

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

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Acronyms and Abbreviations

µg:	microgram
1-HOP:	1-hydroxypyrene
4-NQO:	initiator 4-nitroquinoline-N-oxide
5-HT:	serotonin receptor
ACS:	American Cancer Society
AHA:	American Heart Association
ALS:	amyotrophic lateral sclerosis
AMI:	acute myocardial infarction
AUC:	areas under the curve
B*[a]*P:	benzo[a]pyrene
B*[b]*F:	benzo[b]fluoranthene
B*[k]*F:	benzo[k]fluoranthene
BAT:	British American Tobacco
BMI:	body mass index
BP:	blood pressure
Bq:	Becquerel
B-Se:	selenium in whole blood
CA:	chromosome aberrations
CD:	Crohn's disease
CDC:	US Centers for Disease Control and Prevention
CERP:	cholesterol efflux regulatory protein
CHD:	coronary heart disease
CHO:	Chinese hamster ovary
CI:	confidence interval
CK 10:	cytokeratin 10
Cmax:	mean maximum plasma concentration
CMM:	cutaneous malignant melanoma
CO:	carbon monoxide
CORESTA:	Cooperation Centre for Scientific Research Relative to Tobacco
Con A:	concanavalin A (a mitogenic substance that is used to induce T-cell proliferation)
CP:	Cancer potency

CPD:	[cigs/day]
CS:	cross-sectional study or cross-sectional analysis
CSCC:	cutaneous squamous cell carcinoma
CVD:	cardiovascular disease
CWC:	Construction Worker Cohort
CYP:	cytochrome P450 superfamily
DMSO:	dimethyl sulfoxide
DNA:	deoxyribonucleic acid
DOK:	dysplastic oral keratinocyte cell line
ECL:	enterochromatin-like
EFSA:	European Food Safety Authority
EL-IL-1-β:	elastase-IL-1-β mouse model
ELISA:	enzyme-linked immunosorbent assay
ESTOC:	The European Smokeless Tobacco Council
ET:	endothelin
EU:	European Union
FBN:	free base nicotine
FCTC:	Framework Covention on Tobacco Control
g:	gram
GERS:	gastroesophageal reflux symptoms
GI:	gastrointestinal
GM:	geometric mean
GPCR:	G-protein-coupled receptor
GPX:	blood glutathione peroxidase
Hb:	hemoglobin
HBMA:	4-hydroxybut-2-yl mercapturic acid
HD:	Hodgkin's disease
HDL:	high-density lipoprotein
HEMA:	2-hydroxyethyl mercapturic acid
HIV:	human immunodeficiency virus
Hp:	Helicobacter pylori
HPB:	4-hydroxy-1-(3-pyridyl)-1-butanone

HPHC:	harmful or potentially harmful constituents
HPMA:	3-hydroxypropyl mercapturic acid
HPRT:	hypoxanthine-guanine phosphoribosyl transferase
HPV:	human papillomavirus
HR:	hazard ratio
HSAVEC:	adult normal human endothelial cells
HSV:	herpes simplex virus
HSV-1:	herpes simplex virus-1
IARC:	International Agency for Research on Cancer
IBD:	inflammatory bowel disease
IBS:	irritable bowel syndrome
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
iso-NNAC:	4-(methylnitrosamino)-4-(3-pyridyl)butyric acid
iso-NNAL:	4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol
kg:	kilogram
L:	liter
LCMR:	lung cancer mortality rate
LDL:	low-density lipoprotein
LOD:	limit of detection
LOQ:	limit of Quantitation
LSRO:	Life Sciences Research Office, Inc.
MDPH:	Massachusetts Department for Public Health
MetSy:	metabolic syndrome
mg:	milligram
MGMT:	O6-methylguanine-DNA methyl-transferase
MHBMA:	the sum of 1-hydroxy-2-(N-acetylcysteinyl)-3-butene and 1-(N-acetylcysteinyl)-2-hydroxy-3-butene

MI:	myocardial infarction
MIS:	melanoma in situ
mL:	milliliter
MM:	multiple myeloma
MNBA:	4-(methylnitrosamino) butyric acid
MNPA:	3-(methylnitrosamino) propionic acid
MONICA:	Monitoring of Trends and Determinants in Cardiovascular Disease
mRNA:	messenger ribonucleic acid
MS:	multiple sclerosis
MW:	molecular weight
N:	number
NAB:	*N*-nitrosoanabasine
NAT:	N-nitrosoanatabine
NAzCA:	*N*-nitrosoazetidine-4-carboxylic acid
NDBA:	*N*-nitrosodibutylamine
NDBzA:	*N*-nitrosodibenzylamine
NDEA:	*N*-nitrosodiethylamine
NDELA:	*N*-nitrosodiethanolamine
NDMA:	*N*-nitrosodimethylamine
NDPA:	*N*-nitrosodi-n-propylamine
NDPIPA:	*N*-nitrosodiisopropylamine
NEMA:	*N*-nitrosomethylethylamine; also known as N-nitrosoethylmethylamine
ng:	nanogram
NHANES:	National Health and Nutrition Examination Survey
NHL:	non-Hodgkin's lymphoma
NHPRO:	*N*-nitrosohydroxyproline
NMOR:	*N*-nitrosomorpholine
NNA:	4-(methylnitrosamino)-4-(3-pyridyl)-butanal
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
NNN:	*N*-nitrosonornicotine

NOF: normal human oral fibroblast

NOK: normal human oral keratinocyte

NPICA/NPIC: *N*-nitrosopipecolic acid

NPIP: *N*-nitrosopiperidine

NPRO: N-nitrosoproline

NPYR: *N*-nitrosopyrrolidine

NRT: nicotine replacement therapy

NRU: neutral red uptake

NSAR: *N*-nitrososarcosine

NTCA: *N*-nitrosothiazolidine 4-carboxylic acid

NTP: National Toxicology Program

OGTT: oral glucose tolerance test

OOSCC: oral and oropharyngeal squamous cell carcinoma

OR: odds ratio

PAH: polycyclic aromatic hydrocarbon

PAR: population attributable risk

PBMC: peripheral blood mononuclear cell

PCNA: proliferating cell nuclear antigen

PCR: polymerase chain reaction

pg: pictogram

PM: particulate matter

POB: pyridyloxobutylated

PONV: post-operative nausea and vomiting

ppm: parts per million

PREP: potential reduced-exposure tobacco product

PSOL: portion snus original large

PSW: portion snus white

PSWL: portion snus white large

RPF: relative potency factors

RR: risk ratio

SAH: subarachnoid hemorrhage

SCC: squamous cell carcinoma

SCD:	sudden cardiac death
SCE:	sister chromatid exchanges
SCENIHR:	Scientific Committee on Emerging and Newly-Identified Health Risks
SD:	standard deviation
SES:	socioeconomic status
SGA:	small-for-gestational-age
SIL:	snuff-induced lesions
SM:	Swedish Match
S-NNN:	*S*-enantiomer of NNN
SNP:	single nucleotide polymorphism
SPMA:	S-phenyl mercapturic acid
SRNT:	Society for Research on Nicotine and Tobacco
S-Se:	selenium in serum
ST:	smokeless tobacco
STA:	Svenska Tobaks
STP:	smokeless tobacco product
SWS:	Swedish snus
TD50:	dose at which 50% of animals developed tumors under chronic administration
tk:	thymidine kinase
tmax:	time to maximum concentration
TN:	total normal nucleotides
Total NNAL:	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides
Total NNN:	*N*′-nitrosonornicotine and its glucuronides
TPM:	total particulate matter
TSNA:	tobacco-specific nitrosamine
TSRC:	Tobacco Science Research Conference
UC:	ulcerative colitis
UK:	United Kingdom
ULSAM:	Uppsala Longitudinal Study of Adult Men
US:	United States
USCDC:	US Centers for Disease Control and Prevention
USDHHS:	US Department of Health and Human Services

USEPA:	US Environmental Protection Agency
UST:	US Smokeless Tobacco Company
VNA:	volatile nitrosamine
WC:	waist circumference
WHO:	World Health Organization
WHR:	Waist-to-hip ratio
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, using large cohorts and readily available health outcomes data, 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 fire-cured and fermented, Swedish snus is made from air- or sun-cured tobacco and heat-treated. This difference helps to explain the lower concentrations of certain trace components in Swedish snus, including tobacco-specific nitrosamines (TSNAs) and polycyclic aromatic hydrocarbons (PAHs).

In its initial report, ENVIRON International Corporation (ENVIRON) 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 2011. Since that time, ENVIRON has systematically identified all literature as it is published; this current report is updated to reflect the scientific literature comprehensively through December 31, 2012 and selectively for important new studies through April 2013. The review includes sections on the chemical properties, the manufacturing process, biomarkers of exposure, and epidemiological and toxicological studies of Swedish snus. Relevant studies that provide analyses of Swedish snus in addition to other smokeless tobacco products and novel products marketed as snus are summarized in the Appendices to this report for product chemistry, biomarkers of exposure, and toxicology. The appendices also include a summary of the health risks to Swedish snus users and to smokers compared to those of nontobacco users, as well as a discussion of potential health risks to Swedish snus users who also smoke (so called, dual users) and snus users who were previously smokers (“switchers”).

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. Of a suggested panel of

validated biomarkers relevant for tobacco-related exposure, several were not available specifically for Swedish snus users.

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 fulfill the criteria described in the March 2012 FDA *Guidance to Industry* for determining if a product can be characterized as a modified risk product. This draft Guidance establishes standards for scientific studies needed to determine and characterize risk to be used by companies when applying to FDA for modified risk status.

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. Novel brands may deviate from these values. 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 a quality standard (GothiaTek®), for the entire manufacturing process; including limits for certain "undesired" trace-level components in snus. The current list of "Harmful or Potentially Harmful Constituents (HPHC)" released by the FDA in April 2012 consists of 93 components, 43 of which are thought to originate mainly from combustion processes. In this section, published data available on the remaining 50 components and on additional components in STPs that have been quantified or were considered relevant were discussed. Where available, results from extraction studies were also presented.

Concentrations of TSNAs, traditionally the most frequently analyzed and reported trace-level components in STPs due to their carcinogenic potential in experimental animals have 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. The newest data indicates that TSNA concentrations have continued to decline and combined NNK and NNN concentrations currently appear to be approximately half the limit (2 µg/g dry weight) recommended by the WHO in 2009.

Published data for most other trace-level components other than TSNAs analyzed in STPs and snus have become available (e.g., PAHs, aldehydes, metals, and radioisotopes). PAH

concentrations reported in recent studies demonstrate that B[*a*]P concentrations are generally lower than the limit recommended by the WHO in 2009 (5 ng/g dry weight). Limited data on the presence of other PAHs indicates that only phenanthrene, fluoranthene, pyrene, and possibly naphthalene were detected in higher quantities. Generally, the analytical data from recent published studies on the various components indicate that concentrations in traditional Swedish snus are below the GothiaTek® limits as well as existing WHO-recommended limits.

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, nicotine content and delivery between snus brands, as well as, extraction and absorption of the chemical substances from snus, which all need to be taken into account when conducting an exposure assessment.

A comparison of critical components in traditional Swedish snus with other STPs, such as new products marketed as snus and US-type moist snuff, other factors, including moisture content, pH and resulting free nicotine are provided in Appendix II.

For a risk assessment, patterns of use of any of the STPs 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.

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

Studies of exposure biomarkers in individuals who use various STPs have increasingly been reported in the scientific literature. Biomarkers of exposure may be used to assess the actual internal dose of a tobacco component to which a tobacco user might be exposed. While limitations to the available biomarkers of exposure exist, they can be used to supplement information from product analyses as they reflect total exposure, bypassing differences in routes of exposure and product use behavior. In addition, biomarker levels on a population basis may give an indication of general trends in internal exposure to certain components of a well characterized product. With respect to harm reduction, conclusions from these studies should be interpreted carefully and in the context of additional data from clinical and/or epidemiological studies

A panel of biomarkers of exposure to components in tobacco products has been recently proposed for the use in product regulations. Many biomarkers of exposure are less relevant for non-combusted tobacco products such as snus; however, the panel does include the potentially relevant biomarkers of exposure for snus: nicotine, TSNAs, PAHs, aldehydes, cadmium, and acrylamide. To date, published studies are available that have investigated biomarkers of exposure to nicotine, TSNAs, cadmium, and selenium in regular users of traditional Swedish snus.

Commonly measured biomarkers of nicotine exposure are cotinine in plasma or serum. However, their levels may be impacted by the route of exposure, i.e., first pass metabolism of

nicotine to cotinine via the oral route may result in higher blood concentrations of cotinine that do not necessarily reflect increased exposure to the parent compound, nicotine. Total nicotine equivalents in urine are considered to better represent the total nicotine dose absorbed. Information from nicotine pharmacokinetic parameters is relevant for nicotine delivery, total dose, and abuse liability assessments. The time to maximum plasma nicotine concentrations in snus users appears to be dependent on the usage time, but not on nicotine content or portion size. On the other hand, C_{max} and AUC appear mostly dependent on total nicotine content (per pouch or portion size) as well as pH of the product. Whether the snus was loose or pouched had no influence on these parameters.

A number of studies in regular snus users show that mean or median cotinine levels in plasma or serum range from 137 to 399 ng/mL, depending on the amount of snus consumed (average 11-32 g/day). In the saliva, average levels ranged from 80 to 343 ng/mL. Urinary biomarkers of nicotine measured in regular users of snus were as follows: for nicotine itself, 29 µg/mmol creatinine; for cotinine, approximately 1000–1210 µg/L; for total cotinine, 5926 µg/L; and for nicotine equivalents ranged from 14-36 mg/24 hrs.

TSNAs and their metabolites have been determined in various human bodily fluids, including saliva, blood, and urine, as well as in toenails. Urinary NNAL is the most commonly-measured biomarker of TSNA exposure, and is considered to reflect 12-17% of the NNK dose.

Four studies of TSNA biomarkers in users of Swedish snus were identified. Of those, one publication from 1988 measured TSNA levels in saliva during snus use; snus in the 1980s contained considerably higher TSNA concentrations than more contemporary snus products. More recently, urinary total NNAL was measured in users of conventional US STPs that were switched to *General* snus use. Of the two clinical studies available, only one appears to have a sufficient duration to examine for and detect differences in levels before and after the switch. In this study, total NNAL levels decreased significantly (to half the concentration measured at baseline) by week 4. Importantly, urinary total cotinine levels in this study did not change significantly, indicating the decreased toxicant exposure could not be explained by a decrease in tobacco intake and mean product use was similar to that reported for regular snus users. No studies measuring biomarkers of NNN in snus users were identified. POB-DNA adducts were significantly increased in oral mucosa of Swedish snus based on information provided in a study abstract; however, the importance of these adducts in oral cancer development has been questioned.

With respect to the available studies of biomarkers of metals/metalloids, both levels of cadmium and selenium biomarkers in regular users of traditional Swedish snus were similar to those detected in non-tobacco users.

Toxicological Studies

Swedish snuff/snus has been investigated *in vitro* for genotoxic and cytotoxic endpoints in a variety of cell types, in animal models, including surgical lip canal in rats, cheek pouches in hamsters, and dietary studies in transgenic mice in comparison with wild-type strains. The available *in vitro* studies in cell types relevant to oral, cardiovascular, and immune systems indicate that snus extracts can cause concentration-dependent changes in cell morphology,

viability, and other endpoints, including cell proliferation, gene expression, and expression and function of GPCR receptors. However, it is unknown to what extent the effects seen *in vitro* are relevant for the highly complex *in vivo* situation. In three sets of genotoxicity assays, most snus extracts, at best, showed weak and variable mutagenicity in bacteria, except for a snus extract in methylene chloride that was positive. No pattern of responses indicative of genotoxicity relevant for human snus users was observed in the available studies.

While of invasive nature, the seven experiments involving the surgical lip canal rat model appear to present a route of exposure sufficiently comparable to human use that they are considered informative for human risk assessment, despite several limitations. Although non-malignant oral lesions similar in histopathology to those seen in human snus users ("snus-induced lesions") were observed in snus-treated rats, the incidence of oral cavity tumors in treated animals were not significantly different from controls.

Two studies in wild-type and transgenic mice strains may provide some mechanistic information related to gastrointestinal and pancreatic pathology potentially associated with ingestion of tobacco products. However, limitations in the data, i.e., the differences in exposure route, dose and study duration, make the data difficult to extrapolate to human risks. In the wild-type mouse strains, treatment with snus alone for 6 months did not cause any changes in the stomach wall except for an increased expression of an apoptosis marker and no changes in the pancreas were detected after 15 months.

