 who were first examined in 1990-94 and who returned for follow-up 10 years later. Multivariate regression analyses were performed for separate components of MetSy including obesidy determined by a BMI ≥30. At study initiation, 2.7% of women and 18.9% of men used ≤4 cans of snus/week; 0.4% of women and 5.7% of men used >4 cans of snus/week. In this paper, snuff was defined as Swedish moist snuff.	**Body Mass Index ≥30** Smoking Ex-smoker Daily smoking Use of snus ≤4 cans/week >4 cans/week	Odds Ratio (95% CI) 1.2 (1.04-1.30)* 1.1 (0.98-1.23) 1.0 (0.88-1.20) 1.7 (1.36-2.18)*	**The authors concluded that high use of snus consumption was associated with obesity.** Odds ratios for obesity were adjusted for age, sex, family history of CVD or diabetes, education, exercise, and alcohol use. It is unclear whether they were adjusted for smoking. The study had some strengths: it was large and population-based. However, it appears that people who had the disease of interest were not eliminated at baseline, as is necessary in a cohort study. Consequently, this study cannot demonstrate a temporal relationship. Although the investigators had data on tobacco use at baseline and 10 years later, this analysis only considered tobacco use at baseline. Subjects may have changed their tobacco habits during the long follow-up period, especially since this was an intervention program, in which subjects were advised how to reduce risk of CVD.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix O3

Case-Control Studies of Body Weight

APPENDIX O-3
CASE-CONTROL STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (N=1)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Hergens et al. 2005 Sweden This study assessed whether long-term use of snus increased risk of being overweight (BMI ≥30 kg/m^2). See Appendix J-2 for results on MI and Appendix M-4 for results on diabetes.	Case-control study (population-based) Cases were 1,760 male patients with a first acute MI drawn from two methodologically equivalent case-control studies using identical questionnaires: a study consisting of Swedish men aged 45 to 70 years living in Stockholm County from 1992 to 1993, and a study of men aged 45 to 65 years living in Västernorrland County from 1993 to 1994. 1,432 of these cases provided data on tobacco use (1,173 nonfatal and 259 fatal). Controls consisted of 1,810 men randomly selected after stratification for age and hospital catchment area. Risk factors of MI were also investigated among the controls (including overweight). "Snuff" was defined as Swedish moist snuff.	<u>Snuff Use</u> **Overweight** Never Former Current	<u>Odds Ratio (95% CI)</u> 1.00 (reference) 1.5 (0.79–2.8) 1.9 (1.2–2.9)*	**The authors state that "it is unclear to what extent snuff use could influence some of these risk factors [including overweight]." The authors concluded that this study does not support the hypothesis that smokeless tobacco increases risk of MI.** Being overweight was significantly elevated among current snus users. Adjusting for diabetes, hyperlipidemia, hypertension, overweight, physical inactivity, and job strain had little impact on the risk estimates for MI. Odds ratios of being overweight were adjusted for age, hospital catchment area, and smoking.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix P1

Cohort Studies of Pregnancy Outcomes and Reproductive Effects

APPENDIX P-1
COHORT STUDIES OF PREGNANCY OUTCOMES AMONG SWEDISH SNUS USERS (N=1)

CITATION, LOCATION,	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
England et al. 2003 Sweden This study examined the effects of snus use during pregnancy on birth weight, small-for-gestational-age birth, preterm delivery, and preeclampsia among women who had a live, single birth from 1999 through 2000. Risk among snus users was compared to that among cigarette smokers and nonusers of tobacco.	Cohort study Subjects were 23,524 women who were delivered of singleton, live-born infants in Sweden from 1999 through 2000. Information on birth outcomes (birth weight, preterm delivery, and preeclampsia) and tobacco use was obtained from the Swedish Medical Birth Register. There were 789 daily snuff users (who did not smoke cigarettes), 11,240 smokers (who did not use snuff), and 11,495 nonusers of tobacco. Smokeless tobacco is not defined in this paper, but is assumed to be Swedish snus as the cohort population is women who gave birth in Sweden.	Pregnancy Outcome **Small-For-Gestational-Age Birth (>2 SD below mean weight)** Nonusers of tobacco Snus users Cigarette smokers **Preterm Delivery (<37 weeks gestation)** Nonusers of tobacco Snus users Cigarette smokers **Preeclampsia** Nonusers of tobacco Snus users Cigarette smokers	Odds Ratio (95% CI) 1.00 (reference) 1.25 (0.72-2.17) 2.99 (2.48-3.61)* 1.00 (reference) 1.98 (1.46-2.68)* 1.57 (1.38-1.80)* 1.00 (reference) 1.58 (1.09-2.27)* 0.63 (0.53-0.75)**	**The authors concluded that daily use of snuff during pregnancy was associated with increased risk of preterm delivery and preeclampsia, but not with an increased risk of small-for-gestational age birth.** Adjusted mean birth weight was reduced in snuff users by 39 gms (95% CI:6-72 gms), and in cigarette smokers by 190 gms (95% CI:178-202 gms), compared to nonusers of tobacco. Odds ratios were adjusted for gestational age at delivery (birth weight only), infant sex (birth weight and preterm delivery), maternal age, height, body mass index, and parity (birth weight, small-for-gestational age birth, preterm delivery, and preeclampsia).

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix P2

Descriptive Studies of Pregnancy Outcomes and Reproductive Effects

APPENDIX P-2
DESCRIPTIVE STUDIES OF PREGNANCY OUTCOMES AMONG SWEDISH SNUS USERS (N=1)

CITATION, LOCATION,	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Richthoff et al. 2008 Sweden This study examined the impact of tobacco smoking and snuffing on reproductive characteristics of young males.	Cross-sectional study Subjects were male military conscripts, 217 non-smokers 85 smokers, and 51 snuffers (based on data from 242 conscripts) with a median age of 18 at enrollment. Lifestyle-associated factors including maternal smoking during pregnancy and snuffing, were recorded. All participants filled out a questionnaire regarding smoking and drinking habits, mothers' tobacco smoking during pregnancy, and possible incidence of congenital abnormalities. 15% of non-smokers were snuff users, 22% of smokers used snuff. Overall, 17% of the participants used snuff based on data from 242 of the 302 men. Snuff is not defined in this paper, but is assumed to be Swedish snus as the men live in Sweden.	<u>Reproductive Outcome</u> **Semen parameters** **Seminal biochemical biomarkers** **Hormone levels**	<u>*p*-Value</u> nonsignificant nonsignificant nonsignificant	**The authors concluded that use of snuff did not have any effect on any of the reproductive parameters evaluated; however tobacco smoking was associated with negative impacts. This may suggest that it is not tobacco itself that causes negative impacts on reproductive parameters but rather the compounds which are released by smoking.** Due to the nature of their design, causality cannot be determined from cross-sectional studies since disease and exposure are measured simultaneously.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix Q1

Cohort Studies of Other Health Effects

APPENDIX Q-1
COHORT STUDIES OF OTHER HEALTH EFFECTS (N=3)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Fang et al. 2006 Sweden This study examined the association between cigarette smoking, snuff dipping, and the risk of incident amyotrophic lateral sclerosis (ALS) in a large cohort of Swedish male construction workers.	Cohort study Subjects were 280,558 male Swedish construction workers who underwent periodic preventive health check-ups (with first registration from 1978 to 1993). Information on tobacco use was obtained by personal interviews with nurses. Incidence of ALS was ascertained by linkage to the Swedish Inpatient Register. Follow-up was carried out through linkage with nationwide death and migration registries. Subjects were followed until date of first ALS diagnosis, emigration, death, immigration to a country without or with incomplete Inpatient Register, or December 31, 2004, whichever occurred first. Adjusted relative risks were derived from Cox proportional hazard regression models. At study initiation, 13.6% of subjects were pure snuff dippers and 17.3% were mixed snuff dippers and smokers. The type of snuff used in this population is assumed to be Swedish snus as the cohort consists of Swedish men.	<u>Incidence of ALS</u> Non-tobacco use Pure snuff dipping Mixed snuff dipping/smoking	<u>Relative Risk (95% CI)</u> 1.00 (reference) 0.6 (0.3-1.5) 0.9 (0.6-1.4)	**The authors concluded that their study provides no evidence that smoking or snuff dipping is associated with increased risk of ALS among men.** Relative risks were adjusted for age and county of residence. However, the authors did not adjust for some potential confounders, such as socioeconomic status or alcohol consumption. The study cohort was large, there was a high prevalence of snus use, the follow-up time was long (19.6 years on average), and the follow-up was almost complete. A reanalysis that excluded cases identified during the first 5 years of follow-up (in response to the concern that there may be a long preclinical period before ALS diagnosis) did not yield materially different results. A weakness of this study is that tobacco habits were assessed only at study entry; changes in tobacco habits over time could affect the results. Also, there were few cases of ALS among snus users (6 among pure snuff dippers; 30 among mixed snuff dippers/smokers).