Snus treatment for 6 months combined with hypergastrinemia in a transgenic mouse model of stomach cancer and/or *H. pylori* infection caused histopathological changes in the stomach wall, though the contribution of snus cannot be established due to the lack of a *H. pylori*-infected control group, and the small number of treated animals. Possible preneoplastic changes were observed; however no malignancies were observed,

The toxicology data base for effects of snus exposure to *in vitro* cell systems and various animal models is not large, compared to data for effects of other tobacco products. Nevertheless, the cellular pathology reported in the animal models, as well as the lack of snus-related tumor development, is consistent with the human data base for snus users. Thus, the nonclinical data are useful for informing on snus-related effect mechanisms in humans if care is taken to apply appropriate weight of evidence to the experimental models and the epidemiology data.

Epidemiological Studies

A characteristic type of oral mucosal lesion ("snus-induced lesion") which is localized to the area where the snus is placed, has been observed in epidemiological studies of Swedish snus users; however, the lesions are reversible following cessation of snus use and there is no clinical evidence to suggest that they transform into malignancies. No other effects of snus use on periodontal disease, gingivitis, gingival recessions, and other dental conditions were consistently identified among studies that controlled for important confounders such as socioeconomic status and oral hygiene habits.

Evidence from clinical studies suggests that Swedish snus use acutely increases blood pressure and heart rate, almost certainly due to nicotine. An increased risk of developing

hypertension was observed in the single available prospective cohort study, among Swedish Constructions Workers, but limited to participants with repeated visits, and not the entire cohort. No other consistent associations between biochemical measurements and other risk factors for cardiovascular disease were observed. Single epidemiological studies observed an increased risk of death from myocardial infarction and from one specific stroke type among Swedish snus users; however, multiple additional findings for risk of MI and stroke have consistently shown no association between use of snus and these cardiovascular outcomes.

Well controlled epidemiological evidence indicates that Swedish snus is not associated with oral cancer or with lung cancer. Though the studies are mostly consistent showing no association between Swedish snus use and esophageal cancer, a single recent study did observe an increased risk for this cancer site. 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: laryngeal, stomach, kidney, bladder, skin, colon, anal, rectal, and 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 populations studied; there are inconsistencies between the two studies and the interpretation of the studies has been the topic of much scientific debate. A third analysis that pooled several studies of Western smokeless tobacco and pancreatic cancer did not observe an association with this cancer type. Though it is unlikely that Swedish snuff was a major product used in any of the populations included in the analysis, these results are potentially relevant with respect to Swedish snus in that smokeless tobacco used in North America and other western countries are expected to contain more TSNAs than Swedish snus. TSNAs are thought to be the components of tobacco products that are likely associated with an increased risk of pancreatic cancer.

Multiple studies have examined weight, weight gain, and measures of central adiposity in association with snus and smoking. Because smoking is known to suppress body weight, and many people who quit smoking gain weight, only studies that addressed the potential confounding effect of current or former smoking were examined. Some evidence suggests that snus use may be associated with higher BMI or weight gain in studies that account for past and current smoking. However, overall, the results are mixed; even those of the two studies of consistent snus users are contradictory.

Body weight and composition are important risk factors for type 2 diabetes and metabolic syndrome. One well-conducted prospective study found that use of Swedish snus was not associated with increased risk of diabetes, but two additional epidemiologic studies of the same population concluded that heavy users of moist snuff have an increased risk of type 2 diabetes; each study had significant limitations with respect to study design and sample size. Though a single study has suggested that heavy use of Swedish snus could be associated with increased risk of metabolic syndrome (MetSy), other studies have not observed this outcome, or associations with clinical markers of MetSy, such as insulin reactivity. Other components of MetSy include body, weight, hypertension, and diabetes, which as discussed above, may be associated with snus use. Further research is needed to understand the potential mechanisms and causative factors to determine if snus use increases the risk of these metabolic-related health outcomes.

The literature indicates that use of Swedish snus is not associated with harmful gastrointestinal symptoms or diseases, including peptic ulcer, reflux, dyspepsia, or heartburn, Crohn's disease or ulcerative colitis. One study reported increased risk of altered histology of the esophago-gastric junction among exclusive snus users when examined macroscopically in a subset of study participants. This finding needs to be confirmed in additional studies.

Several epidemiological studies suggest that daily use of Swedish snus during pregnancy is associated with some adverse consequences (a modest reduction in average birth weight and small-for-gestational-age birth, and increased risk of preterm delivery, stillbirth, and neonatal apnea). Daily use of snus during pregnancy is not associated with risk of preeclampsia, gestational hypertension, or antenatal bleeding. 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.

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 health risks known to be associated with chronic use of Swedish snus are benign, snus-induced lesions in some snus users, and acute, reversible cardiovascular effects such as an increase in blood pressure and heart rate, most likely due to nicotine. Overall, the evidence supports a conclusion that current use levels of snus in Sweden are not 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 for long term cardiovascular risks, and possible 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. 2009b). 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

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

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 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 Chapter 2 of this report), and not other kinds of smokeless tobacco, though often data regarding other types of tobacco products are referred to in comparison to data from snus. Data for these other forms of tobacco are summarized in the Appendices to this report, including a chemical analyses and exposure biomarkers of other tobacco products, a summary of the health risks to Swedish snus users and to smokers compared to nontobacco users, as well as a discussion of potential health risks to Swedish snus users who also smoke (so called, dual users) and snus users who were previously smokers ("switchers") (Appendices II, III, VI, VII, and VIII).

It is also not the intent of this report to present a comprehensive 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 cannot abstain from using tobacco. 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; the FDA issued draft guidance to industry, "Modified Risk Tobacco Product Applications", which establishes standards for scientific studies needed to determine and characterize risk 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 (FDA 2012b).

The FDA guidance specifies, that for a risk modification order, the applicant "provide scientific evidence to demonstrate that the product significantly reduces harm" to the individual and benefits the public health as a whole. The guidance recommends product chemical analyses, human studies including clinical, population and epidemiology studies that examine tobacco use behaviors, and epidemiology studies that show the tobacco product's use will result in significant reduction of harm to the individual tobacco users, and suggests that nonclinical and clinical studies that look at intermediate health endpoints and biomarkers of exposure provide evidence of the potential for a product to result in reduced risk. In addition, the Guidance calls for post market studies that may 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.

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

In its initial report, ENVIRON International Corporation (ENVIRON) 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, governmental databases, several additional databases in Dialog® through December 31, 2009. Since that time, ENVIRON has systematically identified all literature as it is published through frequent searches and publication alerts using these same resources. The ENVIRON review summarizes studies of the potential health risks associated with the use of Swedish snus comprehensively through December 2012, and selectively for important new publications as available through April 2013. 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 these 'new products marketed as snus' and discusses if and how they differ from traditional Swedish snus. Furthermore, a distinction between Swedish snus and these new products from US-type oral moist snuff is made, where available data allowed direct comparison. This includes more specific discussions on Swedish Match's *Catch Dry* products, which are novel brands similar to traditional Swedish snus and manufactured under the GothiaTek® standard, 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 literature published from 2004 to 2012. The more recent literature is more likely to contain STP brand names of samples analyzed in the studies, and this information has been included in the present chapter whenever available.

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. Between 1915 and 1992, snus has been manufactured by only one company, the Tobacco Monopoly followed by the state-owned company (STA), which eventually, during the 1990s, became the private company

Swedish Match (SM). First competitors appeared in 1992; however, SM is still the major manufacturer of snus in Sweden with a market share of approximately 85% (Rutqvist et al. 2011).

The manufacturing principles of snus have remained essentially the same as in the 1800s, when fermentation of tobacco was replaced by heat treatment to achieve specific flavor characteristics. In the late 1960s, the temperature during the heat treatment was raised slightly to decrease microbial contamination. Since 1971, snus has been regulated by the Swedish Food Act, which imposed food-grade hygienic requirements and restrictions to ingredients, additives, and containers. In 1982, the manufacturer of snus in Sweden that later became 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 Rutqvist; (Rutqvist et al. 2011). These changes are based on processing the ground tobacco in closed process blenders at much higher temperatures, stabilization of the pH with sodium carbonate, and use of humectants to reduce water activity in the final product (Rutqvist et al. 2011).

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 "undesired" components (see Section 2.3.7) (Swedish Match 2012[3]).

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 2009/2013)[4]. 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 2012). 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 Rutqvist). 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

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[2] Some authors have erroneously reported that the 1981/82 change in processing was a switch from the fermentation to the heat treatment method (Ramström 2000).

[3] Swedish Match. 2012. http://www.swedishmatch.com/en/Snus-and-health/GOTHIATEK/, accessed April 2013.

[4] ESTOC. 2009/2013. http://www.estoc.org/about-smokeless-tobacco/production, accessed November 2009; April 2013.

[5] Swedish Match. 2010. http://www.swedishmatch.com/en/Snus-and-health/Our-quality-standard-GothiaTek/GothiaTek-standards/, accessed February 2010.

slow the normal ageing process and to preserve moisture (Swedish Match, personal communication with Dr. Lars Erik Rutqvist). The snus is filled in tea bag-like pouches (mini-portion or standard portion sachets) or loose in tins or boxes (ESTOC 2009/2013). In Sweden, retailers also keep the product refrigerated until sale (Foulds et al. 2003).

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

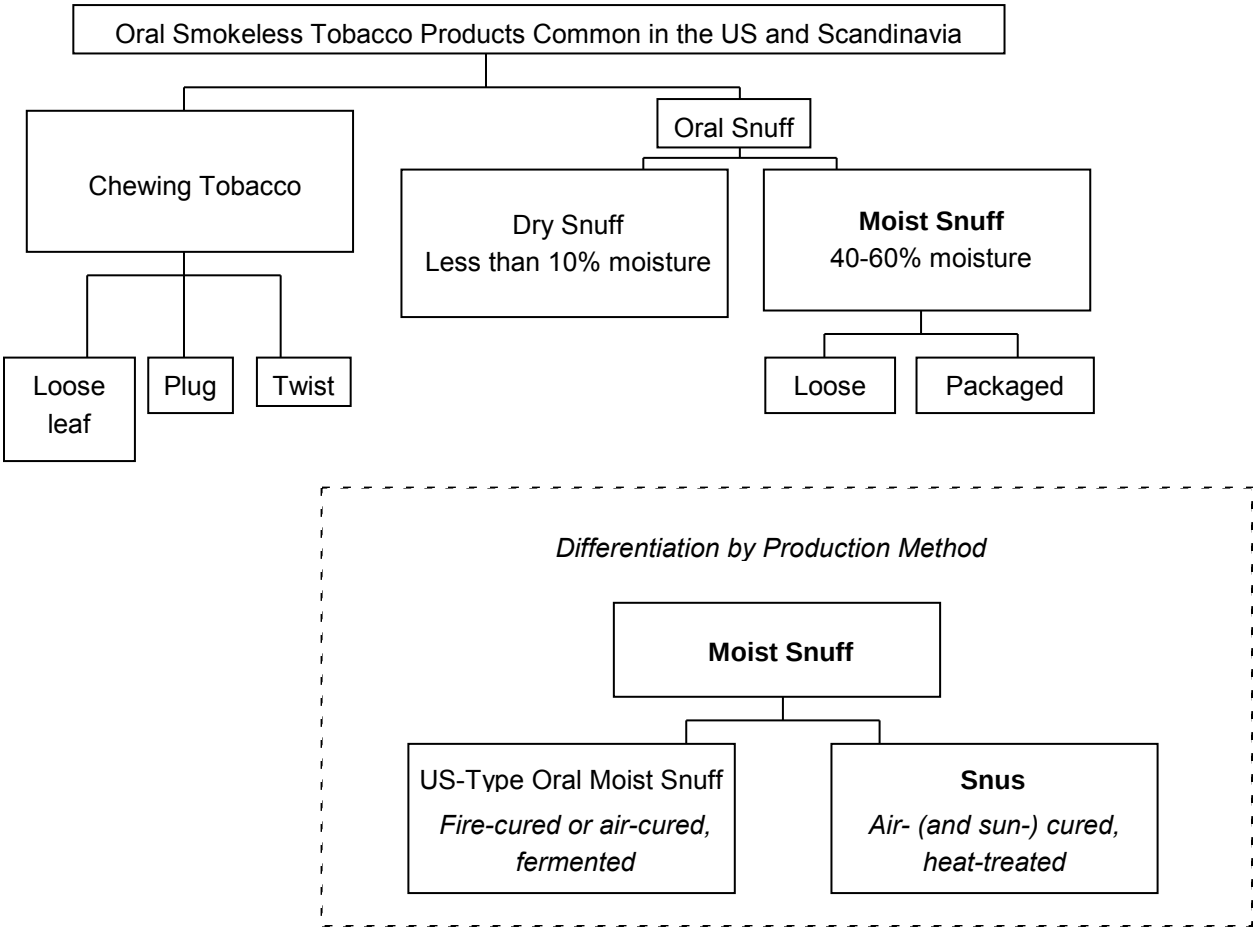
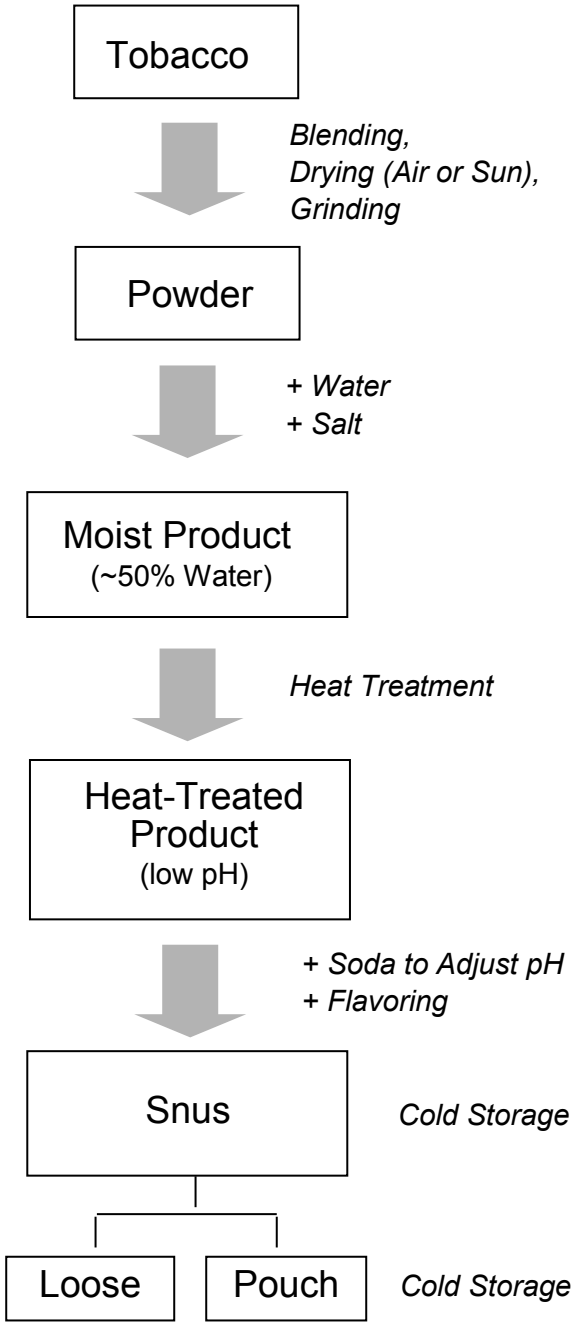


Figure 2-2. Manufacturing Process of Snus (According to ESTOC 2009[6])



[6] ESTOC. 2009/2013. http://www.estoc.org/about-smokeless-tobacco/production, accessed November 2009, April 2013.

2.2 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 2013[7]). 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 2013). Ethanol may serve as a processing aid or solvent (Swedish Match 2013). 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 2013).

[7] Swedish Match. 2013. http://www.swedishmatch.com/en/Our-business/Snus-and-snuff/Ingredients-in-snus/?tab=1, accessed April 2013.

While the Tobacco Control Act of 2009 requires tobacco product manufacturers or importers in the US to submit a listing of all ingredients[8], there is currently no US regulatory requirement to 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 report analyses of additives in oral moist snuff. 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.

As noted above and 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 (see Section 2.1).

2.3 Chemical Analysis of Snus

More than 8,000 different components have been identified in tobacco and tobacco smoke (Rodgman and Perfetti 2009). Information on concentrations of at least 542 components in main stream smoke is available according to a recent publication by Talhout and colleagues (2011). To date, a much smaller number of tobacco/tobacco smoke components has been studied more thoroughly and related to health effects. Because of the extensive number present in tobacco products and the complexity of the mixture, research efforts over the last decades have been made to establish the components that cause or contribute to tobacco-use associated disease and to identify those that contribute most. It is thought that "for many diseases attributable to tobacco use, reducing risk of disease by reducing exposure to tobacco toxicants is feasible" (Institute of Medicine 2012). In this context, several regulatory bodies and researchers have established or proposed priority lists of components for analysis and regulation based on their prevalence and potency to cause disease (e.g., Ayo-Yusuf and Connolly 2011; Burns et al. 2008; Cunningham et al. 2011; Fowles and Dybing 2003).

In April 2012, the U.S. FDA released a list of "Harmful or Potentially Harmful Constituents (HPHC)" that it determined can cause direct or indirect harm. This list is based on lists previously established by other authoritative bodies (e.g., WHO 2009) and currently consists of

[8] 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 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.)

93[9] components, listed as associated with at least one of five different disease outcomes (cancer, cardiovascular disease, respiratory effects, developmental or reproductive effects, and addiction) (FDA 2012a).

Many of the chemicals on the current list are routinely analyzed in tobacco smoke. However, since STPs are not combusted, components yielding from this process are not present in STPs. Accordingly, a substantially lower number of components have been quantified in STPs compared to tobacco smoke. Of the 93 components on the current HPHC list, 43 are thought to originate from combustion of tobacco or have never been quantified comprehensively in STPs. These include aromatic amines[10], volatile hydrocarbons[11], some carbonyls[12] and inorganics[13], phenols[14], heterocyclic aromatic amines[15], chlorinated dioxins and furans, other aromatic components[16], small organic components[17], and some epoxides[18]. These components are not further discussed in the present report.

The remaining 50 components together with additional components that have been quantified or were considered relevant to STPs are discussed in the following sections and data available from the scientific literature for traditional Swedish snus is presented. 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 on the Swedish and US market and traditional US-type oral moist snuff where reported in more recent (2004 to 2012) published studies (Tables A II-1 to A II-7).

2.3.1 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). The American Heart Association (AHA) recommends limiting salt intake to less than 1,500 mg sodium (3.8 g of sodium chloride) per day to reduce the risk of cardiovascular disease[19].

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[9] The count of 93 components is based on the following: stereoisomers of cresol (*o*-, *m*-, *p*-), metals/metalloids and their compounds, as well as chlorinated dioxins and furans were not counted separately.

[10] 4-Aminobiphenyl, 1-aminonaphthalene, 2-aminonaphthalene, *o*-anisidine, *o*-toluidine, 2,6-dimethylaniline

[11] Acrylonitrile, 1,3-butadiene, benzene, ethylbenzene, isoprene, styrene, toluene

[12] Acetone, methyl ethyl ketone, propionaldehyde

[13] Carbon monoxide, hydrogen cyanide

[14] Catechol, cresols (o, m, p), phenol

[15] A-α-C (2-Amino-9H-pyrido[2,3-b]indole), Glu-P-1 (2-Amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole), Glu-P-2 (2-Aminodipyrido[1,2-a:3',2'-d]imidazole), IQ (2-Amino-3-methylimidazo[4,5-f]quinoline), MeA-α-C (2-Amino-3-methyl)-9H-pyrido[2,3-b]indole), PhIP (2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine), Trp-P-1 (3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole), Trp-P-2 (1-Methyl-3-amino-5H-pyrido[4,3-b]indole)

[16] Caffeic acid, coumarin (banned in food), furan, nitrobenzene, quinoline

[17] Acetamide, nitromethane, 2-nitropropane, vinyl acetate, vinyl chloride

[18] Ethylene oxide, propylene oxide

[19] American Heart Association (AHA). 2013. http://www.heart.org/HEARTORG/GettingHealthy/NutritionCenter/HealthyDietGoals/Sodium-Salt-or-Sodium-Chloride_UCM_303290_Article.jsp, accessed April 2013.

Sodium Concentration in Traditional Swedish Snus

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% (35 mg/g wet weight sodium chloride or 13.8 mg/g wet weight sodium).

Sodium Extraction

Lunell and Lunell (2005) 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 30 minutes of use by regular Swedish snus users, and the extracted amount was determined by calculating the difference between both values. This extracted amount, i.e., the amount that would be expected to be ingested by the studied snus users, varied between approximately 5.6 and 10.4 mg sodium chloride per portion (8-11 mg/g wet weight or 3.1-4.3 mg/g wet weight sodium).

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.

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[20], corresponding to 2.75 mg of sodium. With an average consumption of pouched snus of 12 g per day (Digard et al. 2009), the daily intake of sodium would range from approximately 28 to 53 mg for the three different snus brands tested. This is approximately 1.9 to 3.5% of the AHA-recommended upper limit of daily sodium intake.

[20] These authors concluded that it would take daily consumption of approximately 900 sachets of snus to produce an intake of approximately 6 g of sodium chloride, based on previous recommendations with respect to sodium intake (e.g., AHA 2009).

A new study[21] reported approximately 25% of sodium extraction (6.1 mg/1-g pouch) from a snus product under 60 minute use conditions (Digard et al. 2013). The mean amount sodium extracted is slightly higher than what was detected in the study by Lunell and Lunell (2005) and would amount to approximately 73 mg sodium per day (4.8% of the AHA recommended upper limit).

In summary, the available data indicates that the average amount of sodium extracted from traditional Swedish snus ranges from 2 to 4.4 mg per pouch under 30 minute use conditions. Data from a product with higher than the typical sodium chloride content of 3.5% indicate a mean extracted amount of sodium of 6.1 mg per pouch. Thus, the amount of sodium from Swedish snus contributes less than 5% to the recommended daily limit.

2.3.2 Alkaloids Other Than Nicotine

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 minor tobacco alkaloids are anabasine, anatabine, cotinine, nornicotine, and myosmine (Ramström 2000). As discussed in section 2.3.6.1 in more detail, nicotine and other alkaloids can react to form tobacco-specific nitrosamines (TSNA).

The minor alkaloids are thought to be pharmacologically active (Clemens et al. 2009; Dani et al. 2009, as cited in FDA 2010)[22]. There are indications that nornicotine may accumulate in the brain and thus 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). Further, a study in a behavioral model of addiction in rats indicates that minor alkaloids, in particular anatabine, myosmine, and cotinine may increase the reinforcing effects of nicotine (Clemens et al. 2009).

The current FDA list of HPHCs list the alkaloids anabasine and nornicotine based on their potential to contribute or increase addiction (FDA 2012a).

Alkaloids in Traditional Swedish Snus

Concentrations of tobacco alkaloids other than nicotine are not frequently reported in the literature. In a study of new and traditional smokeless tobacco products, Stepanov and colleagues (2008a) provided analyses of one traditional Swedish snus brand (*General*). Nornicotine, anatabine, and anabasine concentrations were reported to be 0.223, 0.367, and

[21] This study by BAT researchers tested the extraction of various components from a *Lucky Strike Original Snus* after 60 minutes of use by regular Swedish snus users (Digard et al. 2013). These authors reported approximately 25 % of sodium extraction (6.1 mg) from a product that had a mean sodium concentration of 24.7 ±1.97 mg per 1-g pouch. This study also showed high inter-subject variability (25%) and overall variability (45%).

[22] FDA. 2010. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/TobaccoProductsScientificAdvisoryCommittee/UCM214299.pdf, accessed April 2012.

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, myosmine, anatabine, anabasine, 2,3'-dipyridyl, and cotinine (Hoffmann et al. 1991a).

2.3.3 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). FDA's current HPHC list categorizes nicotine for its addiction potential and reproductive or developmental toxicity as concerns (FDA 2012a). Other effects of nicotine have been implicated including its potential to affect the cardiovascular system (Benowitz 2009).

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 present in the product, and by other product design features and human usage factors (Lauterbach et al. 2010).

Non-ionized (unprotonated) nicotine, also called 'free nicotine' or 'free-base nicotine' is rapidly absorbed through the mucosal membrane (Armitage and Turner 1970). The amount of non-ionized nicotine in a tobacco product 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)[23], these characteristics 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 cooled conditions.

Tobacco products with a higher pH value have greater non-ionized nicotine content and thus are thought to have the potential to deliver more nicotine to the user and hence may be more addictive. As a result, regulators (e.g., Massachusetts, USCDC, Health Canada, FDA) have mandated to report the free base nicotine (FBN)-content of tobacco products (Lauterbach et al. 2010).

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

Some authors have called the scientific relevance of the determination of product pH value and free base nicotine of STPs into question (Lauterbach et al. 2010). Because the free base nicotine content is not analytically determined but calculated based on the pH and the total nicotine content of whole tobacco samples using the Henderson-Hasselbach equation[24], Lauterbach and colleagues (2010) investigated how different product chemistries can influence the determination of pH-value of aqueous extracts of STPs and subsequently the calculated free base nicotine values. Based on their analysis, Lauterbach and colleagues (2010) questioned the relevance of the pH value of the aqueous extract, as the pH value might be very different under actual use conditions and influenced by different factors; these include product design features, physiological factors, such as the buffering capacity of saliva, and individual usage behavior, e.g. concurrent consumption of acidic beverages.

Other authors observed, however, that for oral tobacco products, the extent and speed of oral nicotine absorption into the systemic circulation is largely dependent on product pH (Richter et al. 2008), e.g., the buffering capacity of moist snuffs were shown to be 10 to 20 times higher than the buffering capacity of human saliva (Ciolino et al. 2001), excluding the potential influence of foods and drinks that influence acidity in the mouth.

Lauterbach and colleagues (2010) also questioned the use of the Henderson-Hasselbach equation, which was developed for a dilute aqueous solution of a base and its conjugate acid. These authors argued that the correct use of the equation requires an aqueous extract with no other acids, bases, and salts present, which is not the case in the aqueous nicotine-containing extract from an STP. They also pointed out that the influence of temperature should be accounted for since the acid dissociation constant pKa, a parameter included in the Henderson-Hasselbach equation is temperature dependent and decreases as the temperature increases. Further, the authors noted that there is little evidence in the clinical literature to support that free base nicotine contents of aqueous extracts of STP correlate with the pharmacokinetics of nicotine absorption.

Moisture, pH, Total and Free Nicotine Concentrations in Traditional Swedish Snus

Seven more recent studies are available in which total nicotine, free nicotine, pH, and moisture in traditional Swedish snus are reported (Borgerding et al. 2012; Digard et al. 2012; Lunell and Curvall 2011; Lunell and Lunell 2005; McNeill et al. 2006; Stanfill et al. 2010; Stepanov et al. 2008a). (Table A II-1a in Appendix II).

Moisture

Moisture levels in traditional Swedish snus are approximately 50% (Table 2- 1).

[24] pH = pKa + log(B/BH+) with pKa: acid dissociation constant (for nicotine pKa = 8.02), B: free-base nicotine, BH+: ionized nicotine. Total FBN (mg/g) = total nicotine (mg/g) x ((B/BH+x100)/ (B/BH++1))/100

An older 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. 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).

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, six months of storage at room temperature decreased the moisture in a sample of traditional Swedish snus (*Ettan*) from approximately 56% to less than 30%.

Consistent with results from the older studies, levels as measured in newer studies were between 45.8 and 56.3% (weight/weight) moisture; (Table A II-1a in Appendix II) (Borgerding et al. 2012; Digard et al. 2012; Digard et al. 2013; McNeill et al. 2006; Stepanov et al. 2008a). Similarly, data on 21 portion snus and 11 loose snus samples from Sweden (sourced in 2008 from seven different manufacturers) presented in a poster by (British American Tobacco) BAT researchers at a Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) conference indicates that the majority of pouched samples and all loose snus samples had a moisture content between 44 and 52% (Faizi et al. 2010). Four pouched samples had less than 40% moisture, indicating that they were either novel brands of traditional snus products or new products marketed as snus.

pH

The target value of the pH in traditional snus is close to 8.5 (Swedish Match 2009)[25]. 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).

Brunnemann and colleagues (Brunnemann et al. 1985) reported values between pH 7.3 and 8.7 in three brands of Swedish moist snuff purchased between 1984 and 1985. In an analysis of three Swedish snuff brands on the market around 1990, the same investigators measured values between pH 7.67 and 7.94 (Hoffmann et al. 1991a). 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 samples of portion-bag and loose snus, respectively.

[25] Swedish Match. 2009. http://www.swedishmatch.com/en/Snus-and-health/Research-on-snus/Snus-nicotine-and-nicotine-addiction/, accessed April 2013.

Consistent with the findings of previous studies, the pH of traditional snus as measured in studies from 2005 through 2012 generally ranged from 7.5 to 8.7 (Table A II-1a in Appendix II) (Borgerding et al. 2012; Digard et al. 2012; Lunell and Curvall 2011; Lunell and Lunell 2005; McNeill et al. 2006; Stepanov et al. 2008a). The highest pH values were reported for samples of *General White Large* and *General Onyx* (Lunell and Curvall 2011). Only one study conducted by researchers from the U.S. Center for Disease Control and Prevention (CDC) reported lower values, which ranged from pH 6.6 to 7.21 in four *General* brands and one *Catch* brand (Stanfill et al. 2010). The reason for these discrepancies is unclear. Different from the early study by Andersson and colleagues (1994), both Borgerding and colleagues (2012) and Stanfill and colleagues (2010) observed a lower pH for *General Loose* compared to pouched *General* brands.

Nicotine

Investigators of the work group of Brunnemann and Hoffmann 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 1980 per dry weight of tobacco were between 1.13 and 1.81% (11.3-18.1 mg/g) and in 1990 between 1.13 and 1.25% (11.3-12.5 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, therefore it is likely to be on an “as is”-basis (wet weight). The nicotine level in one brand of Swedish snus (*Ettan*) purchased in 2000 was reported to be 2.01% (20.1 mg/g) (Brunnemann et al. 2001).

In more recent studies that analyzed total nicotine content in various traditional Swedish snus brands (including several *General* and *Catch* brands and one *Nick and Johnny* brand) concentrations were generally in the range of 7.04 and 11 mg/g wet weight (approximately 14 and 22 mg/g dry weight (Table A II-1a in Appendix II) (Borgerding et al. 2012; Digard et al. 2012; Digard et al. 2013; Lunell and Curvall 2011; Lunell and Lunell 2005; McNeill et al. 2006; Stanfill et al. 2010; Stepanov et al. 2008a). There were only two exceptions, one of which was a traditional Swedish snus product, *Catch Peppermint*, with a slightly higher reported total nicotine concentration of 15.2 mg/g wet weight (approximately 30 mg/g dry weight) (Stanfill et al. 2010).

Free Nicotine

Calculated free nicotine of traditional snus (“general [sic] pouch”, *General*) was reported in two recent studies to be 6.3 and 7.7 mg/g dry weight, respectively (Table A II-1a in Appendix II) (McNeill et al. 2006; Stepanov et al. 2008a). Borgerding and colleagues (2012) reported free nicotine concentration between 4.5 and 5.1 mg/g wet weight (43-61% of total nicotine) in pouched *General* brands. Free nicotine in other snus brands, including *General Loose* and *Nick and Johnny* ranged from 1.6 to 5.7 mg/g wet weight (23-70% of total nicotine) in the same study. Consistent with their reported lower pH values, Stanfill and colleagues (2010), reported free nicotine concentrations in this study ranging from 0.52 to 0.78 mg/g wet weight (6.5-10% of total nicotine) for pouched *General* brands. *General Loose* was reported to have a lower free nicotine concentration (0.29 mg/g wet weight; 3.8% of total nicotine) than pouched *General* brands (Stanfill et al. 2010). Consistent with its high total nicotine content, *Catch Peppermint*

had the highest free nicotine concentration in this study (2.03 mg/g wet weight; 13.3% of total nicotine).

Summary

In summary, it appears that the moisture levels and pH values in traditional Swedish snus range from approximately 45 to 56% and 7.5 to 8.7, and have not varied considerably over time. Only one study reported lower pH values in the snus products tested. Total nicotine concentrations reported in recent studies were between 14 to 21 mg/g dry weight, with only few exceptions with higher nicotine contents. It appears that nicotine concentrations have not varied considerably over time. Free nicotine content varied widely, between 23 and 73% of total nicotine, with a single exception (a study that reported considerably lower free nicotine levels as well as lower pH values; the reason for this exception is unclear).