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX Q-1
COHORT STUDIES OF OTHER HEALTH EFFECTS (N=3) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Lindström et al. 2006 Sweden This study was done to assess the effect of smoking, use of Swedish snus, and obesity on post-operative complications after inguinal hernia surgery.	Cohort study Subjects were male Swedish construction workers who underwent periodic preventive health check-ups. A detailed tobacco consumption history was obtained through self-administered questionnaire. Construction workers who had undergone first-time open inguinal hernia repair were identified (n=12,697) through linkage to the Swedish Inpatient Register. Subjects were followed until December 31, 2004. Post-operative complications occurring within 30 days of hospitalization, as well as length of hospitalization, were recorded. Risk of post-operative complications due to tobacco exposure was estimated in a multiple logistic regression model and length of hospital stay was estimated in a multiple linear regression model. At study initiation, 20.9% of subjects had ever used snus.	<u>Snus Use</u> **Any Complication** Never user of snus Ever user of snus **Mean Length of Hospital Stay** Never user of snus Ever user of snus	<u>Odds Ratio (95% CI)</u> 1.00 (reference) 0.93 (0.71-1.22) <u>Coefficient (95% CI)</u> ---- (reference) 0.02 (0.00-0.04) *p=0.15*	**The authors concluded that use of Swedish snus did not affect either the complication rate or the length of hospitalization after hernia surgery.** In contrast, current smoking was significantly associated with postoperative complications. Odds ratios and regression coefficients were adjusted for age, calendar period, body mass index, and acute surgery. Strengths of this study are its large size and prospectively collected data on tobacco use. The quality of the smoking data has been reviewed and is considered to be high. When answers 2 to 3 years were compared, inconsistencies in the snus data were present for 7% of the workers. However, the authors acknowledge that there was a low overall rate of complications, largely due to a failure of complete registration in the Swedish inpatient register. They do not believe that this should have affected the study results, as any misclassification is most likely nondifferential.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX Q-1
COHORT STUDIES OF OTHER HEALTH EFFECTS (N=3) (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
W-Dahl and Toksvig-Larsen 2007 Sweden This study examined the effect of snuff use and smoking on the time for bone healing.	Cohort study Subjects were 175 male hospital patients comprising of 41 smokers, 21 oral snuff users, and 113 non-smokers/non-snuffers who were operated on for knee deformity by tibial osteotomy between 2000 and 2005. Preoperative tobacco use, postoperative complications, and treatment time in external fixation were documented. The type of snuff used in this population is assumed to be Swedish snus as the cohort consists of Swedish men who use snuff.	<u>Delayed bone healing</u> Smokers vs Snuffers Smokers vs non-smokers/non-snuffers Oral snuffers vs non-smokers/non-snuffers	<u>Difference in time in external fixation (CI)</u> 12 days (0.004-25) *p*=0.05* 6 days (-0.3-13) *p*=0.05* -6.1 days (-12.7-0.5) *p*=0.07	**The authors conclude that the use of snuff does not have the negative effects-such as delayed bone healthing and increased risk of post-operative complications –that cigarette smoking has.** There were no cases of delayed healing among the oral snuff users. These results confirm other findings of another study that delayed bone healing in smokers was the result of smoke components other than nicotine. Some limitations of this study include the fact that there was no information on amount or duration of snus use or smoking, so dose-response analyses were not possible. The results were adjusted for age, size of correction, and simultaneous bilateral surgery.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix Q2

Descriptive Studies of Other Health Effects

APPENDIX Q-2
DESCRIPTIVE STUDIES OF OTHER HEALTH EFFECTS (N=2)

CITATION, LOCATION,	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Ellingsen et al. 2009 Norway This study examined the association between cigarette smoking, snuff use and the biomarkers of selenium status.	Cross-sectional study Subjects were 98 blue-collar, male workers from south Norway. Subjects were interviewed and also submitted biological samples. Alcohol and smoking/snuff use was obtained through questionnaire. At study initiation, 49 of subjects were non-smokers/non-snuff users, while 38 and 11 were smokers and snuff users respectively. The type of snuff used in this population is assumed to be Swedish snus as the cohort consists of Norwegian men.	<u>Selenium Status</u> **Selenium in serum (S-Se)** Non-smokers/non-snuff users Smokers Snuff users *p*-value (between smokers and non-smokers/non-snuff users) **Selenium in whole blood (B-Se)** Non-smokers/non-snuff users Smokers Snuff users *p*-value (between smokers and non-smokers/non-snuff users)	<u>Mean (µmol/L) (range)</u> 1.54 (0.9-2.7) 1.34 (0.7-2.0) 1.55 (1.1-2.1) p<0.05* 1.52 (1.0-2.3) 1.38 (0.9-2.1) 1.50 (1.3-1.9) p<0.05*	**The authors concluded that smoking, not snuff use, is associated with lower concentrations of B-Se and S-Se.** Regression analysis adjusted for mercury exposure, alcohol consumption, number of fish meals/week, prescribed medication and exposure to chloralkali. Snuff users had about the same levels of B-Se and S-Se as the non-smokers/non-snuff users, although they had about the same amount of nicotine metabolites in urine and serum as the smokers. Due to the nature of their design, causality cannot be determined from cross-sectional studies since disease and exposure are measured simultaneously.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

APPENDIX Q-2
DESCRIPTIVE STUDIES OF OTHER HEALTH EFFECTS (N=2) (continued)

CITATION, LOCATION,	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Jakobsson 2008 Sweden This study examined the relationship between tobacco use and pain intensity.	Population-based cross-sectional study Subjects were 384 male and female individuals aged 18-102 years from southern Sweden who reported chronic pain for a duration of at least 3 months. Questionnaires were used to gather data on demographics, subjective health, chronic pain (e.g. intensity, duration, and location), and pain management. Pain intensity was measured using a rating scale from 0 to 6, 6 being "very intense pain". Pain duration was measured in years. At study initiation 12.5% of the population reported ever using moist snuff. The type of snuff used in this population is assumed to be Swedish snus as the cohort consists of Swedish men and women who use moist snuff.	<u>Snus Use</u> Have quit Occasionally Daily	<u>Coefficient (95% CI)</u> 0.959 (0.063-1.856) *p*=0.036* 1.282 (-0.065-2.628) *p*=0.062 -0.039 (-0.740-0.661) *p*=0.912	**The author concluded that there was no significantly higher pain intensity among those who used moist snuff compared with those who did not.** In contrast, smokers experienced higher pain intensity than nonsmokers. This relationship was also found among those who had quit smoking. Regression coefficients were adjusted for age and gender. Due to the nature of their design, causality cannot be determined from cross-sectional studies since disease and exposure are measured simultaneously. Because tobacco is often used for coping with stress, it is possible that occasional smokers resorted to using tobacco more to cope with chronic pain and end up grouped daily smokers.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix Q3

Case Control Studies of Other Health Effects

APPENDIX Q-3
CASE-CONTROL STUDIES OF OTHER HEALTH EFFECTS (N=1)

CITATION, LOCATION,	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Hedstrom et al. 2009 Sweden This study estimated the influence of tobacco smoking and Swedish snuff use on the risk of developing multiple sclerosis (MS).	Case-control study (population-based) Subjects were 902 incident cases of MS, and 1,855 randomly selected controls (male and female) aged 16-70 years old. Information on exposure was collected by questionnaire. The type of snuff used in this population is Swedish snus.	<u>Smoking and Snuff Use</u> **Smoking** Ever-smoker Ex-smoker <5y since stopping ≥5y since stopping Current smoker Pack-years ≤5 6-10 11-15 16+ p Value for trend **Snuff Use** Never smokers (pack-years) Current Snuff users <5 ≥5 p Value for trend Ever smokers (pack-years) <5 ≥5 p Value for trend >15y prior to disease onset (See Hedstrom et al. 2009 for additional analyses)	<u>Odds Ratio (95% CI)</u> 1.5 (1.3-1.8)* 1.4 (1.1-1.8)* 1.5 (1.1-2.0)* 1.0 (0.8-1.3) 1.6 (1.3-1.9)* 1.3 (1.0-1.6) 1.5 (1.1-2.0)* 1.7 (1.2-2.4)* 1.9 (1.4-2.6) <0.0001* 0.8 (0.4-1.3) 0.4 (0.01-13) 0.4 (0.01-18) ---- 0.5 (0.2-1.3) 0.3 (0.1-0.9)** 0.02** 0.3 (0.1-0.8)**	**The authors concluded that smoking among both sexes is associated with an increased risk of MS, while the use of Swedish snuff was not associated with an increased risk of developing MS.** The authors report that there was clear evidence of a dose-response correlation between the cumulative dose of smoking and developing MS. Snuff users on the other hand who had used snuff for 5 or more years had a significantly lower risk of developing MS. Odds ratios for smokers were adjusted for age, ancestry, residential area, and for gender. Among snuff users, never smokers were adjusted for age, sex, ancestry and residential area, while ever smokers were adjusted for age, sex, ancestry, residential area and smoking. Information on cumulative dose for smoking and snuff use was collected so a dose-response analysis could be carried out. Confidence intervals among never-smoking snuff users were wide and imprecise, suggesting there were few cases in these subgroups.