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

Nicotine Extraction

The amount of extractable nicotine from traditional Swedish snus has been reported in four studies (Andersson et al. 1994; Digard et al. 2012; Lunell and Curvall 2011; 2005). This methodology was used to determine the difference in nicotine content between used and unused snus samples.

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. Since the portion-bag snus users also consumed less on a gram-per-day basis, together with a lower product pH, the overall extracted nicotine was lower from pouched snus compared to loose snus (47.6 ±31.4 mg/24 hrs and 94.7 ±67.9 mg/24 hrs, respectively). The authors note, however, the higher nicotine extraction from loose snus was not reflected in higher saliva cotinine levels or a higher systemic dose; the authors noted that this may be due to a higher expectoration rate of tobacco juices (see Section 3.1 for details).

A study by Lunell and Lunell (2005) investigated nicotine extraction and uptake from different snus brands, including the traditional snus products *General* and *Catch* by 12 regular snus users. The mean extracted amounts were 1.55 ±0.18, 2 ±0.11, and 2.74 ±0.18 mg per portion for *Catch Licorice*, *Catch Mini*, and *General* (approximately 3 to 8 mg/g dry weight[26]), resulting in 22, 44, and 31% average extraction of the total nicotine content, respectively. This study also

[26] Values given were on portion basis and had to be adjusted to gram considering portion sizes (*General*: 1 g; *Catch Licorice*: 1 g; *Catch Mini*: 0.5 g) and dry weight assuming 50% moisture (value multiplied by 2)).

tested *Catch Dry Mini*, a novel brand of traditional Swedish snus (mean extracted amount 1.08 ±0.12 mg/portion, portion size 0.3 g, resulting average extraction 22% of total nicotine content).

In a subsequent study, Lunell and Curvall (2011) determined the nicotine extraction from two *General* snus brands (*Onyx* and *White Large*) by 14 smokers (abstinent overnight). The mean amounts extracted from snus were 2.12 ±0.93 and 2.18 ±0.92 mg per portion (approximately 21-25% extraction of the total nicotine content). The authors noted that the mean extracted amount from snus in this study was lower than in their previous study and attributed it to the fact that the smokers in their study were inexperienced in snus use compared to the regular snus users in the previous study.

In a study by BAT researchers, Digard and colleagues (2012), the nicotine extraction from loose and pouched snus products tested with regular snus users who also smoked in a six-way cross-over study, with each testing period after 12-hours of abstinence. The average extracted amount for 1-g pouched or loose snus portions ranged from 3.45 to 4.53 mg per portion (31-32% extraction of the total nicotine content). For 2.5-g portions of loose snus, the extracted amount was 6.42 mg per portion (24% extraction). (See Section 3.1 and Table A III-7 in Appendix III for details on the nicotine systemic doses and biomarkers measured in these studies).

A new study[27] by the same authors reported approximately 33% mean nicotine extraction from a snus product under 60-minute use conditions (Digard et al. 2013). The mean amount nicotine extracted was with 3.2 mg per 1-g pouch consistent with other studies. In addition, a study by researchers from the same group presented as a poster during the annual CORESTA conference investigated the correlation of extraction with usage time (Gale et al. 2011). These researchers observed an increase of nicotine extraction with time (5% at 5 min, 18% at 30 min, 31% at 60 min, and 51% at 120 min).

In summary, the average percentage of nicotine extraction from different snus products ranged from 21 to 49% (1.55 to 4.5 mg per 1-g portions) and was influenced by portion size (higher for smaller portion sizes and vice versa), user experience, and use time as well as by total nicotine content and pH.

2.3.4 Nitrate and Nitrite

Nitrate is another endogenous tobacco component and nitrate values alone allow differentiation of STPs into three separate classes, (1) moist snuffs, including snus; (2) low moisture snuff, and (3) other products (Rickert et al. 2009). Air-cured tobaccos tend to be high in nitrate (Rickert et

[27] Digard (2013) tested the extraction of various components from a *Lucky Strike Original Snus* after 60 minutes of use by regular Swedish snus users. These authors reported approximately 33 % of nicotine extraction (3.2 mg) from a product that had a mean nicotine concentration of 9.6 ±0.90 mg per 1-g pouch. This study also showed approximately 30% overall variability in extraction.

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 is therefore discussed here, although it would be considered a trace level component (see Section 2.3.6). 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).

Neither nitrate nor nitrite is part of the current HPHC list of the FDA (FDA 2012a).

Nitrate and Nitrite Concentrations in Traditional Swedish Snus

Nitrate concentrations are monitored in snus, however, a GothiaTek® Standard limit is available for nitrite at 7 µg/g dry weight (see more on GothiaTek® Standard limits in Table 2- 3 and Section 2.3.6).

Nitrate levels reported in an early study by Brunnemann and colleagues (1985) as measured in three brands of Swedish moist snuff purchased in 1984/85 per dry weight of tobacco were between 2.13 and 2.62% (21.3-26.2 mg/g).

Only limited newer peer-reviewed published data are available (Table A II-1b in Appendix II). 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. In another study that measured nitrite content in "general [sic] pouch", the nitrite concentrations were below the LOD (limit of detection) of 0.2 µg/g dry weight (McNeill et al. 2006).

Data on 21 portion snus and 11 loose snus samples from Sweden (sourced in 2008 from seven different manufacturers, but no brands specified) presented by BAT researchers as poster at a CORESTA conference indicates that all samples had nitrate contents that were approximately between 0.1 and 0.2% wet weight (1-2 mg/g wet weight) (Faizi et al. 2010). In agreement with this, Digard and colleagues (2013) reported the mean nitrate concentration 1.2 mg per 1-g pouch of a snus product.

Borgerding and colleagues (2012) reported nitrite concentrations between below the LOD (4.72 and 0.57 µg/g per wet weight "as received" for 2006 and 2007, respectively) up to 5.6 µg/g per dry weight in traditional Swedish snus brands, with *General* brands either below the LOD or below the limit of quantitation (LOQ, 15.7 and 1.89 µg/g per wet weight "as received" for 2006 and 2007, respectively).

In summary, the limited data seems to indicate a decline in nitrate content in snus since 1980s. Data from recent studies indicate that nitrite concentrations in traditional Swedish snus are

below the GothiaTek® Limit of 7 µg/g per dry weight. Swedish Match (2013)[28] reported the average content in its snus brands manufactured in 2011 to be 2 µg/g dry weight (range, ± 2 standard deviations, <1.0-3.8 µg/g dry weight), which is in agreement with the data reported in recent peer-reviewed publications.

Nitrate Extraction

Digard and colleagues (2013) reported[29] approximately 27% mean nitrate extraction and the mean amount nitrate extracted was given as 0.323 mg per 1-g pouch under 60-minute use conditions. No other extraction data for nitrate was identified.

2.3.5 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 (For details see Table A II-1b in Appendix II).

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[30] (Stepanov et al. 2008a). Chloride concentrations were also analyzed by Borgerding and colleagues (2012). These researchers reported chloride concentrations between 58.5 to 93.2 mg/g dry weight for traditional Swedish snus samples, including four *General* brands (69.5-93.2 mg/g dry weight chloride) and one *Nick and Johnny* product. Consistent results were also reported in a new study by Digard and colleagues (2013), in which the mean chloride concentration in a snus product was 35.3 ±2.23 mg per 1-g pouch, i.e. wet weight (assuming 50% moisture, this would be approximately 70 mg/g dry weight).

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). Digard and colleagues (2013) reported mean concentrations of ammonia and propylene glycol of 1283.7 ±98.10 µg/1-g pouch and 31.1 ±1.97 mg/1-g pouch in a snus product.

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[28] Swedish Match. 2013. http://www.swedishmatch.com/en/Snus-and-health/GOTHIATEK/GOTHIATEK-standard/, accessed April 2013.

[29] This new study by BAT researchers tested the extraction of various components from a *Lucky Strike Original Snus* after 60 minutes of use by regular Swedish snus users (Digard et al. 2013). These authors reported approximately 27 % of nitrate extraction (0.323 mg) from a product that had a mean nitrate concentration of 1215.3±88.13 mg per 1-g pouch (wet weight). This study also showed high inter-subject variability (24%) and overall variability (40%).

[30] *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).

2.3.6 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 no quantitative information on many of these compounds in the published literature up until the late 2000s. Only the more recent published studies measured a wider range of analytes (Borgerding et al. 2012; several posters by BAT researchers, Pappas et al. 2008; Rickert et al. 2009; Stepanov et al. 2008a; Stepanov et al. 2010).

One company, Swedish Match, has developed limits for certain components in STPs that must not be exceeded (GothiaTek® Standard Limits, see Table 2- 3). Rutqvist (2011) noted that some components (e.g., α- or β-angelica lactones, coumarin, hydrazine, volatile aldehydes, and ethyl carbamate (urethane)) were not included in the standard, because “they were found to be non-detectable or present only in trace amounts in snus, that robust analytical methods were unavailable at the time, or that technical developments of the production were not expected to result in decreased levels of the constituent.”

Table 2-3: GothiaTek® Limits for ”Undesired Components”	
Component	**Limit (µg/g dry weight)**
Nitrite	7
NNN + NNK (previously Tobacco-specific nitrosamines*)	2 (10)
N-nitrosodimethylamine (NDMA)	0.01
Benzo[*a*]pyrene (B[*a*]P)	0.005 (previously 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/GOTHIATEK/GOTHIATEK-standard/, accessed April, 2013 * Total TSNAs	

In a recent publication, Ayo-Yussuf and Connolly (2011) attempted to provide a basis for a scientific discussion on an appropriate regulatory approach for STPs using an existing toxicological assessment framework. These authors conducted risk assessments for several components with carcinogenic potential for which GothiaTek® standard limits have been set (Table 2-3) to evaluate implications for product regulations. The authors stated that sufficiently comparable data was only available for TSNAs, B[*a*]P, cadmium, and lead, but not for arsenic and chromium. They calculated cancer risk estimates for these individual components using published concentrations in various STPs including “Swedish snus”, as well as using the GothiaTek® standard limits. It should be noted that the data used for “Swedish snus” was taken from a publication by Rickert et al. (2009) and these authors only analyzed *Du Maurier* snus, a new product marketed as snus. Calculated average lifetime daily exposures were estimated using the concentrations multiplied by the average daily use of the STPs, for 30 years of use, over a total of 70 years average lifetime, for a 70 kg body weight[31]. The cancer potency was

[31] Cancer risk estimate =
concentration of component in STP (ng/g) x average daily use of STP (g) x 30 yrs/70 yrs x 1/70 kg x CP.
CP: Cancer potency = $1/TD_{50}$ (1/mg/kg b.w./d)

based on TD_{50}[32] values as reported in the Cancer Potency Data Base of the University of California at Berkeley. The product total risk, the sum of the cancer risks for the four individual components, was reported by the authors as between 8 and 9 $x10^{-3}$ (with or without adjusting for extraction) for STPs at the GothiaTek® Standard limits and 1.4 $x10^{-3}$ for "Swedish snus" (*Du Maurier* snus). In comparison, the total cancer risk for medicinal nicotine gum was 3.6 $x10^{-9}$, based only on risk calculated for B[*a*]P equivalents. The authors concluded, that "except for the medicinal nicotine tested, all the STP types, including the relatively lower tobacco specific nitrosamine (TSNA)-containing snus, were found to carry an 'unacceptable' cancer risk." The main contributors to the risk were TSNAs and cadmium. In this study, no adjustments were made for interspecies extrapolations considering that carcinogenic potency was based on TD_{50} values from animal experiments.

A subsequent publication by Haussmann (2012) used cancer slope factors established by the California Environmental Protection Agency instead of TD_{50} values to recalculate the cancer risk from the six components, keeping all other assumptions as proposed by Ayo-Yussuf and Connolly (2011). Based on results in this analysis, the total theoretical incremental lifetime cancer risk for these components in STPs at the GothiaTek® Standard limits would be 4.3 $x10^{-3}$. Main contributors to the risk were NNK (90%), NNN (8%), cadmium (1%), and chromium (1%).

2.3.6.1 *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. 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

[32] TD_{50}: Dose at which 50% of animals developed tumors under chronic administration

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). Based on studies that analyzed the enantiomeric composition of NNN in tobacco, it has been hypothesized that the major precursor of NNN is nornicotine, not nicotine (Carmella et al. 2000; Stepanov et al. 2012b). These researchers observed that the *S*-enantiomer of NNN (*S*-NNN) was the predominant enantiomer in a variety of different tobacco products with means for different product categories ranging between 57% and 75%. This might indicate it was more likely to result from nitrosation of nornicotine, which contains 70% to 96% of its *S*-enantiomer, while nicotine contains more than 99% of its *S*-enantiomer. These studies did not analyze traditional Swedish snus (for more details see Appendix II, Section A II 2.3.6.1.1).

In addition to these commonly-reported TSNAs, two other nitrosation products of nicotine and cotinine acid, (4-(methylnitrosamino)-4-(3-pyridyl)-butanal (NNA) and 4-(methylnitrosamino)-4-(3-pyridyl)butyric acid (*iso*-NNAC), respectively), as well as reduction products of NNK and NNA (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (*iso*-NNAL) can be detected in tobacco (Hoffmann et al. 1995; as cited in IARC 2007).

Since snus is produced with a heat-treatment instead of a fermentation step, it is expected that the resulting elimination or reduction of bacteria results in TSNA concentrations that are lower in finished products than concentrations in fermented STPs.

NNK and NNN are considered to be higher-priority TSNAs because of their 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; IARC 2012d). The IARC classification was "*inadequate evidence* in humans for the carcinogenicity of tobacco-specific *N*-nitrosamines". For its overall evaluation, however, IARC took mechanistic evidence into consideration[33]. IARC stated that *S*-NNN was shown to be more favorably activated via the 2´-hydroxylation pathway, a metabolic pathway that is thought to be important for the formation of carcinogenic active metabolites and was more tumorigenic in rat esophagus and oral mucosa than *R*-NNN (Stepanov et al. 2012b).

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

[33] IARC took the following mechanistic evidence into consideration: NNK and NNN "are the most abundant strong carcinogens in smokeless tobacco; their uptake and metabolic activation has been clearly documented in smokeless tobacco users. Combined application NNN and NNK to the oral mucosa of rats 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 have been detected in users of tobacco." (IARC 2012d)

NNA, *iso*-NNAC and *iso*-NNAL were shown to be inactive as tumorigens in mice studies, and *iso*-NNAC was reported to be inactive in an *in vitro* Deoxyribonucleic acid (DNA) repair assay (Hecht 1998). Due to the limited data available for these substances, they were not evaluated by IARC (IARC 2007). NNAL has been shown to produce tumors in laboratory animals. IARC concluded that there was "sufficient evidence in experimental animals for the carcinogenicity of NNK and its metabolite, NNAL (IARC 2007)." To date, NNA, *iso*-NNAC, NNAL, and *iso*-NNAL have not been classified by IARC.

The current HPHC list of the FDA contains NNK and NNN based on concerns about their carcinogenic potential (FDA 2012a).

Recently, the World Health Organization (WHO) Study Group on Tobacco Product Regulation, in its Report on the Scientific Basis of Tobacco Product Regulation, 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).

TSNA Concentrations in Traditional Swedish Snus

As presented in Table 2-3, the GothiaTek® Standard was previously set for total TSNAs (NNN, NNK, NAB, and NAT) to 10 µg/g dry weight, but has recently been adjusted to 2 µg/g dry weight for NNN and NNK combined in agreement with the WHO recommendation.

Analyses conducted in the early 1980s showed that TSNA levels in Swedish moist snuff products ranged from 7 to 17 ppm (µg/g)[34].

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).

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) under varying storage 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*), these storage conditions led to an increase in TSNA levels between 30 and 130% in two leading US snuff brands. While some authors have reported that refrigeration of the finished snus product was introduced to

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

prevent further bacterial growth and thus TSNA formation, the MDPH study found 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)[35].

Total TSNAs

Using analyses of published studies from 1983 to 1992 and their own results from snus on the market in 2001 and 2002, Österdahl and colleagues (2004) from the Swedish National Food Administration demonstrated a decrease in TSNA concentrations in snus between the 1980s to 2000s. 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[36]). 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 had declined by about 85% since the 1980s.

Analyses of samples of 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 United Kingdom (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). In a recent study, researchers from the U.S. Centers for Disease Control and Prevention (CDC) presented the results of analyses of five TSNAs in three pouched and one loose *General* snus brands, as well as a *Catch* snus brand. They reported that total TSNA concentrations, including NNAL, ranged from 0.601-0.723 μg/g wet weight

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

[36] 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%".

(approximately 1.20-1.45 μg/g dry weight) (Stanfill et al. 2010). Total TSNA concentrations excluding NNAL were in the range of approximately 1.2 to 1.4 μg/g dry weight. Borgerding and colleagues (2012), researchers from Reynolds, analyzed TSNAs in various moist snuff brands on the Swedish market 2006/2007 (including *General* and *Nick and Johnny*), but did not report TSNA concentrations as total TSNAs analyzed. Results for the individual TSNAs (NNK, NNN, NAB, and NAT) are reported in the section below. The mean total TSNA concentration reported in a snus product in a study by BAT researchers was 0.83 μg per 1-g pouch (1.66 μg/g dry weight (Digard et al. 2013). (For details see Table A II-2a in Appendix II).

Individual TSNAs

With respect to trends in concentrations of the individual TSNAs, mean NNK and NNN concentrations in 2002 as reported in the review by Österdahl and colleagues (2004) 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. These data suggest that the combined NNK and NNN concentrations in traditional Swedish snus may have declined by 85% from the 1980s to the 2000s (means, 1983 - 9.2 μg/g dry weight, 2002 - 1.36 μg/g dry weight). More recently-conducted studies have reported NNK and NNN concentrations in traditional Swedish snus (including *General*) perhaps even lower, 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). In the recent study by Stanfill and colleagues (2010), NNK and NNN in five *General* and *Catch* snus brands were in the range of 0.0845 to 0.105 μg/g wet weight and 0.267 to 0.345 μg/g wet weight, respectively (approximately 0.17-0.21 and 0.53-0.69 μg/g dry weight, respectively). In the analysis by Borgerding and colleagues (2012) NNK concentrations for brands of Swedish snus, including *General* and *Nick and Johnny,* were reported as below the limit of quantification (LOQ; 0.109 μg/g wet weight in 2007, 0.272 μg/g wet weight in 2006). NNN concentrations were in the range of 0.601 to 0.885 μg/g dry weight. NNN concentrations were similar, although slightly higher than those reported by Stanfill and colleagues (2010) for brands measured in both studies (i.e., *General Original*, *General White* Portion, and *General* Loose), when converted to dry weight (assuming 50% moisture content). NNN and NNK concentrations reported in the new study by Digard and colleagues (2013) were 0.192 and 0.344 μg per 1-g pouch (approximately 0.384 and 0.689 μg/g dry weight, respectively (Digard et al. 2013).

Thus, combined NNK and NNN concentrations in traditional Swedish snus as reported in studies by different investigators from 2003 up to 2008 range between 1.4 and 2.32 μg/g dry weight. The differences might be due to inter-laboratory variability in analytical methods. Data from the 2010 study conducted in the CDC Tobacco Analysis Laboratory yielded combined NNK and NNN concentrations in traditional Swedish snus brands of approximately 0.71 to 0.88 μg/g dry weight (Stanfill et al. 2010). Similarly, combined NNK and NNN concentrations as measured by Borgerding and colleagues (2012) as well as those measured by Digard and colleagues (2013) were below 1.2 μg/g dry weight. This appears to indicate that NNK and NNN concentrations in Swedish snus have remained lower since the 1980s.

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 lower compared to levels 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 the recent study by Stanfill and colleagues (Stanfill et al. 2010), NAB and NAT in five *General* and *Catch* snus brands were in the range of 0.0134 to 0.0208 μg/g wet weight and 0.214 to 0.248 μg/g wet weight, respectively (~0.027-0.042 and 0.43-0.50 μg/g dry weight, respectively). In the analysis by Borgerding and colleagues (2012) NAB concentrations for brands of Swedish snus, including *General* and *Nick and Johnny,* were reported as below the limit of detection (LOD; 0.0124 μg/g wet weight in 2007, 0.031 μg/g wet weight in 2006) in three General brands, and below the LOQ (0.0412 μg/g wet weight in 2007, 0.103 μg/g wet weight in 2006) in the other five snus brands. NAT concentrations were below the LOQ (0.085 μg/g wet weight in 2007, 0.213 μg/g wet weight in 2006) in *General* Loose and in the range of 0.422 to 0.754 μg/g dry weight in the other snus brands. NAT concentrations were similar, although slightly higher than those reported by Stanfill and colleagues (2010) for two brands measured in both studies (i.e., *General Original*, *General White* Portion), when converted to dry weight (assuming 50% moisture content). Consistent with the results from Stanfill and colleagues (2010), NAB and NAT concentrations reported by Digard and colleagues (2013) were 0.025 and 0.269 μg per 1-g pouch (approximately 0.050 and 0.537 μg/g dry weight, respectively (Digard et al. 2013).

Two more recent studies also analyzed NNAL, NNA and *iso*-NNAL in snus (McAdam et al. 2011; Stanfill et al. 2010, Appendix II, Table A II-2b). Stanfill and colleagues (2010) reported NNAL concentrations in five snus brands ranging between 0.00857 and 0.0131 μg/g wet weight (approximately 0.017-0.026 μg/g dry weight). In a study by BAT researchers presented at the 2011 Society for Research on Nicotine and Tobacco (SRNT) meeting, NNAL concentrations in Swedish pouched and loose snus (unspecified brands[37]) were reported as close to the LOD (0.0084 μg/g wet weight) or LOQ (0.028 μg/g wet weight) with a slightly higher upper range of approximately 0.08 μg/g wet weight (~0.16 μg/g dry weight) in pouched snus compared to loose snus (~0.06 μg/g wet weight) (McAdam et al. 2011).

iso-NNAL reported by these researchers was below the LOQ (0.029 μg/g wet weight) in all but one of the Swedish snus products. For this one pouched product, the level reported was approximately 0.32 μg/g wet weight *iso*-NNAL (approximately 0.64 μg/g dry weight). In an older

[37] Since BAT manufactures Lucky Strike Snus brands as Swedish snus, data from this study might refer to these brands.

study by researchers of the German Cancer Research Center in which five samples of Swedish moist snuff were analyzed, the average *iso*-NNAL concentration was 0.027 μg/g[38] (range, below detection limit-0.08 μg/g) (Tricker and Preussmann 1989).

NNA in Swedish snus could not be detected (<0.345 μg/g wet weight) in loose and some of the pouched snus product samples, and was below the quantitation limit (1.151 μg/g wet weight) in the remaining pouched snus samples (McAdam et al. 2011).

Summary

In summary, the total as well as the individual (commonly analyzed) TSNA concentrations in traditional Swedish snus decreased considerably from the 1980s to the 2000s, with an apparent additional decrease as shown in the latest published analyses from 2010 through 2013. Data from the newest studies shows that mean total TSNA concentrations are in the range of 1.2 to 1.7 μg/g dry weight. In these latest analyses, combined NNN and NNK averages are in the range of 0.7 to 1.2 μg/g dry weight, respectively. NAB and NAT averages range from below LOD/LOQ to 0.05 μg/g dry weight and from below LOQ to 0.75 μg/g dry weight, respectively. Swedish Match (2013)[39] reported the average combined NNN and NNK content in snus brands manufactured in 2011 to be 0.8 μg/g dry weight (range, ± 2 standard deviations, 0.6-1.22 μg/g dry weight), which is consistent with the newest data reported in peer-reviewed publications.

Therefore, total TSNA concentrations for traditional Swedish snus reported in recent studies are consistently below the previous GothiaTek® Standard limit of 10 μg/g dry weight. Based on the most recent analyses published, total TSNAs concentrations in snus were more than six times lower than this standard. As can be seen the mean combined concentrations in 2002 in traditional Swedish snus were below the 2 μg/g dry weight value and analytical results from recent studies were below or close to this value, with the newest data indicating NNK plus NNN concentrations of approximately half this value.

Few newer studies have analyzed additional TSNAs (NNAL, iso-NNAL, NNA), which were, if detected in traditional Swedish snus, generally present at concentrations below 1 μg/g wet weight.

TSNA Extraction from Snus

Two studies with information on TSNA extraction from traditional Swedish snus are available (Andersson et al. 1994; Österdahl and Slorach 1988). It should be noted that the TSNA concentrations in the snus products used in these studies were considerably higher than those reported in recent analyses of Swedish snus. (See Section 3.2.1 for details on biomarkers of

[38] This study did not specify if measurements were given as per wet or dry weight, therefore it is likely that it was "as is" (per wet weight)

[39] Swedish Match. 2013. http://www.swedishmatch.com/en/Snus-and-health/GOTHIATEK/GOTHIATEK-standard/, accessed April 2013.

exposure measured in these studies). Additional information is available from studies on products marketed as snus, or unpublished studies.

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). The total TSNA content (NNK, NNN, and NAT) was determined to be 9.2 µg/g[40] in the snuff pouches used by three of the snuff dippers. After 30 minutes of 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 (up to 10% total TSNA extraction), which was mainly due to decreases in NNK and NNN content (up to 30% NNK and 20% NNN extraction, respectively). 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.

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 (Andersson et al. 1994). Subjects used their regular brands in an unrestricted manner but recorded overall usage time and amount used per day (13.1 hours/day and 14.4 g/day for portion-bag and 12.3 hours/day and 20.8 g/day for loose snus users, respectively). The used portions provided by the study participants on one of the study days were analyzed for residual TSNA content. The total TSNA content before use ranged from 3.7 to 6.0 µg/g for snus in portion bags and from 6.1 to 7.7 µg/g in loose snus. Averaged over 24 hours, the degree of TSNA extraction was lower from portion-bag snus (55.7 ±20.5%) than from loose snus (64.1 ±16.4%). When this information is combined with lower product use, the portion-bag snus users extracted overall significantly less TSNA from snus (44.5 ±25.7 µg/24 hours) than the loose snus users (125.3 ±115.5 µg/24 hours).

A more recent study[41] reported approximately 36% mean TSNA extraction from a snus product under 60-minute use conditions, with similar results for the individual TSNAs (35-38%). The extracted amount was approximately 0.3 µg per 1-g pouch (Digard et al. 2013). Combined extracted amounts of NNN and NNK were approximately 0.2 µg per 1-g pouch. In addition, a study by researchers from the same group presented as a poster during the annual CORESTA conference investigated the correlation of extraction with usage time (Gale et al. 2011; Gale et

[40] It was not specified if this value was given per wet or dry weight, but it is likely to be on an "as is"- basis.

[41] This new study tested the extraction of various components from a *Lucky Strike Original Snus* after 60 minutes of use by regular Swedish snus users (Digard et al. 2013). The mean amount of total TSNAs extracted was given as 0.298 µg per 1-g pouch (approximately 0.596 µg/g dry weight) from a product that had a mean total TSNA concentration of 0.83 µg per 1-g pouch. Extracted amounts of NNK, NNN, NAB, and NAT were 0.073, 0.123, 0.009, and 0.093 µg per 1-g pouch. There was approximately 21-24% total variability in extraction results based on both inter- and intra-individual variability.

al. 2012). These researchers observed an increase of TSNA extraction with time (6% at 5 min, 18% at 30 min, 30% at 60 min, and 49% at 120 min).

Average extraction rates from unpublished new studies with traditional Swedish snus were between 14 and 41% for total TSNAs after 30-minute usage time (unpublished data, personal communication with Dr. Margareta Curvall) and are in the range of the in the other recent studies described above.

In summary, these studies indicate that TSNA extraction varies significantly between individuals, depending on the type of snus used, usage time, and ranged from 18 to 64% for 30 to 60 minute usage times. The variability may be due to the higher TSNA content in older studies, characteristics of the study participants and difference in usage times, unrestricted versus restricted study conditions, or differences in analytical methods.

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). Four of eleven identified *N*-nitrosamino acids have been classified as carcinogens in experimental animals, i.e., *N*-nitrososarcosine (NSAR) (classified in 1987 by IARC as Group 2B carcinogen[42]), *N*-nitrosoazetidine-4-carboxylic acid (NAzCA), 3-(methylnitrosamino)propionic acid (MNPA), 4-(methylnitrosamino)butyric acid (MNBA) (Brunnemann and Hoffmann 1992; IARC 2007). The non-carcinogenic *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).

The current HPHC list of the FDA lists NSAR categorized based on its carcinogenic potential (FDA 2012a).

N-Nitrosamino Acids in Traditional Swedish Snus

No recent published studies that analyzed traditional Swedish snus for *N*-nitrosamino acids were identified, and from the limited amount of older data, a trend over time could not be identified (see also Appendix II, Table A II-2c). A recent poster presented by BAT researchers indicates that *N*-nitrosamino acids are considered in newer analyzes of STPs by tobacco companies, but actual data were not presented (Essen et al. 2011).

[42] 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)

NSAR

Tricker and colleagues (1989; 1991) detected NSAR concentrations between 0.008 and 0.031 μg/g [43] (mean, 0.019 μg/g) in five samples of Swedish moist snuff commercially available 1987/88. 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). Subsequent studies reported NSAR concentrations in moist snuff brands from Sweden as ranging from 0.01 to 0.68 μg/g dry weight (1989-1991) and 0.03-0.68 μg/g dry weight (1990/1991) (Brunnemann and Hoffmann 1992; Hoffmann et al. 1991a).

Other *N*-Nitrosamino Acids

Tricker and colleagues (1989; 1991) detected NAzCA only in heavily cured/fermented tobaccos, but not in the Swedish moist snuff samples (Tricker and Preussmann 1989; Tricker and Preussmann 1991).

MNPA and MNBA[44] 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 2.9 to 4.4 μg/g dry weight and from not detected to 0.24 μg/g dry weight, respectively. In five samples of Swedish moist snuff commercially available 1987/88, Tricker and Preussmann (1989; 1991) determined MNPA and MNBA concentrations to range from 1.04 to 1.82 μg/g (mean, 1.34 μg/g) and from 0.053 to 0.094 μg/g (mean, 0.07 μg/g), respectively. MNPA and MNBA concentrations in moist snuff from Sweden on the market between 1989 and 1991 were between 1.0 and 3.3 μg/g dry weight and 0.05 and 0.23 μg/g dry weight, respectively (Brunnemann and Hoffmann 1992; Hoffmann et al. 1991a).

The NPRO concentrations in snuff from Sweden with approximately 50% moisture ranged from 6.21 to 29.5 μg/g dry weight (Ohshima et al. 1985). Brunnemann and colleagues (1985) detected NPRO concentrations in the range of 3.1 to 8.2 μg/g dry weight in three brands of moist snuff from Sweden on the market 1984 and 1985. Tricker and Preussmann (1989; 1991) reported NPRO concentrations were between 0.63 and 1.82 μg/g (mean 1.10 μg/g) in five moist snuff brands from Sweden. In their subsequent studies, Brunnemann and colleagues (Brunnemann and Hoffmann 1992; Hoffmann et al. 1991a) reported NPRO concentrations between 0.63 and 8.33 μg/g dry weight.

Several of these studies also reported concentrations for four additional *N*-nitrosamino acids[45] (see Appendix II, Table A II-2c).

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

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

[45] 4-(*N*-methylnitrosamino)-4-(3-pyridinyl)-1-butyric acid (iso-NNAC), *N*-nitrosohydroxyproline (NHPRO), *N*-nitrosopipecolic acid (NPICA/NPIC), *N*-nitrosothiazolidine 4-carboxylic acid (NTCA).

Summary

No recent published analyses of *N*-Nitrosoamino acids in traditional Swedish snus were identified. Data from the late 1980s and early 1990s indicate NSAR concentrations up to approximately 0.7 μg/g dry weight. Of the three other *N*-Nitrosoamino acids that have been classified as carcinogenic in animals, only two have been detected in traditional Swedish snus, with MNPA and MNBA concentrations up to approximately 4 and 0.2 μg/g dry weight, respectively. NAzCA was not detected in traditional Swedish snus in the single study that reported this analyte. Swedish Match recently analyzed some of its snus brands for NSAR concentrations but results have not been published (personal communication with Dr. Margareta Curvall).

Volatile N-Nitrosamines

Volatile amines (VNAs) naturally present in tobacco can undergo nitrosation forming a variety of volatile *N*-nitrosamines (Tricker and Preussmann 1989). The only VNA frequently analyzed in STPs has been *N*-nitrosodimethylamine (NDMA). Several older studies have also measured *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). Further, *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 found to be carcinogenic in laboratory animals, and classified in 1987 by IARC as Group 2A[46] (NDMA) and 2B (NPYR, NPIP, and NMOR) carcinogens (IARC 2009[47]).