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix R

In-Vitro Investigations

APPENDIX R
IN-VITRO INVESTIGATIONS OF SWEDISH SNUS (N=10)

CITATION	*IN-VITRO* TEST	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Andersson et al. 2006	This study compared the effect of extracts of Swedish moist snuff (Ettan Gothia Tobak AB) and American snuff (Kentucky reference snuff) on growth of periodontal ligament cells. The cells were isolated from 3 healthy volunteers and grown in culture; snuff extract was added in varying concentrations (0.3%, 1%, 3%). There was also a negative control (culture medium only). After 24 hours, the cell cultures were analyzed for growth (number of viable cells) and morphology and the cell suspensions were evaluated for the production of alkaline phosphatase (which is related to cell differentiation).	Cells maintained in control medium generally increased in number after 24 hours. By comparison, cells maintained in medium containing snuff extracts at low concentrations (0.3% and 1.0%) showed variable effects (unchanged cell numbers or increased cell numbers). At the highest concentration of either the Swedish or American snuff extracts (e.g., 3%), cell numbers were reduced. Production of alkaline phosphatase was also significantly decreased after exposure to 3% of either Swedish or American snuff extract.	The authors concluded that smokeless tobacco has biological effects on periodontal tissues, in terms of reduced cell growth and production of alkaline phosphatase. Similar effects were seen with both Swedish and American snuff extracts. The authors noted that additional study is needed to understand the effects of snuff on periodontal tissues.

APPENDIX R
IN-VITRO INVESTIGATIONS OF SWEDISH SNUS (continued)

CITATION	*IN-VITRO* TEST	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Costea et al 2009	Aqueous extracts prepared from moist Toombak and Swedish snuff (Ettan, Bothia Tobak AB) that were added in serial dilutions to primary normal human oral keratinocyte (NOK) and fibroblast (NOF) cells isolated from superfluous tissues of clinically healthy buccal mucosa and commercially available dysplastic oral keratinocytes (DOK cells). Cell viability, morphology and growth, DNA double-strand breaks, apoptosis, and cell cycle were assessed after various exposure time periods.	Significant decreases in cell number, DNA double-strain breaks, morphological and biochemical signs of apoptosis were detected in all cell types exposed to clinically relevant dilutions of Toombak extract, although to a lesser extent in normal oral fibroblasts and dysplastic keratinocytes. Cell cycle arrest was also detected in normal oral keratinocytes and fibroblasts. Swedish snuff extract had less adverse effects on oral cells, mainly at non-clinically relevant (high dose) dilutions. Continuous exposure for 6 days to the aqueous extract of Swedish snuff showed, at clinically relevant concentrations, inhibited the growth and induced DNA strand breaks in NOK cells, but not in DOK cells.	The investigators concluded that this study indicates a greater potential for Toombak to induce adverse effects on normal oral mucosal cells than Swedish snuff.

APPENDIX R
IN-VITRO INVESTIGATIONS OF SWEDISH SNUS (continued)

CITATION	*IN-VITRO* TEST	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Curvall et al. 1987	Standard *Salmonella typhimurium* reverse mutagenicity test (i.e., Ames test) "Snuff" was not defined in this paper. The authors did state that urine was collected from users of "Swedish wet snuff."	Mutagenic activity of the urine samples was detected only in the presence of S9. Urine samples from 8 smokers showed a significant ($p<0.001$) mutagenic effect and were within the range of 4.2×10^3 revertants per 24-h urine to 17.6×10^3 revertants per 24-h urine. In comparison, urine samples from 8 snuff users had mutagenic activities between 0.3×10^3 revertants per 24-h urine and 2.5×10^3 revertants per 24-h urine, and for the 6 non tobacco users the range was 0.4×10^3 to 2.2×10^3. In addition, 6 of the 8 snuff users abstained from snuff use for 1 week and collected urine over 24-h at the end of this period. Mutagenic activity in the urine samples from the 6 abstinent snuff users ranged from 0.5×10^3 revertants per 24-h urine to 2.4×10^3 revertants per 24-h urine.	The authors detected no significant difference in mutagenic activity between urine from the snuff users and urine from the non-tobacco users. They concluded that consumption of Swedish snuff does not elevate levels of urinary mutagens.

APPENDIX R
IN-VITRO INVESTIGATIONS OF SWEDISH SNUS (continued)

CITATION	*IN-VITRO* TEST	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hasseus et al. 1997	Functional assays using spleen cells, epithelial cells, and T-cells from 8 to 10 week old Lewis rats "Snuff" was defined as Swedish snuff in this paper (brand name: Röda Lacket).	Statistically significant ($p < 0.05$) inhibition of spleen cell proliferation was noted at a Swedish snuff (SS) extract concentration of 0.8%. Spleen cells recovered from cytoxicity of the SS extract at concentrations below 6%. Inhibition of epithelial cell and T-cell proliferation occurred at a 12.5% concentration of SS extract, with T-cell proliferation reduced by 50% at a 4% concentration. As with spleen cells, both epithelial cells and T-cells recovered from cytotoxicity at concentrations below 6%. When T-cells and oral epithelial cells were pretreated with 50% concentrations of SS extract, significant and irreversible inhibition of T-cell proliferation was observed.	Swedish snuff derivatives ANNA, NAB, NNN, NNK, and NDMA did not cause significant stimulation or inhibition of spleen cell proliferation. Incubation of epithelial cells and T-cells with various alkaloids and TSNAs showed no effect on cell proliferation. In the absence of con A, neither the alkaloids, TSNAs, nor Swedish snuff extract demonstrated mitogenic capacity.

APPENDIX R
IN-VITRO INVESTIGATIONS OF SWEDISH SNUS (continued)

CITATION	*IN-VITRO* TEST	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Ibrahim et al. 1996	Immunohistochemistry was used to examine the expression of p53 in premalignant oral lesions and oral squamous-cell carcinomas (SCCs) from Swedish and Sudanese snuff dippers, and non-snuff-dippers from the Sudan, Sweden and Norway. There were biopsy specimens obtained from oral lesions of 15 Swedish snuff dippers, 22 Sudanese snuff dippers, and non-snuff dippers (number not reported). There were a total of 15 and 114 SSCs from Sudanese snuff dippers and non-snuff dippers, respectively. There were a total of 15 fibroepithelial lesions from Swedish snuff dippers and 8 and 22 fibroepithelial or pre-malignant oral lesions from Sudanese snuff dippers and non-snuff dippers, respectively. "Snuff" was not defined in this paper. Specific brands of Swedish snua are not identified.	Of the 14 SCCs from Sudanese snuff-dippers, 21% (3/14) expressed p53. Of the 14, 60, and 41 SCCs from non-snuff dippers from the Sudan. Sweden and Norway, 64% (9/14), 65% (39/60) and 68% (28/41) expressed p53, respectively. A statistically significant difference in expression of p53 was found in SCCs from Sudanese snuff dippers compared to those from non-snuff-dippers None of the suspected pre-malignant oral lesions from Sudanese snuff dippers or non-snuff-dippers expressed p53. Only 2 of 15 (13%) oral fibro-epithelial hyperplasias from Swedish snuff-dippers expressed p53.	Mutation in the p53 tumor suppressor gene occurred at a relatively low level in biopsy samples from Swedish snuff users. This study suffers from major weaknesses, including the lack of a suitable control population, absence of a statistical analysis, and failure to control for confounders (*e.g.*, concomitant use of smoked tobacco products and/or alcohol)..

APPENDIX R
IN-VITRO INVESTIGATIONS OF SWEDISH SNUS (continued)

CITATION	*IN-VITRO* TEST	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Jansson et al. 1991	Ames *Salmonella typhimurium* reverse mutagenicity test, chromosome aberration test, sister chromatid exchange test, HPRT test, micronucleus test "Snuff" was not defined in this paper. Authors referred to product as "Swedish moist oral snuff."	An Ames test on aqueous snuff extract demonstrated no toxicity to the Salmonella bacteria. The methylene chloride extract, however, demonstrated clear mutagenic effects in the presence of metabolic activation in this assay. Induction of chromosome aberrations in V79 Chinese hamster cells was noted with the aqueous extract, in addition to a low but significant level of chromosome aberrations induced by the methylene chloride extract in the presence of S9. Both the aqueous and methylene chloride extracts produced significant, dose-related increases in sister-chromatid exchange in human lymphocytes. Neither the aqueous nor the methylene chloride extract induced gene mutations in the HPRT locus of Chinese hamster cells. In the micronucleus test, neither extract caused any bone marrow toxicity. No significant increases in the recessive lethal mutation frequencies in Drosophila were caused by the methylene chloride extract.	*In-vitro* data in Salmonella bacteria indicate that aqueous- and solvent-extracts of Swedish snuff are mutagenic. However, neither aqueous- or solvent-extracts of Swedish snuff were mutagenic to mammalian cells. *In-vitro* test data in mammalian cells indicate that aqueous- and solvent-extracts of Swedish snuff-induced chromosome breaks. However, *in vivo* test data identified no clastogenic effect from exposure to aqueous or solvent-extracts of Swedish snuff. Carcinogenic potential of Swedish snus should be considered to be low.