In addition to these four VNAs (NDMA, NPYR, NPIP, and NMOR), the current HPHC list of the FDA contains *N*-nitrosodiethylamine (NDEA; IARC Group 2A) and N-nitrosomethylethylamine (also sometimes as *N*-nitrosoethylmethylamine, NEMA; IARC Group 2B) based on concerns about their carcinogenic potential (FDA 2012a).

Volatile N-Nitrosamines in Traditional Swedish Snus

Limited information on the presence of VNAs in snus is available (see also Appendix II, Table A II-2d). A recent poster presented by BAT researchers indicates that *N*-nitrosamines are considered in newer analyses of STPs by tobacco companies (McAdam et al. 2010a). In addition to the six VNAs on the HPHC list, these researchers analyzed for the presence of four other VNAs[48] in various STPs, including Swedish pouched and loose snus. McAdam and colleagues (2010a) noted that the majority of STPs samples studied had VNA concentrations

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[46] 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)

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

[48] *N*-Nitrosodi-n-propylamine (NDPA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosodiisopropylamine (NDPIPA), *N*-nitrosodibenzylamine (NDBzA)

below their LOQ or LOD, with the exception of NDMA, NPYR, NMOR, and *N*-nitrosodi-n-propylamine (NDPA). In a review for the IARC in 1991, Österdahl from the Swedish National Food Administration stated that levels of VNAs in Swedish snuff decreased considerably since 1979 (Österdahl 1991).

NDMA

The GothiaTek® standard limit for NDMA is set to 10 ng/g dry weight (Table 2-3).

Of all VNAs, NDMA has been analyzed most frequently. Österdahl (1991) reported a mean concentration of NDMA in 67 samples of snuff on the Swedish market 1983-86 to be 0.7 (approximately 1.4 ng/g dry weight, assuming 50% moisture content). A study by Brunnemann and colleagues (1985) of 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. 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). 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 concentrations in the range of 51 and 63 ng/g dry weight (Hoffmann et al. 1991a; Hoffmann et al. 1991b). The authors did no comment on the discrepancy in these concentrations compared to their earlier study.

More recent studies reported that most of the concentrations were below the LOD or LOQ. McNeill and colleagues (McNeill et al. 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.

On their poster, McAdam and colleagues (2010a) reported quantifiable (LOQ, 3.9 ng/g wet weight ("as received")) amounts of NDMA in a small proportion of pouched Swedish snus samples; however, NDMA concentrations, were below the GothiaTek® standard limit of 10 ng/g dry weight. In Swedish loose snus samples, NDMA was detected (LOD, 1.98 ng/g wet weight ("as received")), but was not quantifiable. The snus brands were not specified.

Borgerding and colleagues (2012), did not detect (LOD, 1.17-3.54 ng/g wet weight ("as received")) or could not quantify (LOQ, 3.9-11.7 ng/g wet weight ("as received")) NDMA in most[49] moist snuff brands on the market in Sweden 2006/2007, including four *General* brands and one *Nick and Johnny* snus brands.

[49] Two brands of *Rocker* snus (manufacturer Rocker Production AB), did contain quantifiable NDMA concentrations (19.8-24.5 ng/g dry weight).

Other volatile *N*-Nitrosamines

In the study by Österdahl (1991) the mean concentration of NPYR was 5.1 ng/g wet weight, respectively (approximately 10.2 ng/g dry weight, assuming 50% moisture content). Both, NPIP and NMOR were detected only at trace levels. Brunnemann and colleagues (1985) reported concentrations of NPYR and NMOR ranged between 12.2 and 22.1 ng/g dry weight and from below the LOD to 9.1 ng/g dry weight, respectively. Tricker and colleagues (Tricker and Preussmann 1989; 1991) reported NPYR and NMOR concentrations between 4.5 to 6.0 ng/g (mean, 5.0 ng/g) and LOD up to 1.0 ng/g, respectively. NPIP was not detected. Hoffmann and colleagues (1991a; 1991b) reported NPYR concentrations to be below the LOD of 0.01 and up to 155 ng/g dry weight. In another publication, Brunnemann and Hoffmann (1992) reported concentrations of NPYR and NMOR in moist snuff from Sweden on the market 1981-1990 to be below the LOD up to 95 ng/g dry weight and up to 44 ng/g dry weight, respectively.

Newer published data was not available on these VNAs. In their analysis that included 10 VNAs, McAdam and colleagues (2010a) reported quantifiable amounts in addition to NDMA of only NMOR (LOQ, 1.77 ng/g wet weight ("as received")) and NDPA (LOQ, 5.05 ng/g wet weight ("as received")) in a small proportion of pouched snus samples. Other VNAs were detected but not quantifiable in both pouched and loose snus samples.

Summary

In summary, the recent data indicates that NDMA concentrations in traditional Swedish snus were frequently below the quantification or detection limit. Swedish Match (2013)[50] reported the average content in snus brands manufactured in 2011 to be <0.6 ng/g dry weight (range, ± 2 standard deviations, <0.6-0.8 ng/g dry weight). Limited data from recent studies on other VNAs indicates that in most snus samples, VNAs were present at concentrations below their respective LODs or LOQs. In addition to NDMA, Swedish Match recently analyzed some of its snus brands for concentrations of eight other VNAs, including NPYR, NPIP, NMOR, and NDPA, but results have not been published (personal communication with Dr. Margareta Curvall).

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).

NDELA has been classified as Group 2B carcinogen by IARC (2000) and is on the current HPHC list of the FDA based on concerns about its carcinogenic potential (FDA 2012a).

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[50] Swedish Match. 2013. http://www.swedishmatch.com/en/Snus-and-health/GOTHIATEK/GOTHIATEK-standard/, accessed April 2013.

NDELA in Traditional Swedish Snus

As with the other non-tobacco-specific N-nitroso compounds, investigators have not focused on the analysis of NDELA in STPs since the early 1990s and only limited data on its presence in snus is available (see also Appendix II, Table A II-2d).

NDELA concentrations detected in Swedish moist snuff products appear to have decreased based on studies published in 1982, 1985 and 1991. Two 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.

The only recent publication that presented NDELA concentrations in snus was a study reported by McAdams and colleagues (2010a). In that study, NDELA was not detected (LOD, 0.784 ng/g wet weight ("as received")) in either pouched or loose Swedish moist snuff brands.

2.3.6.2 Polycyclic Aromatic Hydrocarbons

PAHs are formed during the incomplete combustion or pyrolysis processes of organic matter (EFSA 2008; IARC 2010b). High levels of PAHs are present in tobacco smoke. 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).

PAHs occur generally in complex mixtures, so human toxicity data for individual components are mostly unavailable. Many individual 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). Of those, B[*a*]P is the only component currently classified as a known human carcinogen (Group 1) by IARC (IARC 2006; 2009)[51]. At high levels of exposure to PAHs in the occupational setting, lung, bladder and skin cancers have been reported (CDC 2009).

[51] 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.

The HPHC list of the FDA currently contains 17 PAHs[52], based mostly on the carcinogenic potential (FDA 2012a). Of these, 16 are classified as carcinogens by IARC (Groups 1 or 2). To date, eight of these PAHs (benzo[*j*]aceanthrylene, benzo[*b*]furan, benzo[*c*]phenanthrene, cyclopenta[*c,d*]pyrene, dibenzo[*a,e*]pyrene dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene) have not been quantified in STPs. In addition, more than 10 other PAHs have been analyzed in STPs by researchers (McAdam et al. 2010b; Stepanov et al. 2010). PAHs for which data were available are listed in Table A II-3b and c.

In addition to their carcinogenic potential, PAHs have been associated to the pathophysiology of cardiovascular disease, for example with increased atherosclerosis in experimental animals (as reviewed in USDHHS 2010). Benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and chrysene are currently listed as cardiovascular toxicants on the FDA HPHC list.

While occupational exposures with PAH mixtures are well studied, only limited toxicity data is presently available for PAHs mixtures taken up via dietary exposure (IARC 2010b). In their risk characterization, the Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) focused on PAHs for which oral carcinogenicity data were available and concluded that eight PAHs (B[*a*]P, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, chrysene, dibenz[*a,h*]anthracene, indeno[*1,2,3-cd*]pyrene) were "currently the only possible indicators of the carcinogenic potency of PAHs in food" (EFSA 2008). Since B[*a*]P is well studied, it is often used as index chemical for the potency of other PAHs and as indicator for their presence[53]. However, the panel concluded that B[*a*]P "is not a suitable indicator for the occurrence of PAHs in food"[54] and instead proposed that four or all eight of the above listed PAHs (PAH4[55] or PAH8), which are including B[*a*]P, are the most suitable indicator (EFSA 2008). A recent WHO Study Group on Tobacco Product Regulation recommended that "the concentration of benzo[*a*]pyrene in smokeless tobacco should be limited to 5 ng/g dry weight of tobacco" (WHO 2009).

Using the average daily consumption of 12 g of pouched snus or 29 g of loose snus with approximately 50% moisture, the WHO recommendation would result in a daily exposure of 30 or 72.5 ng B[*a*]P, which is in the range of exposure expected from foods, and 3 to 8 times less

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[52] Benz[*a*]anthracene, benz[*j*]aceanthrylene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*b*]furan, benzo[*a*]pyrene, benzo[*c*]phenanthrene, chrysene, cyclopenta[*c,d*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[*1,2,3-cd*]pyrene, 5-methylchrysene, naphthalene

[53] 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). 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). Naphthalene (Group 2B) was not included the RFP approach by US Environmental Protection Agency (USEPA) (EPA IRIS 2010).

[54] In 30% of samples analyzed for the 15 priority PAHs identified by EFSA, carcinogenic and genotoxic PAHs were detected despite testing negative for B[*a*]P (EFSA 2008).

[55] PAH4: B[*a*]P, chrysene, B[*a*]A, B[*b*]F; PAH8: PAH4, B[*k*]F, B[*ghi*]P, DB[*ah*]A, I[*123cd*]P

than the European Union (EU) median exposure for the average population reported by EFSA (2008).

PAH Concentrations in Traditional Swedish Snus

A limited number of published studies have presented analyses of PAHs in snus; B[*a*]P was usually the only member analyzed (Table A II-3a in Appendix II). 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 potentially carcinogenic and present in cigarette smoke. In their study, Stepanov and colleagues (2010) analyzed different oral moist snuff products for 23 PAHs[56], but did not include any traditional Swedish snus products. Twenty-two PAHs were detected, of which, in addition to B[*a*]P, nine are classified by IARC as potential carcinogens; these are part of the FDA HPHC list. In a study by BAT researchers presented at the 2010 American Cancer Society (ACS) Fall Meeting, 21 PAHs[57] were quantified in Swedish pouched and loose snus and various US STPs (McAdam et al. 2010b), thought except for B[*a*]P, the data for individual PAHs was not presented.

B[*a*]P

The GothiaTek® Standard limit for B[*a*]P has recently been adjusted to reflect the WHO recommendation (see Table 2- 3). The previous GothiaTek® Standard limit was 20 ng/g dry weight and currently is 5 ng/g.

As noted above, few historical data are available on PAHs, including B[*a*]P, in traditional Swedish snus. In a review, Ramström (2000) 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.

In more recent studies, lower B[*a*]P concentrations were reported. McNeill and colleagues (McNeill et al. 2006) reported a B[*a*]P concentration of 1.99 ng/g dry weight in "snus (general [sic] pouch) from Sweden". Stepanov and colleagues (2008a) analyzed eight different PAHs in new and traditional STPs. These investigators did not detect[58] B[*a*]P in traditional Swedish snus (*General*). In the study by McAdams and colleagues (2010b) all but one of the pouched snus

[56] 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

[57] Naphthalene, 1-methylnaphthalene, 2-methylnapthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*j*]fluoranthene, benzo[*a*]pyrene, benzo[*e*]pyrene, perylene, indeno[*1,2,3-cd*]pyrene, dibenz[*a,h*]anthracene and benzo[*g,h,i*]perylene

[58] LOD was not provided.

samples and all of the loose snus samples tested had B[a]P concentrations below 5 ng/g dry weight. In the analysis by Borgerding and colleagues (2012) B[*a*]P concentrations for several brands of Swedish snus, including *General* and *Nick and Johnny*, were below the recommended 5 ng/g dry weight. *General* brands (2006/2007) had concentrations between 0.3 and 1.1 ng/g dry weight. The B[*a*]P concentration in *Nick* and *Johnny* snus was 2.1 ng/g dry weight.

All Other PAHs

McAdams and colleagues (2010b) reported the total concentration of all 21 PAHs in Swedish snus ranged from approximately 50 to 700 ng/g for pouched snus, and 50 to 500 ng/g tobacco for loose snus; it is unclear if these concentrations were given on a wet weight or dry weight basis. No concentrations for the individual PAHs were presented. The authors noted that the relative content of individual PAHs (ratio of individual PAH to total PAHs) was comparable among product categories, except for naphthalene. The PAHs present at the highest levels in all STPs analyzed in this study were phenanthrene, naphthalene, fluoranthene, and pyrene. This is consistent with results from studies by Stepanov and colleagues (Stepanov et al. 2008a; Stepanov et al. 2010). McAdams and colleagues (2010b) noted that the naphthalene concentrations measured in their study across products were 15 times lower than those reported by Stepanov and colleagues (2010) but did not have an explanation for this difference.

Of the seven PAHs in addition to B[*a*]P that Stepanov and colleagues (2008a) quantified in their study, the two other potential carcinogens, B[*b*]F) and B[*k*]F, were not detected in the traditional snus sample (*General*) investigated. The content of phenanthrene was highest, followed by fluoranthene and pyrene concentrations, with 55.3, 31.1 and 29.7 ng/g dry weight, respectively. The acenaphthylene concentration was reported to be 1.70 ng/g dry weight. Anthracene was not detected.

Summary

Few studies that provide historical data on levels of PAHs in traditional Swedish snus are available. Recent analyses of snus brands demonstrate that B[a]P concentrations are generally lower than the 5 ng/g dry weight limit recommended by WHO (WHO 2009). Swedish Match (2013)[59] reported the average content in snus brands manufactured in 2011 to be 0.8 ng/g dry weight (range, ± 2 standard deviations, <0.6-2 ng/g dry weight), consistent with the data reported in recent peer-reviewed publications. Limited available published data on other PAHs indicates that the members with the highest quantities were phenanthrene, naphthalene, fluoranthene, and pyrene, with the exception of naphthalene, substances categorized by IARC as "*Not classifiable as to its carcinogenicity to humans*" (Group 3). Limited quantitative data on phenanthrene, fluoranthene, and pyrene in snus indicates these PAHs to be in the range of 30 to 55 ng/g dry weight.

[59] Swedish Match. 2013. http://www.swedishmatch.com/en/Snus-and-health/GOTHIATEK/GOTHIATEK-standard/, accessed April 2013.

PAH Extraction from Snus

No published studies were identified that measured extraction of PAHs from traditional Swedish snus. A recent study[60] on brands of new products marketed as snus showed that the mean B[*a*]P extraction (proportion removed from product) was approximately 29% under unrestricted use conditions (mostly 10-30 min usage) (Caraway and Chen 2012).

2.3.6.3 Aldehydes

Volatile aldehydes are widely present in the human environment (Stepanov et al. 2008a), found in foods as well as in STPs. The levels in these products have not been widely quantified (IARC 2007; Stepanov et al. 2008a).

Stepanov and colleagues (2008a) analyzed for four aldehydes in different STPs: formaldehyde and acetaldehyde, which are classified by IARC as known (Group 1) and probable human carcinogens (Group 2B), respectively, as well as acrolein and crotonaldehyde, both classified by IARC as "Not classifiable as to its [their] carcinogenicity to humans" (IARC 2009[61]).

The current HPHC list of the FDA contains all four aldehydes (FDA 2012a). They are listed regarding their carcinogenic potential (acetaldehyde, crotonaldehyde, formaldehyde), and potential toxicity to the cardiovascular system (acrolein), or to the respiratory system (acetaldehyde, acrolein, formaldehyde). Acetaldehyde is also listed for its potential to contribute to addiction.

Aldehyde Concentrations in Traditional Swedish Snus

To date, few studies have reported aldehyde concentrations in traditional Swedish snus (Table A II-4 in Appendix II).

The concentrations of formaldehyde, acetaldehyde, acrolein, and crotonaldehyde in traditional Swedish snus (*General*) detected by Stepanov and colleagues (2008a) were 8.49, 31.7, 1.01, and 1.05 μg/g dry weight, respectively. 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.

Faizi and colleagues (2009), researchers from BAT, investigated 70 different STPs for their aldehyde contents, including samples of Swedish pouched and loose snus from seven different manufacturers. Their presentation indicated lower concentrations than those detected by

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[60] In their recent study, Caraway and Chen (2012), researchers from Reynolds, investigated the extraction of different components from *Camel Snus* brands for 53 regular US users under unrestricted use conditions, who collected their used pouches during the 7-day study period. The average amount used was 3.3 pouches/day (5.4 pouches/day for snus only users, 2.8 pouches/day for dual users who also smoked). The majority (47%) used each pouch for 10-30 minutes. The mean B[*a*]P extraction (proportion removed from product) was approximately 29% for all four *Camel Snus* brands analyzed.

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

Stepanov and colleagues (2008a). Because Faizi and colleagues (2009) did not provide brand names, it cannot be determined if these samples were mostly traditional Swedish snus or included novel brands or new products marketed as snus. Formaldehyde concentrations in pouched and loose snus were reported to range from 0.83 to 3.89 μg/g dry weight (median, approximately 1.4 μg/g dry weight) and 0.80 to 1.80 μg/g dry weight (median, approximately 1.2 μg/g dry weight), respectively. Acetaldehyde concentrations were given to be between 0.9 and 10 μg/g dry weight (median, approximately 4.5 μg/g dry weight) and 3.15 to 10.61 μg/g dry weight (median, approximately 6 μg/g dry weight), for pouched and loose snus, respectively. Both acrolein and crotonaldehyde concentrations were below their respective LOQs (0.033 and 0.024 μg/g dry weight, respectively) in all snus samples analyzed, with the exception of one portion snus sample, in which approximately 0.35 μg/g dry weight acrolein was detected.

In summary, the limited data on aldehyde concentrations in Swedish snus indicates formaldehyde, acetaldehyde, acrolein, and crotonaldehyde concentrations of 8.49, 31.7, 1.01, and 1.05 μg/g dry weight, respectively, or lower. Swedish Match also analyzes its snus brands for its aldehyde content, but results have not been published (personal communication with Dr. Margareta Curvall).

2.3.6.4 Metals and Metalloids

Tobacco plants, like most plants, 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. 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.

Metals and metalloids on the FDA HPHC list are arsenic, beryllium, cadmium, chromium, cobalt, lead, mercury, nickel, and selenium (FDA 2012a). Both the elemental form of the metals and metal-bound compounds are referred to in this list. Aside from their carcinogenic potential, arsenic, cobalt, and lead are considered potential cardiovascular toxicants (FDA 2012a). Selenium and selenium compounds are "*Not classifiable as to its [their] carcinogenicity to humans*"(Group 3) (IARC 1987). The HPHC list classifies selenium based on its potential effects on the respiratory system; cadmium, chromium, and nickel are also listed as respiratory toxicants. Arsenic, cadmium, chromium, lead, and mercury are listed as potential reproductive and developmental toxicants (FDA 2012a).

In addition to potential systemic effects due to the uptake of metals and their compounds from STPs, metals and their compounds have the potential to cause local oral sensitization, irritation, and inflammation (Pappas 2011). Based on dental studies, oral sensitization has been in observed with cobalt, mercury, nickel, and other metals present in dental materials. In a recent review, Pappas considers leukoplakia and lichen planus lesions caused by metals alone or by STP to be "quite similar". Exposure to metals from STP may contribute to various oral inflammatory lesions observed in STP users (Pappas 2011). In his recent review, Pappas

(2011) considers the epithelial tissue of the oral cavity to have high proximal transfer potential, permitting absorption and transfer of metals from STPs across the epithelia tissue. No study investigating the absorption of metals from snus through the oral mucosa was identified.

Metal/Metalloid Concentrations in Traditional Swedish Snus

The GothiaTek® Standard limits are available for cadmium, lead, arsenic, nickel and chromium (see Table 2- 3); standards have not been set for other analytes, such as beryllium, cobalt, barium, and selenium.

A limited number of studies on metal and metalloid concentrations in STPs, and in particular in snus, were identified in the peer-reviewed published literature. One study has reported levels of arsenic, chromium, lead, and nickel in oral tobacco products in the UK, including Swedish snus ("general [sic] pouch") (McNeill et al. 2006). More recently, Borgerding and colleagues (2012) analyzed arsenic, cadmium, chromium, lead, and nickel in STPs on the market 2006/2007, including traditional Swedish snus products, such as four *General* brands and one *Nick and Johnny* brand.

A poster by BAT researchers presented at the SRNT Annual Meeting 2010, presented results of analytical data graphically for eight different metals/metalloids in a variety of STPs from the US and Sweden obtained in 2008 (from all major manufacturers, representing 80 to 90% of the market shares in both the US and Sweden) (McAdam et al. 2010c). These products included pouched and loose snus, but the source of the snus products (i.e., from Sweden) was not specified. Despite this uncertainty, this data is discussed in this section. An additional uncertainty is whether the assumption of 50% moisture content to extrapolate from wet weight to dry weight is valid. Additional data on heavy metals in different STPs presented in the form of graphs is available from a presentation by Philip Morris USA at the Life Sciences Research Office, Inc. (LSRO) Reduced Risk Review Meeting in 2007 (Fisher 2007).

In addition to reporting concentrations of various metals and metalloids in STPs, McAdam et al. (2010c) compared their relative contents to each other. For all products investigated, the nickel concentration was highest of all elements analyzed, followed by chromium or cadmium. In most STPs, including pouched snus, cadmium ranked third based on its amount, followed by lead, arsenic, selenium, and beryllium. In loose snus, the lead content was higher than the cadmium. These results are consistent with an analysis done by Rickert et al. (2009), but these authors did not analyze traditional Swedish snus. Rickert et al. (2009) also found the contribution of nickel to the overall metal content to be greatest or similar to chromium in most STPs investigated. In Swedish-type snus (*du Maurier*, a new product marketed as snus), the chromium content was greater than the nickel content. In most STPs, cadmium content ranked third, followed by lead and arsenic content.

<u>Arsenic</u>

McNeill and colleagues (2006) detected 0.3 μg/g per dry weight arsenic in "snus (general [sic] pouch) from Sweden".

Data presented by Philip Morris USA at the LSRO Reduced Risk Review Meeting in 2007 (Fisher 2007), indicates arsenic concentrations in Swedish snus (no brands specified) in a

similar range (approximately 0.15-0.2 μg/g dry weight). In the recent BAT analysis, arsenic concentrations in pouched and loose snus products ranged from approximately 0.04 up to 0.275 μg/g wet weight and approximately 0.03 to 0.90 μg/g wet weight, respectively (McAdam et al. 2010c). If 50% moisture is assumed, the maximum, as measured in one pouched snus product, resulted in maximum arsenic concentrations per dry weight slightly above the GothiaTek® Limit of 0.5 μg/g per dry weight. All other pouched snus products were below this limit. The maximum measured in loose snus brands resulted in concentrations more than 3 times higher than the GothiaTek® limit. However, as noted above, it is not known if other new products marketed as snus, which often have considerably lower moisture levels, were included in the analysis. Therefore, assuming 50% moisture might result in an overestimation of the arsenic concentration in these products.

Borgerding and colleagues (2012) reported concentrations of 0.078 to 0.160 μg/g per dry weight arsenic in traditional Swedish snus brands, with *General* brands ranging from 0.084 to 0.153 μg/g per dry weight.

In summary, reported levels from recent studies indicate that arsenic concentrations in traditional Swedish snus appear to be below the GothiaTek® Limit of 0.5 μg/g per dry weight. Swedish Match (2013[62]) reported the average content in snus brands manufactured in 2011 to be <0.1 μg/g dry weight (range, ± 2 standard deviations, <0.10-0.22 μg/g dry weight), which is consistent with the data reported in recent peer-reviewed publications.

Beryllium

Beryllium concentrations in pouched snus were mostly in the range of approximately 0.002 to 0.02 μg/g wet weight with the exception of two samples, which had concentrations up to approximately 0.087 μg/g wet weight (McAdam et al. 2010c). Beryllium concentrations in loose snus ranged from approximately 0.006 to 0.014 μg/g wet weight, with the exception of one sample that had a beryllium concentration of approximately 0.062 μg/g wet weight. If 50% moisture is assumed, beryllium concentrations in snus samples were generally equal or less than 0.04 μg/g dry weight, with the maximum concentrations as measured in pouched and loose snus samples below 0.18 and 0.12 μg/g dry weight (see above for limitations of this assumption regarding the moisture content).

Cadmium

Data presented by Philip Morris USA at the LSRO Reduced Risk Review Meeting in 2007 (Fisher 2007), showed lower maximum cadmium concentrations in Swedish snus (range, approximately 0.3-0.75 μg/g dry weight). The recent BAT analysis showed cadmium concentrations in pouched and loose snus products in the range of approximately 0.15 to 0.73

[62] Swedish Match. 2013. http://www.swedishmatch.com/en/Snus-and-health/GOTHIATEK/GOTHIATEK-standard/, accessed April 2013.

μg/g wet weight and approximately 0.15 to 0.41 μg/g wet weight, respectively (McAdam et al. 2010c). If 50% moisture is assumed, the maximum in pouched snus products resulted in maximum cadmium concentrations per dry weight exceeding the GothiaTek® Limit of 1 μg/g per dry weight (see above for limitations of this assumption regarding the moisture content).

In contrast, measurements presented by Borgerding and colleagues (2012) for cadmium concentrations in traditional Swedish snus products, including four *General* brands and one *Nick and Johnny* brand, ranged from 0.355 to 0.615 μg/g per dry weight.

In summary, data from recent studies indicate that cadmium concentrations in traditional Swedish snus are generally below the GothiaTek® Limit of 1 μg/g per dry weight. Swedish Match (2013)[63] reported the average content in snus brands manufactured in 2011 to be 0.4 μg/g dry weight (range, ± 2 standard deviations 0.2-0.6 μg/g dry weight), which is consistent with data reported in one recent peer-reviewed publication.

Chromium

McNeill and colleagues (2006) detected 1.54 μg/g per dry weight chromium in "snus (general [sic] pouch) from Sweden".

Data presented by Philip Morris USA at the LSRO Reduced Risk Review Meeting in 2007 (Fisher 2007), indicates chromium concentrations in Swedish snus in a similar range (approximately 0.45-1.70 μg/g dry weight). In the recent BAT analysis, chromium concentrations in pouched snus products ranged from approximately 0.40 up to 1.10 μg/g wet weight, with the exception of one sample that showed a higher concentration, approximately 4.4 μg/g wet weight. In loose snus samples, chromium concentrations ranged from approximately 0.4 to 1.6 μg/g wet weight (McAdam et al. 2010c). If 50% moisture is assumed, the maximum as measured in most pouched snus samples per dry weight were below the GothiaTek® Limit of 3 μg/g per dry weight, concentrations in loose snus samples were at the limit, and one pouched snus sample exceeded the limit by almost three times (see above for limitations of this assumption regarding the moisture content).

In contrast, measurements presented by Borgerding and colleagues (2012) reported chromium concentrations in traditional Swedish snus products, including four *General* brands and on *Nick and Johnny* brand, in the range of 0.870 to 1.822 μg/g per dry weight.

In summary, data from recent studies indicate that chromium concentrations in traditional Swedish snus are generally below the GothiaTek® Limit of 3 μg/g per dry weight. Swedish Match (2013)[64] reported the average content in snus brands manufactured in 2011 to be 0.6

[63] Swedish Match. 2013. http://www.swedishmatch.com/en/Snus-and-health/GOTHIATEK/GOTHIATEK-standard/, accessed April 2013.
[64] Swedish Match. 2013.

μg/g dry weight (range, ± 2 standard deviations 0.2-1.2 μg/g dry weight), which is lower than data reported in two recent peer-reviewed publications.

Cobalt

No recent data on cobalt concentrations in traditional Swedish snus or similar products were identified.

Lead

McNeill and colleagues (2006) detected 0.5 μg/g per dry weight lead in "snus (general [sic] pouch) from Sweden".

Data presented by Philip Morris USA at the LSRO Reduced Risk Review Meeting in 2007 (Fisher 2007), indicates lead concentrations in Swedish snus in a similar range (approximately 0.35-0.50 μg/g dry weight). In the recent BAT analysis, lead concentrations in most pouched snus products ranged from approximately 0.12 up to 0.36 μg/g wet weight, with the exception of two samples that showed concentrations of up to approximately 1.3 μg/g wet weight. In loose snus, lead concentrations ranged from approximately 0.18 to 0.28 μg/g wet weight, with the exception of one sample, which had a concentration of 0.5 μg/g wet weight (McAdam et al. 2010c). If 50% moisture is assumed, the lead concentrations per dry weight in all snus samples analyzed in this study were below the GothiaTek® Limit of 2 μg/g per dry weight, with the exception of one pouched snus sample that exceeded the limit slightly (see above for limitations of this assumption regarding the moisture content).

In contrast, measurements presented by Borgerding and colleagues (2012) reported lead concentrations in traditional Swedish snus products in the range of 0.157 to 0.244 μg/g per dry weight. Concentration in four *General* brands ranged from 0.180 to 0.209 μg/g per dry weight.

In summary, data from recent studies indicate that lead concentrations in traditional Swedish snus are generally well below the GothiaTek® Limit of 2 μg/g per dry weight. Swedish Match (2013)[65] reported the average content in snus brands manufactured in 2011 to be 0.2 μg/g dry weight (range, ± 2 standard deviations <0.08-0.4 μg/g dry weight), which is consistent with data reported in two recent peer-reviewed publications.

Mercury

Mercury concentrations in snus were only determined in the recent BAT analysis and appear to have been analyzed a relatively low number samples (McAdam et al. 2010c). Concentrations in three pouched snus products ranged from approximately 0.0105 to 0.0145 μg/g wet weight. In one loose snus sample, the mercury concentration was approximately 0.0105 μg/g wet weight.

[65] Swedish Match. 2013. http://www.swedishmatch.com/en/Snus-and-health/GOTHIATEK/GOTHIATEK-standard/, accessed April 2013.

If 50% moisture is assumed, the measured mercury concentrations were below 0.03 μg/g per dry weight (see above for limitations of this assumption regarding the moisture content).

Nickel

McNeill and colleagues (2006) detected 2.59 μg/g per dry weight nickel in "snus (general [sic] pouch) from Sweden"(McNeill et al. 2006)(McNeill et al. 2006).

Data presented by Philip Morris USA at the LSRO Reduced Risk Review Meeting in 2007 (Fisher 2007), indicate somewhat lower nickel concentrations in Swedish snus (approximately 0.20-1.90 μg/g dry weight). In the recent BAT analysis, nickel concentrations in most pouched snus products ranged from approximately 0.60 up to 1.70 μg/g wet weight, with the exception of one sample that had a concentration of approximately 2.3 μg/g wet weight (McAdam et al. 2010c). In loose snus, nickel concentrations ranged from approximately 0.60 to 1.95 μg/g wet weight. If 50% moisture is assumed, the nickel concentrations per dry weight in all snus samples analyzed in this study were below the GothiaTek® Limit of 4.5 μg/g per dry weight, with the exception of one pouched snus sample that slightly exceeded the limit (see above for limitations of this assumption regarding the moisture content).

Borgerding and colleagues (2012) reported nickel concentrations in traditional Swedish snus products including four *General* brands and one *Nick and Johnny* brand, in the range of 1.182 to 2.781 μg/g per dry weight.

In summary, data from recent studies indicate that lead concentrations in traditional Swedish snus are generally below the GothiaTek® Limit of 4.5 μg/g per dry weight. Swedish Match (2013)[66] reported that the average content in snus brands manufactured in 2011 was 1.4 μg/g dry weight (range, ± 2 standard deviations <0.6-2.0 μg/g dry weight), which is consistent with data reported in two recent peer-reviewed publications.

Selenium

Data on selenium concentrations in snus were only identified in the recent BAT analysis (McAdam et al. 2010c). Concentrations in most pouched snus products ranged from approximately 0.055 up to 0.10 μg/g wet weight, with the exception of two samples that showed concentrations of up to approximately 0.125 μg/g wet weight. In loose snus selenium concentrations ranged from approximately 0.05 to 0.09 μg/g wet weight. If 50% moisture is assumed, the measured selenium concentrations were below 0.3 μg/g per dry weight (see above for limitations of this assumption).