APPENDIX R
IN-VITRO INVESTIGATIONS OF SWEDISH SNUS (continued)

CITATION	*IN-VITRO* TEST	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Merne et al. 2002	Immunohistochemistry was performed to evaluate expression of proteins of cell proliferation, cell cycle regulation, keratins, and collagen type IV in biopsies of oral lesions from snuff users. Histology was assessed by light microscopy. Biopsy specimens were obtained from the oral mucosa of 14 men with snuff dippers' lesions. The snuff used was exclusively Scandinavian moist snuff. The average time of snuff use was 5.9 years (range 2 to 15 years), and the mean frequency of use was 6.8 times per day (range 2 to 10). Five of these men were also current smokers. Control biopsy samples were also obtained from 12 people (8 men) with normal buccal mucosa who had never used tobacco. "Snuff" in this study refers to non-fermented, Scandinavian-type moist snuff.	Biopsy specimens from snuff-induced lesions were characterized by a thick hyperkeratinized surface layer. The epithelium was thickened, but no changes compatible with dysplasia were seen. In contrast, the control samples showed normal epithelial structure with a non-keratinized surface. Expression of cellular proliferation proteins (PCNA and Ki-67) was lower in snuff lesions than in controls ($p<0.001$). Expression of cell cycle proteins (p53, p21 did not differ between snuff lesions and controls. p53 levels were increased in only 2 of 14 snuff users' lesions and p21 was increased in 7/14 – both of these values were not statistically significant.	The authors concluded that lesions seen among snuff users are associated with suppressed cellular proliferation and infrequent p53 dysfunction. This helps to explain why dysplastic changes are seldom seen in mucosal lesions induced by Swedish snuff. These data suggest that the epithelium in snuff-induced lesions is not thickened as a result of increased cellular proliferation; rather, it is due to protracted turnover of differentiating cells.

APPENDIX R
IN-VITRO INVESTIGATIONS OF SWEDISH SNUS (continued)

CITATION	*IN-VITRO* TEST	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Merne et al. 2004	This study was conducted to evaluate the mechanisms by which snuff could affect the growth and differentiation of oral mucosal tissues. The authors used a three-dimensional cell culture that permitted cell-to-cell and cell-to-matrix interactions, which they considered to be an appropriate model for studying pathogenic effects of exogenous agents. The cells (a line of skin epithelium) were grown for 6 to 18 days in the presence of 1% commercial moist Swedish snuff (Ettan®; Gothia Snus, Sweden) and were then examined histochemically and compared to control cultures.	Exposure to snuff for more than 12 days resulted in morphologic changes such as cellular damage (intercellular dyskeratosis and cellular vacuolization, lack of basal cell layer) and impaired cellular adhesions and disturbances in the differentiation process. However, cellular proliferation, as detected by Ki-67 staining, was not increased in the snuff-treated group compared to controls.	The authors concluded that snuff extract caused morphologic changes and that long-term snuff exposure does not increase epithelial cell proliferation activity, but causes disturbances in the differentiation process.

APPENDIX R
IN-VITRO INVESTIGATIONS OF SWEDISH SNUS (continued)

CITATION	*IN-VITRO* TEST	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Rickert et al. 2009	The objective for this study was to characterize several types of STP available on the Canadian market using the modifications of the Official Health Canada chemical and toxicological methods developed for cigarettes. The samples tested included 7 types of smokeless tobacco products: 1) fine-cut moist snuff reportedly made in US by U.S. Smokeless Tobacco Company (UST) and imported into Canada; 2) long-cut moist snuff also made by UST and imported; 3) pouched moist snuff also made by UST and imported; 4) low-moisture snuff reportedly manufactured by McChrystal's in the UK and imported into Canada; 5) loose-leaf and plug chewing tobacco reportedly made in US by Swedish Match North America and imported into Canada, 6) pouched snus, reportedly made in Sweden and imported into Canada by Imperial Tobacco Canada; and 7) a gutkha-type product imported from India. Different doses of 11 sample brands with vehicle controls (saliva, dimethyl sulfoxide, or dichloromethane) were evaluated for mutagenicity, cytotoxicity, and clastogenicity. Extracts of various products were evaluated for mutagenicity in several assays using the Ames assay with salmonella tester strains TA98, TA100, TA102, TA1535, and TA1537. Sample brands were also evaluated for cytotoxicity using the neutral red uptake assay and clastogenicity using the micronucleus assay.	Several types of moist snuff samples tested had TSNA and benzo(a)pyrene levels slightly above the GothiaTek® standard while samples of Swedish snus, low-moisture snuff (McChrystal's), and US-style chewing tobacco (made by Swedish Match) did not exceed the standard. The Manikchand Gutkha sample had the highest cytotoxicity based on the NRU assay and the lowest clastogenicity with the micronuclei test. No other differences were detected between the remaining samples tested, including the snus sample. Most of the cytotoxicity assays did not reach the 50% cytotoxicity target and none of the Ames assays reached the two-fold rule for a positive mutagenicity response. According to the investigators, the use of *in vitro* mutagenicity assays to assess STP toxicity was of limited utility in distinguishing STP product types.	Many of the products had toxicant levels below or near the levels specified in the GothiaTek standard. Attempts to use bioassays of cytotoxicity, clastogenicity, and mutagenicity to distinguish among the different types of STP tested were not overly successful because of weak inherent activity and the possibility of yet to be identified interferences present in the products. Consequently, it is likely the procedures currently in place for the study of smoked tobacco products will require further investigation before they can be applied routinely to STP.

APPENDIX R
IN-VITRO INVESTIGATIONS OF SWEDISH SNUS (continued)

CITATION	*IN-VITRO* TEST	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Wedenberg et al. 1996	Immunohistochemistry performed on biopsy specimens obtained from oral lesions of 15 Swedish snuff-dippers. Controls comprised four non-Swedish snuff users with normal oral mucosa. Study subjects (but not controls) were also studied by Ibrahim et al. 1996. "Snuff" was not defined in this paper. Authors did not identify specified brands of Swedish snuff used by snuff dippers.	P53 over-expression and increased Ki-67 staining were observed among biopsy samples from oral lesions of Swedish snuff users compared to biopsy samples from Swedish non-snuff users.	p53 over-expression among biopsy samples from Swedish snuff users suggests that Swedish snuff induced mutations in the p53 tumor suppressor gene. Higher incidence of Ki-67 staining among biopsy samples from Swedish snuff users suggests that increased cell proliferation took place. This study design has weaknesses, including the absence of a statistical analysis, failure to control for confounders (*e.g.*, concomitant use of smoked tobacco products and/or alcohol), and small sample size (15 snuff users).

Appendix IV

Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users that Present Effect Estimates

Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users that Present Effect Estimates

Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users that Present Effect Estimates		
Reference	**Comparison**	**Effect Estimate (95% CI)**
Acute Effects on Heart Rate		
Associations examined but effect estimates were not reported by: Bolinder and de Faire 1998; Eliasson et al. 1991; Bolinder et al. 1997a,b; Hirsch et al. 1992		
Acute Effects on Blood Pressure		
Associations examined but effect estimates were not reported by: Bolinder and de Faire 1998; Eliasson et al. 1991; Bolinder et al. 1997a,b; Hirsch et al. 1992		
Hypertension		
Bolinder et al. 1992	**Hypertension (46-65y age group)**	3.0 (1.9-4.9)*
	Diastolic BP >90 mmHg	
	Age	
	16-35	1.3 (1.0-1.7)
	36-45	1.3 (1.0-1.6)
	46-55	1.8 (1.5-2.1)*
	56-65	1.3 (1.1-1.4)*
	Systolic BP >160 mmHg	
	Age	
	16-35	1.0 (0.5-1.7)
	36-45	1.3 (0.8-2.1)
	46-55	1.7 (1.3-2.1)*
	56-65	1.2 (1.1-1.4)*
Hergens et al. 2005	**Snuff use**	
	Former	0.98 (0.58-1.6)
	Current	1.8 (1.3-2.5)*
Hergens et al. 2008	**Healthy baseline**	
	Ever snuff use	1.08 (0.89-1.29)
	Former snuff use	0.78 (0.43-1.41)
	Current snuff use	1.10 (0.91-1.33)
	<12.5 g day^{-1}	1.03 (0.74-1.43)
	12.5-24.9 g day^{-1}	1.15 (0.88-1.49)
	25-49.9 g day^{-1}	1.15 (0.79-1.69)
	>50 g day^{-1}	1.03 (0.59-1.79)
	Healthy baseline with repeated measurements	
	Ever snuff use	1.36 (1.07-1.72)*
	Former snuff use	0.85 (0.40-1.79)
	Current snuff use	1.43 (1.12-1.83)*
	<12.5 g day^{-1}	1.18 (0.77-1.82)
	12.5-24.9 g day^{-1}	1.43 (1.01-2.02)*
	25-49.9 g day^{-1}	1.77 (1.08-2.90)*
	>50 g day^{-1}	1.76 (0.90-3.42)
Norberg et al. 2006	**Hypertension**	
	≤4 cans/week	0.9 (0.84-1.05)
	>4 cans/week	1.2 (0.99-1.46)
Associations examined but effect estimates were not reported by: Janzon and Hedblad 2009; Angman and Eliasson 2008		
Atherosclerosis		
Associations examined but effect estimates were not reported by: Bolinder et al. 1997a; Wallenfeldt et al. 2001		

Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users that Present Effect Estimates		
Reference	**Comparison**	**Effect Estimate (95% CI)**
	Cholesterol	
Hergens et al. 2005	**Hyperlipidemia**	
	Snuff use	
	Former	1.1 (0.63-2.0)
	Current	0.99 (0.66-1.5)
Norberg et al. 2006	**Low HDL**	
	≤4 cans/week	1.0 (0.86-1.18)
	>4 cans/week	1.1 (0.82-1.42)
Associations examined but effect estimates were not reported by: Bolinder et al. 1997a; Wallenfeldt et al. 2001		
	Triglycerides	
Norberg et al. 2006	**Triglycerides ≥1.7**	
	≤4 cans/week	1.2 (1.05-1.35)*
	>4 cans/week	1.6 (1.30-1.95)*
Associations examined but effect estimates were not reported by: Bolinder et al. 1997a; Wallenfeldt et al. 2001; Eliasson et al. 1995; Eliasson et al. 1991		
	Fibrinolytic	
Associations examined but effect estimates were not reported by: Bolinder et al. 1997a; Eliasson et al. 1995; Eliasson et al. 1991		
	Glucose Levels	
Persson et al. 2000	**Impaired glucose tolerance**	
	Moist snuff	
	Former	0.7 (0.4-1.2)
	Current	0.8 (0.4-1.4)
	No. of boxes of snuff week^{-1} in current snuffers	
	≤2	0.7 (0.4-1.4)
	3+	0.8 (0.4-1.4)
Norberg et al. 2006	**f- P-glucose ≥5.6 or diabetes**	
	≤4 cans/week	1.0 (0.86-1.08)
	>4 cans/week	0.8 (0.69-1.02)
Eliasson et al. 2004	**Impaired glucose tolerance**	
	Consistent exclusive snus users	0.23 (0.03-1.80)
	Ex-snus users	0.75 (0.16-3.57)
	Smokers who switched to snus	1.18 (0.51-2.74)
	Pathological glucose tolerance	
	Consistent exclusive snus users	0.45 (0.10-2.04)
	Ex-snus users	1.85 (0.60-5.70)
	Smokers who switched to snus	1.05 (0.46-2.44)
Associations examined but effect estimates were not reported by: Bolinder et al. 1997a; Wallenfeldt et al. 2001; Eliasson et al. 1995; Eliasson et al. 1991		
	Insulin Reactivity	
Persson et al. 2000	**HOMA (resistance), highest third**	
	Moist snuff	
	Former	0.4 (0.1-1.3)
	Current	0.9 (0.4-2.0)
	No. of boxes of snuff week^{-1} in current snuffers	
	≤2	0.5 (0.2-1.6)
	3+	0.7 (0.3-1.7)
	2 h insulin response, lowest third	
	Moist snuff	
	Former	2.2 (1.1-4.4)*

Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users that Present Effect Estimates		
Reference	**Comparison**	**Effect Estimate (95% CI)**
	Current	1.2 (0.5-2.8)
	No. of boxes of snuff week^{-1} in current snuffers	
	≤2	2.1 (1.1-4.1)*
	3+	1.2 (0.5-2.9)
Associations examined but effect estimates were not reported by: Bolinder et al. 1997a; Wallenfeldt et al. 2001; Eliasson et al. 1995; Eliasson et al. 1991; Eliasson et al. 2004		
C-reactive protein		
An association was examined but an effect estimate was not reported by: Wallenfeldt et al. 2001		
Thromboxane A_2		
An association was examined but an effect estimate was not reported by: Wallenfeldt et al. 2001		
O_2 Uptake/Work Capacity		
Bolinder and de Faire 1998	**Physical capacity, low (O_2 uptake)**	1.1 (0.3-3.6)
Associations examined but effect estimates were not reported by: Bolinder et al. 1997b; Wennmalm et al. 1991		
Impaired Endothelial Function		
An association was examined but an effect estimate was not reported by: Rohani and Agewall 2004		
MetSy		
Norberg et al. 2006	**Metabolic Syndrome**	
	Univariate model	
	≤4 cans/week	1.1 (0.90-1.27)
	>4 cans/week	1.8 (1.36-2.30)*
	Multivariate model	
	≤4 cans/week	1.0 (0.85-1.22)
	>4 cans/week	1.6 (1.26-2.15)*
Wandell et al. 2008	**Metabolic Syndrome**	
	Ex-smokers, current snuffers	
	ATP III	1.14 (0.71-1.82)
	EGIR	1.29 (0.78-2.14)
	IDF	1.18 (0.76-1.83)
	Ex-snuffers	
	ATP III	0.69 (0.14-3.28)
	EGIR	0.97 (0.20-4.67)
	IDF	0.48 (0.10-2.26)
	Current snuffers	
	ATP III	1.55 (0.52-4.62)
	EGIR	0.71 (0.16-3.24)
	IDF	1.81 (0.65-5.02)
	Current smokers and snuffers	
	ATP III	1.46 (0.63-3.41)
	EGIR	0.47 (0.14-1.63)
	IDF	0.85 (0.36-2.02)
Diabetes		
Persson et al. 2000	**Type 2 diabetes**	
	Moist snuff	
	Former	0.8 (0.3-2.0)
	Current	1.5 (0.8-3.0)
	No. of boxes of snuff week^{-1} in current snuffers	
	≤2	0.2 (0.0-2.0)
	3+	2.7 (1.3-5.5)*
Hergens et al. 2005	**Diabetes**	
	Snuff use	
	Former	1.1 (0.40-3.3)
	Current	1.5 (0.76-2.9)

Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users that Present Effect Estimates		
Reference	**Comparison**	**Effect Estimate (95% CI)**
Wandell et al. 2008	**Diabetes** Ex-smokers, current snuffers Model 1 Ex-snuffers Model 1 Current snuffers Model 1 Current smokers and snuffers Model 1 Snuff, low consumers (<3 cans/week) Model 2 Snuff, high consumers (≥3 cans/week) Model 2	 1.71 (0.67-4.35) 3.10 (0.36-26.84) 2.12 (0.25-17.71) 2.48 (0.52-11.82) 1.30 (0.49-3.40) 1.80 (0.67-4.85)
Eliasson et al. 2004	Prevalence Results **Known Type 2 diabetes** Ever snus use (exclusive) Current snus user Ex-snus user Incidence Results **Known Type 2 diabetes** Consistent exclusive snus Ex-snus users Smokers who switched to snus Among 513 men with normal OGT at baseline **Type 2 diabetes** Consistent exclusive snus Ex-snus users Smokers who switched to snus	 1.21 (0.59-2.49) 1.06 (0.43-2.64) 1.45 (0.54-3.87) 0 cases 1.72 (0.20-14.8) 3.25 (0.78-13.6) 0.91 (0.10-8.01) 3.97 (0.86-18.33) 0 cases
BMI, Waist-Hip Ratio (WHR)		
Hergens et al. 2005	**Overweight** Snuff use Former Current	 1.5 (0.79-2.8) 1.9 (1.2-2.9)*
Saarni et al. 2004	**Recurrent intentional weight loss** Lifetime frequency of snuff use Men 2-50 times >50 times Women 2-50 times >50 times	 1.51 (1.08-2.13)* 1.41 (0.91-2.19) 1.63 (0.98-2.70) ----
Sundbeck et al. 2009	**BMI ≥30 kg/m^2** All snuff users ≤4 cans/week >4 cans/week All Current exclusive snuff users ≤4 cans/week >4 cans/week All	 1.27 (0.73-2.20) 1.18 (0.50-2.79) 1.24 (0.75-2.06) 0.67 (0.24-1.82) 1.36 (0.36-5.10) 0.83 (0.36-1.90)

Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users that Present Effect Estimates		
Reference	**Comparison**	**Effect Estimate (95% CI)**
	Current snuff users who quit smoking	
	≤4 cans/week	1.65 (0.90-3.01)
	>4 cans/week	1.13 (0.39-3.25)
	All	1.51 (0.87-2.63)
	WHR ≥1.0	
	All snuff users	
	≤4 cans/week	0.96 (0.48-1.94)
	>4 cans/week	1.32 (0.46-3.80)
	All	1.04 (0.55-1.95)
	Current exclusive snuff users	
	≤4 cans/week	0.77 (0.25-2.37)
	>4 cans/week	Too few subjects
	All	0.60 (0.20-1.82)
	Current snuff users who quit smoking	
	≤4 cans/week	1.06 (0.48-2.37)
	>4 cans/week	2.29 (0.75-6.97)
	All	1.31 (0.66-2.61)
Rodu et al. 2004	Prevalence of Overweight at Study Entry	
	Tobacco Use	
	Current exclusive smoking	0.87 (0.73-1.03)
	Current exclusive snus use	1.20 (1.01-1.42)*
	Current combined use	1.25 (1.03-1.63)*
	Development of Overweight During Follow-up Among Men Not Overweight at Entry	
	Tobacco Use At Entry/At Follow-Up	
	Smoking/snus	80 (22-205)
	Snus/snus	120 (84-167)
	Snus/no tobacco	142 (78-264)
Bolinder et al. 1992	**BMI<22**	
	Age (years)	
	≤35	1.0 (0.9-1.1)
	36-45	1.0 (0.7-1.2)
	46-55	1.0 (0.8-1.3)
	≥56	1.1 (0.9-1.3)
	BMI>26	
	Age (years)	
	≤35	1.1 (0.9-1.2)
	36-45	1.3 (1.1-1.5)*
	46-55	1.5 (1.3-1.7)*
	≥56	1.2 (1.1-1.4)*
Nafziger et al. 2007	**Weight non-gain**	
	Snuff use	0.83 (0.74-0.92)*
Associations examined but effect estimates were not reported by: Norberg et al. 2006; Bolinder et al. 1997a,b; Eliasson et al. 1995; Wallenfeldt et al. 2001; Bolinder and de Faire 1998; Eliasson et al. 1991		
Incidence of Myocardial Infarction (fatal or nonfatal)		
Hergens et al. 2005	**All Cases**	
	Snuff use	
	Former	1.1 (0.78-1.5)

Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users that Present Effect Estimates		
Reference	**Comparison**	**Effect Estimate (95% CI)**
	Current	0.98 (0.77-1.3)
	Nonfatal Cases	
	Snuff use	
	Former	1.1 (0.79-1.6)
	Current	0.98 (0.76-1.3)
	Fatal Cases	
	Snuff use	
	Former	1.1 (0.54-2.1)
	Current	1.9 (0.65-1.6)
Huhtasaari et al. 1992	**Snuff Use --Cans/Week**	
	<2 cans weekly	0.63 (0.41-0.98)**
	≥2 cans weekly	0.93 (0.61-1.41)
	Snuff Dippers Vs. No Tobacco (by Age Group of Snuff Dippers)	
	35-54 years	0.96 (0.56-1.67)
	55-64 years	1.24 (0.67-2.30)
	All subjects	0.89 (0.62-1.29)
Huhtasaari et al. 1999	**Fatal and nonfatal acute MI**	
	Regular use of snuff	0.58 (0.35-0.94)**
	Regular smoking	3.53 (2.48-5.03)*
Wennberg et al. 2007	**MI**	
	Never smoked, current snuff	0.82 (0.46-1.43)
	Former smoker, current snuff user	1.25 (0.80-1.96)
	Current smoker, current snuff user	2.14 (1.28-3.60)*
	Never smoked, former snuff user	0.66 (0.32-1.34)
	Former smoker, former snuff user	1.34 (0.84-2.12)
Hergens et al. 2007	MI risk among never smokers	
	Total MI	
	Current snuff users	1.02 (0.92-1.14)
	Former snuff users	0.76 (0.55-1.05)
	MI - Nonfatal	
	Current snuff users	0.94 (0.83 -1.06)
	Former snuff users	0.70 (0.48-1.02)
	Total MI – by snuff use	
	≤ 12.5 g/day	1.12 (0.95-1.30)
	12.5-24.9 g/day	0.93 (0.79-1.09)
	25-49.9 g/day	0.95 (0.73-1.24)
	≥ 50 g/day	1.24 (0.89-1.73)
	MI – Nonfatal – by snuff use	
	≤ 12.5 g/day	1.02 (0.84-1.22)
	12.5-24.9 g/day	0.85 (0.70-1.03)
	25-49.9 g/day	0.95 (0.71-1.29)
	≥ 50 g/day	1.06 (0.71-1.58)
Janzon and Hedblad 2009	First ever MI	
	Males – risk factor adjusted	
	Snuff user, never smoker	0.75 (0.3-1.8)
	Females	

Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users that Present Effect Estimates		
Reference	**Comparison**	**Effect Estimate (95% CI)**
	Snuff user	0 cases
Fatal MI; Sudden Cardiac Death (SCD)		
Hergens et al. 2007	**MI - Fatal**	
	Current snuff users	1.32 (1.08-1.61)*
	Former snuff users	1.00 (0.54-1.88)
	MI – Fatal – by snuff use	
	≤ 12.5 g/day	1.45 (1.09-1.93)*
	12.5-24.9 g/day	1.22 (0.90-1.65)
	25-49.9 g/day	0.95 (0.54-1.69)
	≥ 50 g/day	1.96 (1.08-3.58)*
Huhtasaari et al. 1999	**Fatal acute MI only**	
	Regular use of snuff	1.50 (0.45-5.03)
	Regular smoking	8.57 (2.48-30.3)*
Wennberg et al. 2007	**Fatal MI within 28 Days**	
	Never smoked, current snuff	1.12 (0.38-3.29)
	Former smoker, current snuff user	1.24 (0.44-3.53)
	Current smoker, current snuff user	1.11 (0.34-3.69)
	Never smoked, former snuff user	0.64 (0.13-3.18)
	Former smoker, former snuff user	0.60 (0.18-2.02)
	SCD with Survival <24 Hr	
	Never smoked, current snuff	1.18 (0.38-3.70)
	Former smoker, current snuff user	1.39 (0.44-4.42)
	Current smoker, current snuff user	0.75 (0.17-3.28)
	Never smoked, former snuff user	0.70 (0.14-3.64)
	Former smoker, former snuff user	0.50 (0.12-2.03)
	SCD with Survival <1 Hr	
	Never smoked, current snuff	0.38 (0.08-1.89)
	Former smoker, current snuff user	2.67 (0.52-13.80)
	Current smoker, current snuff user	0.13 (0.01-2.10)
	Never smoked, former snuff user	0.35 (0.03-4.56)
	Former smoker, former snuff user	----

* denotes statistically significant increase in risk
** denotes statistically significant decrease in risk

Appendix V

Smokeless Tobacco Reviews and Meta-analyses: Objectives and Author Conclusions

Smokeless Tobacco Reviews and Meta-analyses: Objectives and Author Conclusions

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Reference	**Objective**	**Author(s) Conclusion**
Boffetta P, Hecht S, Gray N, Gupta P, and Straif K. 2008. Smokeless tobacco and cancer. Lancet Oncol 9:667-675.	To describe trends and patterns of use of smokeless tobacco for the USA, Sweden and India and to conduct a quantitative review of the epidemiology studies of smokeless tobacco and oral, pancreatic, esophageal, and lung cancer.	Cancer risk of smokeless tobacco users is probably lower than that of smokers, but higher than that of non-tobacco users. The risk of cancer depends on the type of product consumed, and the concentration of nitrosamines is the strongest factor to determine product-specific risk; the risk of cancer, especially that of oral and lung cancer, is probably lower in smokeless tobacco users in the USA and northern Europe than in smokers; and the risk of cancer is higher in smokeless tobacco users than in nonusers of any form of tobacco.
Boffetta P and Straif K. 2009. Use of smokeless tobacco and risk of myocardial infarction and stroke: systematic review with meta-analysis. BMJ 339:b3060.	To assess whether people who use smokeless tobacco products are at increased risk of myocardial infraction and stroke by conducting a systematic review with meta-analysis.	In conclusion, in studies carried out in the United States and Sweden we detected an association between use of smokeless tobacco products and risk of fatal myocardial infarction and fatal stroke, which is not readily explained by chance. Confounding and other sources of bias, however, cannot be completely excluded on the basis of available data, although we found no strong evidence for their effect.
Broadstock M. 2007. Systematic review of the health effects of modified smokeless tobacco products. New Zealand Health Technology Assessment 10:1-110.	To conduct a systematic review of the epidemiological evidence for reduced harm relating to health effects of using modified smokeless tobacco products compared with conventional combustible tobacco.	The evidence from this review suggests that the harm of using snus relative to non-tobacco use is significantly less than found for smoking with respect to cancers of the head, neck and gastro-intestinal region, and cardiovascular disease events.
Critchley JA and Unal B. 2003. Health effects associated with smokeless tobacco: a systematic review. Thorax 58:435-443.	To conduct a systematic review of the epidemiology studies relating to health effects associated with smokeless tobacco.	Chewing betel quid and tobacco is associated with a substantial risk of oral cancers in India. Most recent studies from the US and Scandinavia are not statistically significant, but moderate positive associations cannot be ruled out due to lack of power. Further rigorous studies with adequate sample sizes are required, especially for cardiovascular disease.

Smokeless Tobacco Reviews and Meta-analyses: Objectives and Author Conclusions		
Reference	**Objective**	**Author(s) Conclusion**
Critchley JA and Unal B. 2004. Is smokeless tobacco a risk factor for coronary heart disease? A systematic review of epidemiological studies. *Eur J Cardiovasc Prev Rehabil* 11:101-112.	To conduct a systematic review of epidemiology studies relating to the potential relationship of coronary heart disease risk and smokeless tobacco use.	There may be an association between ST use and cardiovascular disease. However, further rigorous studies with adequate sample sizes are required. Most ST products are probably considerably lower risk than cigarette smoking (taking all the potential health effects, particularly cancers, into account). Switching to ST may reduce risks of major death and illness for some nicotine-addicted cigarette smokers.
Colilla SA. 2010. An epidemiologic review of smokeless tobacco health effects and harm reduction potential. *Regul Toxicol Pharmacol* 56:197-211.	To conduct an epidemiological review of the health effects of smokeless tobacco and its relevance to harm reduction.	While the current epidemiologic literature does not provide much evidence for significant health risks with ST use, particularly when compared to the health risks associated with cigarette smoking, whether ST products would be an effective smoking cessation tool (either as a replacement product or for tapering off all tobacco use) has not been well investigated. Politics aside, if the majority of inveterate smokers were to switch to ST use, and the majority of them quit smoking, it seems certain that public health overall would benefit.

Smokeless Tobacco Reviews and Meta-analyses: Objectives and Author Conclusions		
Reference	**Objective**	**Author(s) Conclusion**
Foulds J, Ramstrom L, Burke M, and Fagerstrom K. 2003. Effect of smokeless tobacco (snus) on smoking and public health in Sweden. Tob Control 12:349-359.	To review the evidence on the effects of snus on smoking and ill health in Sweden.	Significant proportions of smokers are capable of transferring their nicotine dependence from an ultra-fast nicotine delivery product (a cigarette) to a medium rate nicotine delivery product (snus) so long as it delivers comparable amounts of nicotine, and so long as it is competitive on price, accessibility, and long term availability. It appears to be extremely unlikely that nicotine is capable of stimulating cancer under normal use conditions. Snus is certainly not harmless. It can cause reversible lesions in the mouth, it most likely causes harmful effects to the unborn fetus when used by a pregnant woman, and long term use may contribute to cardiovascular disease (although most of the available evidence suggests that cardiovascular risks are not increased by snus). Snus is clearly less harmful to the individual user than smoked tobacco, and also less harmful than the types of smokeless tobacco used in some other parts of the world. Snus availability in Sweden appears to have contributed to the unusually low rates of smoking among Swedish men by helping them transfer to a notably less harmful form of nicotine dependence.
International Agency for Research on Cancer (IARC). 1986. Tobacco: A major international health hazard. IARC Scientific Publications No. 74. Lyon, France.	To highlight the scientific deliberations of an International Meeting organized by IARC regarding the public health implications of tobacco use (smoking and chewing).	The tobacco companies, faced with lower sales of cigarettes in the developed countries are now, despite clear evidence of the carcinogenicity of the habit, promoting the use of chewing snuff, the product being sold in the form of sachets for oral use (Cameron, 1985). If the sale of these products, which do not carry any health warning, is allowed to continue, the toll of periodontal disease and oral cancer will be high.

Smokeless Tobacco Reviews and Meta-analyses: Objectives and Author Conclusions		
Reference	**Objective**	**Author(s) Conclusion**
International Agency for Research on Cancer (IARC). 1999. Carcinogenic hazard evaluation. IARC Scientific Publ. No. 146. Lyon, France.	To evaluate the predictive value of short- and medium-term carcinogenicity assays with end-points of neoplasia or lesions that are precursors to neoplasia, as surrogates for lifetime studies in which neoplasms are end-points. Also, to define the role of data from genetic toxicology in the prediction of carcinogenic hazard (distinguish the more useful tests and end-points from those that are less useful in this regard).	Past experience has shown that data for certain types of genetic and related effects, which are commonly summarized in the Monographs, are not suitable for classifying or predicting carcinogenic hazard. Newer assays which could provide additional information include the Comet assay, mutations in transgenic animals, fluorescent in-situ hybridization and cell transformation.
International Agency for Research on Cancer (IARC). 2004. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 83, International Agency for Research on Cancer, Lyon, France.	To critically review data on the carcinogenicity of tobacco smoke and involuntary smoking in terms of human risk.	Use of smokeless tobacco and/or alcohol in combination with tobacco smoking greatly increases the risk of oral cancer.
International Agency for Research on Cancer (IARC). 2007. Smokeless tobacco and some tobacco-specific N-nitrosamines. 89. Lyon, France.	To critically review data on the carcinogenicity of smokeless tobacco and some tobacco-specific n-nitrosamines in terms of human risk.	There is *inadequate evidence* in humans for the carcinogenicity of tobacco-specific N-nitrosamines.

Smokeless Tobacco Reviews and Meta-analyses: Objectives and Author Conclusions		
Reference	**Objective**	**Author(s) Conclusion**
Kallischnigg G, Weitkunat R, and Lee PN. 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.	To conduct a systematic review of the relation between smokeless tobacco and non-neoplastic oral diseases.	Detailed assessment of the overall risks and benefits of ST use to the public health would require consideration of the whole spectrum of its possible health effects and is beyond the scope of this review. However, we do note that there are numerous reports, including our own publications on oral cancer and on circulatory disease, which support the risks of smoking-related diseases from ST as being generally much less than those from smoking. This review confirms the strong relationship of oral mucosal lesions to ST use, shows that prevalence and severity is related to the type and amount of the product used, and that the lesion is reversible on quitting. The evidence relating other oral lesions to ST use is less clear. A causal relationship of snuff use with gingival recession seems probable, but not certain. The relationships between CT use and dental caries and between ST use and attachment loss are less clear, and the evidence here may be regarded only as suggestive of a causal relationship. There seems no real indication that ST use affects gingivitis (or gingival bleeding). Data are too limited to draw reliable conclusions for other endpoints, including oral pain.
Klus H, Kunze M, Konig S, and Poschl E. 2009. Smokeless Tobacco - An Overview. Beiträge zur Tabakforschung International/Contributions to Tobacco Research 23:248-276.	To present an overview on different types of smokeless tobacco, and to review the chemical composition and toxicological properties of smokeless tobaccos of Europe and North America. Also, to summarize the epidemiological evidence concerning a wide range of health outcomes.	While many of the epidemiological studies have weaknesses and data are often inconsistent it is quite obvious that smokeless tobacco use is much less risky for consumers than smoking. In fact, for modern forms of European moist snuff such as Swedish snus, which is subject to strict quality standards, there is evidence for – if any – only very limited serious health risk.

Smokeless Tobacco Reviews and Meta-analyses: Objectives and Author Conclusions		
Reference	**Objective**	**Author(s) Conclusion**
Lee PN. 2007. Circulatory disease and smokeless tobacco in Western populations: a review of the evidence. Int J Epidemiol 36:789-804.	To conduct a systematic review and meta-analysis of the relationship between circulatory disease and smokeless tobacco in Western populations.	The overall evidence on use of snuff taken from a substantial number of studies in Sweden does not demonstrate any increase in the risk of circulatory disease (CID), any chronic effect on blood pressure or any increased risk of a range of other risk factors relevant to CID. More evidence is needed to confirm whether Swedish oral snuff causes an acute rise in blood pressure. It may increase risk of Raynaud-type symptoms. The evidence of a possible effect of ST as used in the US is more compelling. However, the overall evidence is limited.
Lee PN and Hamling J. 2009. 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.	To comment on the differences between the conclusions of two reviews (Lee and Hamling 2009; Boffetta et al. 2008) of smokeless tobacco and cancer in Northern Europe and North America.	When conducting meta-analyses, all relevant data should be used, with clear rules governing the choice between alternative estimates. A systematic meta-analysis using pre-defined procedures and all relevant data gives a lower estimate of cancer risk from smokeless tobacco (probably 1-2% of that from smoking) than does the previous review by Boffetta et al 2008.
Lee PN and Hamling JS. 2009. Systematic review of the relation between smokeless tobacco and cancer in Europe and North America. BMC Med 7:36.	To conduct a systematic review and meta-analysis of the epidemiology studies of smokeless tobacco and cancer, and to compare the effects of smokeless tobacco and smoking (attributable risk).	An increased risk of oropharyngeal cancer is evident most clearly for past smokeless tobacco use in the USA, but not for Scandinavian snuff. Effects of smokeless tobacco use on other cancers are not clearly demonstrated. Risk from modern products is much less than for smoking. Risk from ST products as used in North America and Europe is clearly very much less than that from smoking, and is not evident at all in Scandinavia.
Levy DT, Mumford EA, Cummings KM, Gilpin EA, Giovino G, Hyland A, Sweanor D, and Warner KE. 2004. The relative risks of a low-nitrosamine smokeless tobacco product compared with smoking cigarettes: estimates of a panel of experts. Cancer Epidemiol Biomarkers Prev 13:2035-2042.	To convey expert opinions of mortality risks associated with the use of low-nitrosamine smokeless tobacco as compared with smoking cigarettes.	In comparison with smoking, experts perceive at least a 90% reduction in the relative risk of LN-SLT use. The risks of using LN-SLT products therefore should not be portrayed as comparable with those of smoking cigarettes as has been the practice of some governmental and public health authorities in the past. Importantly, the overall public health impact of LN-SLT will reflect use patterns, its marketing, and governmental regulation of tobacco products.

Smokeless Tobacco Reviews and Meta-analyses: Objectives and Author Conclusions		
Reference	**Objective**	**Author(s) Conclusion**
Levy DT, Mumford EA, Cummings KM, Gilpin EA, Giovino GA, Hyland A, Sweanor D, Warner KE, and Compton C. 2006. The potential impact of a low-nitrosamine smokeless tobacco product on cigarette smoking in the United States: estimates of a panel of experts. Addict Behav 31:1190-1200.	To predict the impact on tobacco use in the US on cigarette smoking of a "harm reduction" policy that requires that the smokeless tobacco product meet low nitrosamine standards, but could be marketed with a warning label consistent with the evidence of relative health risks.	An overall consensus was reached that the introduction of a new LN-SLT product under strict regulations would increase SLT use, but reduce overall smoking prevalence. This reduction would likely yield substantial health benefits, but uncertainties surround the role of marketing and other tobacco control policies.
Phillips CV. 2003. Smokeless tobacco and oral cancer, the curious history of a "fact". Atlanta, GA. Poster Presentation. 2003 Society for Epidemiologic Research Meeting	Position paper on the perceived risk of smokeless tobacco in relation to oral cancer.	Most public health experts, clinicians, and lay people “know” that use of smokeless tobacco (such as snuff dipping) causes oral cancer. This strong belief, widespread among experts and non-experts, is curious given that the evidence for this relationship is, at most, limited and highly equivocal.
Phillips CV, Sargent C, Rabiu D, and Rodu B. 2006. Calculating the comparative mortality risk from smokeless tobacco vs. smoking. Am J Epidemiol 163:S189.	To estimate the mortality risks from smokeless tobacco use compared with smoking.	Our results suggest it is very difficult to justify a comparative risk estimate for premature mortality from ST as high as 5% that from cigarettes. Despite the emphasis on cancer risk in discussions of ST, the uncertainty is dominated by CVD risk, likely from nicotine (it is not clear there is any such risk from ST, but some studies suggest it).Absent CVD risk, plausible estimates based on cancer risk alone yield values under 1%.
Phillips CV, Guenzel B, and Bergen P. 2006. Deconstructing anti-harm-reduction metaphors; mortality risk from falls and other traumatic injuries compared to smokeless tobacco use. Harm Reduct J 3:15.	To estimate the mortality risks from smoking and smokeless tobacco using a metaphor based on the available literature on mortality from falls. Position paper on metaphors used by anti-harm-reduction advocates.	If there are substantive arguments to be made against a harm reduction proposal, they should certainly be introduced into open debate. But exaggerated metaphors do not qualify as substantive arguments and violate the ethical duty (incumbent on all who claim some mantle of expertise and provide health advice) to provide people with accurate health information rather than trying to mislead or manipulate them.
Phillips CV and Rodu B. 2007. Tobacco. The Encyclopedia of Epidemiology. www.tobaccoharmreduction.org/overview.htm	To describe the health risks associated with cigarette smoking, other tobacco smoking, and environmental tobacco smoke, and contrast these to the effect of nicotine in itself and to the use of smokeless tobacco.	The epidemiologic evidence does not definitively demonstrate an association between ST use and any life-threatening disease. Extensive modern epidemiology has consistently shown that ST use causes very little or no risk of oral cancer (clearly much less than the substantial risk of oral cancer from smoking), or of any other life-threatening disease.

Smokeless Tobacco Reviews and Meta-analyses: Objectives and Author Conclusions		
Reference	**Objective**	**Author(s) Conclusion**
Phillips CV. 2008. Commentary: Lack of scientific influences on epidemiology. Int J Epidemiol 37:59-64.	Commentary on the lack of scientific influences on epidemiology.	Only with an improved science that is not the tool of one group of organized interests will it be possible to establish a professional identity that defends the science and the scientists against manipulation and political threats from advocates of all stripes.
Phillips CV and Heavner KK. 2009. Smokeless tobacco: the epidemiology and politics of harm. Biomarkers 14:79-84.	To review the epidemiology and politics of harm reduction as related to non-combustion tobacco products.	Epidemiological evidence suggests that smokeless tobacco causes about one one-hundredth the health risk of smoking. Despite the practice of harm reduction being widely accepted in public health, however, THR (tobacco harm reduction) has faced fierce opposition from anti-tobacco activists. These activists have effectively misled the public about what aspect of smoking cigarettes causes the harm, convincing them that nicotine and tobacco themselves are harmful, ignoring the smoke. In the interests of promoting public health and rescuing science from politics, experts on inhalation hazards and health could play an important role in educating the public and policy makers about THR.
Rodu B and Cole P. 1995. Excess mortality in smokeless tobacco users not meaningful. Am J Public Health 85:118-119.	Commentary on the Bolinder et al. 1994 study on smokeless tobacco use and excess cardiovascular mortality.	There is a reasonable non-biological explanation for the apparent excess of cardiovascular and all-cause deaths in young smokeless tobacco users: it is that members of the comparison group, nonusers of tobacco, are exceptionally healthy. We suggest that the unselected general population is the appropriate comparison group for smokeless tobacco users. From that perspective smokeless tobacco users have no meaningful excess mortality.
Rodu B, Stegmayr B, Nasic S, and Asplund K. 2002. Impact of smokeless tobacco use on smoking in northern Sweden. J Intern Med 252:398-404.	To examine the prevalence and interaction of cigarette smoking and use of snus in the population of northern Sweden.	The major finding in this study is that the prevalence of smoking amongst men in northern Sweden was very low, falling from 23% in 1986 to 14% in 1999. Recent epidemiologic studies have shown that Swedish snus is not associated with oral cancer or other smoking-related cancers. Furthermore, snus does not appear to be a strong risk factor for cardiovascular diseases. Thus, the balance of tobacco use in northern Sweden amongst men – and perhaps incipiently amongst women – may confer substantial health advantages compared with smoking-dominated societies.

Smokeless Tobacco Reviews and Meta-analyses: Objectives and Author Conclusions		
Reference	**Objective**	**Author(s) Conclusion**
Rodu B and Jansson C. 2004. Smokeless tobacco and oral cancer: a review of the risks and determinants. Crit Rev Oral Biol Med 15:252-263.	To review research relevant to the association of smokeless tobacco use and oral cancer including epidemiology studies, studies of tobacco contaminants, and possible cancer inhibitors.	The available epidemiologic studies indicate that the use of chewing tobacco and American moist snuff is associated with minimal risk for oral cancer, while the use of Swedish moist snuff is associated with no demonstrable risk.
Rodu B and Godshall WT. 2006. Tobacco harm reduction: an alternative cessation strategy for inveterate smokers. Harm Reduct J 3:37.	To describe an approach to smoking cessation, tobacco harm reduction, involving alternative sources of nicotine, including modern smokeless tobacco products. To describe traditional and modern smokeless tobacco products, review the epidemiology evidence for low health risks associated with smokeless use, both in absolute terms and in comparison to smoking and describe evidence that smokeless tobacco has served as an effective substitute for cigarettes among Swedish men.	Smokeless tobacco has served as an effective substitute for cigarettes among Swedish men, who consequently have among the lowest smoking-related mortality rates in the developed world. The established health risks associated with ST use are vastly lower than those of smoking.
Roth HD, Roth AB, and Liu X. 2005. Health risks of smoking compared to Swedish snus. Inhal Toxicol 17:741-748.	To review epidemiology studies that provide quantitative risk estimates associated with Swedish snus and cigarette smoking in a single population, using a common reference group.	Our review of the literature indicates that, for certain health outcomes, the health risks associated with snus are lower than those associated with smoking. Specifically, this is true for lung cancer (based on one study, Bolinder et al., 1994), for oral cancer (based on one study, Schildt et al., 1998), and for gastric cancer (based on one study, Ye et al., 1999). Three of four studies showed this for cardiovascular disease (Bolinder et al., 1994; Hergens et al., 2005; Huhtasaari et al., 1992). Although both snus and cigarette smoking were associated with increased risk of all-cause mortality, the risk was significantly greater with cigarette smoking (Bolinder et al., 1994; $p < .05$). Neither snus nor cigarettes were linked to increased risk of two forms of inflammatory bowel disease (Persson et al., 1993).

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Reference	**Objective**	**Author(s) Conclusion**
Royal College of Physicians. 2007. *Harm reduction in nicotine addiction. Helping people who can't quit. A report by the Tobacco Advisory Group of the Royal College of Physicians*. http://www.tobaccoprogram.org/pdf/4fc74817-64c5-4105-951e-38239b09c5db.pdf	To review harm reduction strategies to protect smokers.	On toxicological and epidemiological grounds, some of the Swedish smokeless (snus) products appear to be associated with the lowest potential for harm to health. Swedish smokeless products appear to increase the risk of pancreatic cancer, and possibly cardiovascular disease, particularly myocardial infarction. In Sweden, the available low-harm smokeless products have been shown to be an acceptable substitute for cigarettes to many smokers, while 'gateway' progression from smokeless to smoking is relatively uncommon.
Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR)). 2008. Scientific opinion on the health effects of smokeless tobacco products. http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_013.pdf	To evaluate the health effects of smokeless tobacco products.	All STP cause localized oral lesions and a high risk for development of oral cancer has been shown for various STP but has not been proven for Swedish moist snuff (snus). There is some evidence for an increased risk of fatal myocardial infarction among STP users. Some data indicate reproductive effects of smokeless tobacco use during pregnancy but firm conclusions cannot be drawn. Based on the available evidence it is difficult to identify overall relative risk estimates for the various adverse health effects from oral tobacco products as a whole because the products and conditions of use (e.g. frequency, duration, mode of use, other lifestyle factors) vary widely. There is sufficient evidence that the use of a wide variety of STP causes cancer in humans. Overall, in relation to the risks of the major smoking-related diseases, and with the exception of use in pregnancy, STP are clearly less hazardous, and in relation to respiratory and cardiovascular disease substantially less hazardous, than cigarette smoking.
Sponsiello-Wang Z, Weitkunat R, and Lee PN. 2008. Systematic review of the relation between smokeless tobacco and cancer of the pancreas in Europe and North America. BMC Cancer 8:356.	To conduct a systematic review and meta-analysis of the relationship between pancreatic cancer and use of smokeless tobacco in North America and Europe.	At most, the data suggest a possible effect of smokeless tobacco on pancreatic cancer risk. More evidence is needed. If any risk exists, it is highly likely to be less than that from smoking.
Weitkunat R, Sanders E, and Lee PN. 2007. Meta-analysis of the relation between European and American smokeless tobacco and oral cancer. BMC Public Health 7:334.	To conduct a systematic review and meta-analysis of the relationship between oral cancer and use of smokeless tobacco in America and Europe.	Smokeless tobacco, as used in America or Europe, carries at most a minor increased risk of oral cancer. However, elevated risks in specific populations or from specific products cannot definitely be excluded.