[66] Swedish Match. 2013. http://www.swedishmatch.com/en/Snus-and-health/GOTHIATEK/GOTHIATEK-standard/, accessed April 2013.

Barium

Pappas and colleagues (2008) analyzed commercial moist snuff, but not snus, and detected barium levels significantly higher than those of the other metals examined. No published studies were identified that investigated barium in traditional Swedish snus.

Summary

In summary, there was data from two peer-reviewed publications on arsenic, cadmium, chromium, lead, and nickel. These data indicate that cadmium and nickel concentrations in the traditional Swedish snus that were sampled were below the current GothiaTek® Limits of 1 and 4.5 μg/g per dry weight, respectively. Arsenic and chromium concentrations in these samples were almost half of the current GothiaTek® Limits of 0.5 and 3 μg/g per dry weight, respectively; lead concentrations were about 25% of the GothiaTek® Limit of 2 μg/g per dry weight. Data for beryllium, mercury, and selenium were available from a poster presentation, which indicated the respective concentrations were below approximately 0.2, 0.03, and 0.3 μg/g per dry weight. No data were available for cobalt or barium concentrations.

Metal Extraction from Snus

No data from the published literature were available for metal extraction from traditional Swedish snus. There is indication from Pappas and colleagues (2008) that metal extraction from STPs is significantly less than 100%. These researchers investigated the amount of arsenic, beryllium, cobalt, cadmium, chromium, lead, nickel, and barium extractable from various STP brands using artificial saliva to mimic human use and uptake[67].

A recent study[68] on brands of new products marketed as snus indicates that mean metal (cadmium and nickel) extraction rates (proportions removed from product) were approximately 10% under unrestricted use conditions (mostly for 10-30 min) (Caraway and Chen 2012). Unpublished data of an extraction study with traditional Swedish snus indicates that cadmium

[67] Based on the results by Pappas and colleagues (2008), the amount of cadmium extracted from US moist snuff samples was in the range of 21-47% of the cadmium content in the products. The respective extraction rates of cobalt and nickel were 38-65% and 26-46%. Only one of six STPs analyzed had detectable extracted amounts of beryllium and lead (11% and 8%, respectively). The extracted concentrations for the other samples were below the detection limit. Due to elevated arsenic and chromium background in the artificial saliva used, no additional extraction of these elements could be detected. Barium content was extractable between 2 and 21% and Pappas et al. (2011) noted that the net mass of the extractable barium was the highest of all the metals examined.

[68] In their recent study, Caraway and Chen (2012), researchers from Reynolds, investigated the extraction of different components from *Camel Snus* brands for 53 regular US users under unrestricted use conditions, who collected their used pouches during the 7-day study period. The average amount used was 3.3 pouches/day (5.4 pouches/day for snus only users, 2.8 pouches/day for dual users who also smoked). The majority (47%) used each pouch for 10-30 minutes. The mean cadmium and nickel extraction rates (proportion removed from product) were approximately 11 and 9%, respectively, for all four *Camel Snus* brands analyzed. Negative extraction amounts were reported for arsenic, chromium, and lead. The authors attributed this to variability in constituent levels, e.g., regional variations in tobacco constituent levels in *Camel Snus* was reported by Stepanov and colleagues (2012a).

was extracted to 3 to 11%, while lead extraction was negligible (personal communication with Dr. Margareta Curvall, Swedish Match). Other metals were not tested in this study.

2.3.6.5 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.

Polonium-210 emits α-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).

In addition to polonium-210, the current HPHC list of the FDA contains uranium-235 and uranium-238 based on concerns about their carcinogenic potential (FDA 2012a). All three isotopes are Group 1 carcinogens (IARC 2001). FDA lists the latter two also for their potential to be respiratory toxicants.

Radioisotope Concentrations in Traditional Swedish Snus

As reported in a 1989 Swedish review and risk assessment, levels of polonium-210 in snus ranged from 11 to 60 becquerels (Bq) per kg wet weight (0.011-0.060 Bq/g or 0.022-0.120 Bq/g dry weight) (Samuelsson 1989).

According to Samuelsson (1989), 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.

Recent data on radioactive element content in STPs, including traditional snus, was reported in poster presentations by BAT researchers at the 2009 Tobacco Science Research Conference (TSRC) and the 2010 SRNT Annual Meeting (McAdam et al. 2010c; Mola et al. 2009) (Table A II-6 in Appendix II).

In addition to sampling US STPs, Mola and colleagues (2009) analyzed 22 pouched and 10 loose snus samples from seven different Swedish manufacturers. Since the brands were not specified, these samples may consist of some novel brands or non-traditional snus ("new products marketed as snus") products. The researchers reported combined α-activity for the measured radioisotopes (polonium-210, uranium-234, -235, and -238, radium-226, thorium-232, -230, -228) ranging from below 0.012 to 0.050 Bq/g wet weight for both Swedish snus products and US STPs. Approximately 50% of the activity was due to polonium-210, with medians[69]

[69] The data for each radioisotope and product group was reported in the form of box-plots.

around 0.005 and 0.003 Bq/g wet weight for pouched and loose snus, respectively. Both uranium-235 and uranium-238 were not detected in any of the products tested. Only one thorium radioisotope (thorium-228) was detected in Swedish pouched and loose snus products, with median activities of approximately 0.0015 and 0.002 Bq/g wet weight, respectively. Median radium-226 activities were approximately 0.0018 and 0.004 Bq/g wet weight for pouched and loose snus, respectively. Some β-activity from lead-210 was detected, which was generally not more than 0.02 Bq/g wet weight for snus samples.

McAdam and colleagues (2010c) reported analytical data for the same nine radioisotopes in a variety of STPs from the US and Sweden obtained in 2008 (from all major manufacturers, representing 80-90% of the market shares in both the US and Sweden); results were presented in the form of graphs (McAdam et al. 2010c). These products included pouched and loose snus, but it was not specified if the snus products were exclusively from Sweden. As with the study by Mola and colleagues (2009), brands were not specified for these samples and hence may consist of some non-traditional snus products. McAdam and colleagues (2010c) concluded that α-radioactivity of STPs was dominated by polonium-210, radium-226, and thorium-228, but did not report the activity levels separately for the different product classes. The authors noted that the quantities of these isotopes were very low (10^{-12}-10^{-17} g/g), with uranium-238 and thorium-232, isotopes of lower radioactivity, comprising the highest quantity. In pouched and loose snus, both radium-226 and lead-210 concentrations were in the femtogram range (fg, 10^{-15} g), while both thorium-228 and polonium-210 concentrations were in the atogram range (ag, 10^{-18} g; polonium-210 ~10-45 and ~10-30 ag/g wet weight for pouched and loose snus, respectively). Uranium-238, uranium-235, and thorium-230 were not detected in the snus samples. Uranium-234 was detected in only one pouched snus sample (~9.5 pg/g wet weight).

In summary, the only recent data on radioisotopes in snus products is provided in poster presentations. This data indicates that total combined α-radioactivity of eight different radioisotopes measured was similar to the radioactivity attributed to polonium-210 reported in 1989 for Swedish snus. Based on the recent studies, radioactivity from polonium-210 constitutes approximately 50% of the total radioactivity measured (up to a median of 0.005 Bq/g wet weight). In addition, radioactivity from thorium-228 and radium-226 were also detected in the snus samples. Neither uranium-235 nor uranium-238 was detected. Some β-activity from lead-210 was also detected (generally <0.02 Bq/g wet weight).

2.3.6.6 Other Trace-Level Components

Other carcinogenic compounds, including acrylamide, ethyl carbamate (urethane) and hydrazine, and mycotoxins can also be present in STPs at trace concentrations.

Acrylamide can be formed when foods rich in carbohydrates are subjected to high-temperature cooking processes; it is also formed during tobacco smoking. Acrylamide is classified as Group 2A carcinogen by IARC (1994) and is part of the current HPHC list of the FDA based on concerns about its carcinogenic potential (FDA 2012a).

Ethyl carbamate (urethane) is formed during fermentation processes. It is classified as a Group 2A carcinogen by IARC (2010a) and is part of the current FDA HPHC list based on concerns

about its carcinogenic potential, as well as for potential reproductive and developmental toxicity (FDA 2012a).

Hydrazines can be found in both air- and fire-cured tobaccos (IARC 2007). Hydrazine is classified as Group 2B carcinogen by IARC (IARC 1999) and is part of the current FDA HPHC list based on concerns about its carcinogenic potential, as well as potential to be a respiratory toxicant (FDA 2012a).

The presence of bacteria, mold, and microbial toxins in tobacco products and their potential to induce chronic inflammation is discussed in one recent review (Pauly and Paszkiewicz 2011). Aflatoxins are classified as Group 1 carcinogens by IARC (2012a). Aflatoxin B_1 is part of the current HPHC list of the FDA based on concerns about its carcinogenic potential (FDA 2012a).

Concentrations of Other Trace-Level Components in Traditional Swedish Snus

No published scientific literature on the acrylamide content in traditional Swedish snus was identified. Swedish Match analyzes snus for its acrylamide content but results have not been published (personal communication with Dr. Margareta Curvall).

No peer-reviewed published studies were identified that reported ethyl carbamate concentrations in traditional Swedish snus. Data on 21 portion snus and 11 loose snus samples from Sweden (sourced in 2008 from seven different manufacturers) were presented by BAT researchers as poster at a CORESTA conference. Their analysis indicated that ethyl carbamate concentrations ranged from below the reporting limit (20 ng/g wet weight) to 155 ng/g dry weight in pouched snus samples, and 74 ng/g dry weight in loose snus samples (Faizi et al. 2010). Swedish Match analyzes snus for its ethyl carbamate content but results have not been published (personal communication with Dr. Margareta Curvall).

No recent studies that investigated hydrazine in traditional Swedish snus were identified.

While no published scientific literature on mycotoxin content in traditional Swedish snus was identified, aflatoxin content of snus is regulated under the Swedish National Food Agency Directive (Swedish Match 2013)[70] and therefore, Swedish Match analyzes snus samples for this mycotoxin. In addition to aflatoxins, Swedish Match analyzes their snus brands for ochratoxin but results have not been published (personal communication with Dr. Margareta Curvall). Bacteria content and bacterial growth are strictly monitored under the GothiaTek® standard sanitation requirements in manufacturing.

[70] Swedish Match. 2013. http://www.swedishmatch.com/en/Snus-and-health/GOTHIATEK/GOTHIATEK-standard/, accessed April 2013.

In summary, no published literature was available that reported concentrations of acrylamide, hydrazine, and mycotoxins. Some data on ethyl carbamate are available from a poster presentation, indicating concentrations of up to 155 ng/g dry weight.

2.3.7 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).

2.4 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. Novel brands may deviate from these values. 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 a quality standard (GothiaTek®), for the entire manufacturing process; including limits for certain "undesired" trace-level components in snus. The current list of "Harmful or Potentially Harmful Constituents (HPHC)" released by the FDA in April 2012 consists of 93 components, 43 of which are thought to originate mainly from combustion processes. In this section, published data available on the remaining 50 components and on additional components in STPs that have been quantified or were considered relevant were discussed. Where available, results from extraction studies were also presented.

Concentrations of TSNAs, traditionally the most frequently analyzed and reported trace-level components in STPs due to their carcinogenic potential in experimental animals; have 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. The newest data indicates that TSNA concentrations have continued to decline and combined NNK and NNN concentrations currently appear to be approximately half the limit (2 µg/g dry weight) recommended by the WHO in 2009.

Published data for most other trace-level components other than TSNAs analyzed in STPs and snus have become available (e.g., PAHs, aldehydes, metals, and radioisotopes). PAH concentrations reported in recent studies demonstrate that B[*a*]P concentrations are generally lower than the limit recommended by the WHO in 2009 (5 ng/g dry weight). Limited data on the presence of other PAHs indicates that only phenanthrene, fluoranthene, pyrene, and possibly

naphthalene were detected in higher quantities. Generally, the analytical data from recent published studies on the various components indicate that concentrations in traditional Swedish snus are below the GothiaTek® limits as well as existing WHO-recommended limits.

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, nicotine content and delivery between snus brands, as well as, extraction and absorption of the chemical substances from snus, which all need to be taken into account when conducting an exposure assessment.

A comparison of critical components in traditional Swedish snus with other STPs, such as new products marketed as snus and US-type moist snuff, other factors, including moisture content, pH and resulting free nicotine are provided in Appendix II.

For a risk assessment, patterns of use of any of the STPs 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 Snus

Biomarkers of exposure may be used to assess the actual internal dose of a tobacco component to which a tobacco user might be exposed. A biomarker of exposure to a chemical or component is defined as, "The chemical, or its metabolite, or the product of an interaction between a chemical and some target molecule or cell that is measured in a compartment in an organism" (Institute of Medicine 2012). Because biomarkers of exposure represent the integrated exposure from all routes, use of exposure biomarkers reduces uncertainties in the assessment of exposures that are based on the concentrations if components in tobacco products coupled with extraction and uptake of these components via different routes, e.g., oral tobacco use versus smoking or due to different use patterns may be bypassed (Institute of Medicine 2012). Exposure biomarkers for tobacco components may also be contributed to by other exposure sources, however, such as diet, automobile exhaust, and occupational exposure, with the exception of tobacco-specific biomarkers.

Biomarker levels vary between individuals, due to potential differences in product use behavior, genetic polymorphisms and other host differences, and differences in the characteristics of products used. Comparisons of biomarker levels on a population basis, however, provide an indication of general trends in internal exposure to certain components/constituents due to use of a specific well-characterized product. In its report, *Scientific Standards for Studies on Modified Risk Tobacco Products*, the IOM (2012) concluded "In summary, biomarkers can provide a more realistic assessment of the consumer's exposure to carcinogens and toxicants in tobacco products than simple analyses of the products because laboratory analyses cannot fully duplicate human use conditions. In most cases, the general trend of laboratory results is reflected in the biomarker data."

Aside from inter-individual variation, there are other limitations to the use of biomarkers to assess exposure to certain components from tobacco products. First, even though a large number of components have been quantitated in various tobacco products, to date only a limited number of exposure biomarkers have been measured and validated in tobacco users. Furthermore, downstream metabolites, such as those measured in urine, may reflect not only differences in exposure to the component of origin, but also a potential change in upstream metabolism (Hecht et al. 2010) (e.g., impact of genetic polymorphisms, other components competing for metabolizing pathways).

While some studies have shown associations between exposure biomarkers and risk of specific health endpoints, the specific tobacco components that might ultimately be responsible for tobacco-related diseases has not been established. As pointed out by the IOM (2012), "it is possible that constituents that play a decisive role in disease causation are simply not being measured, […]". Furthermore, mixture effects due to "potential interactive effects among components that are critical in disease etiology" may not have been taken into account in the analyses.

Thus, due to all these limitations, conclusions from these studies with respect to harm reduction should be interpreted carefully and in the context of additional data from clinical or epidemiological studies. The IOM (2012) noted "If the panel of biomarkers presented were

decreased to the levels found in nonsmokers, it is likely that there would be a beneficial effect on health, but this has not been proven."

Hecht and colleagues (2010) recently suggested a panel of carcinogen and toxicant biomarkers that could be used in product regulations. The panel consists of analytically validated exposure biomarkers, most of which have been analyzed in multiple studies on large number of smokers and non-smokers (Hecht et al. 2010). These authors also point out that all tobacco components that were identified as priority components in mainstream cigarette smoke for regulation under the Framework Convention on Tobacco Control (FCTC) by the WHO are included in their suggested panel (Burns et al. 2008). The panel includes the following biomarkers of exposure that are likely more relevant for exposure to smokeless tobacco, including snus:

- Urinary biomarkers of nicotine (nicotine equivalents[71]), NNK (total NNAL[72]), NNN (total NNN[73]), PAHs (1-HOP[74]), acrolein (HPMA[75]), crotonaldehyde (HBMA[76]), and cadmium
- Hemoglobin adducts of acrylamide (carbamoylethylvaline)
- Leukocyte DNA adducts of formaldehyde (N^6-hydroxymethyl-deoxyadenosine) and acetaldehyde (N^2-ethylidene-deoxyguanosine)

The sources of the following biomarkers of tobacco-related exposure on the panel suggested by Hecht and colleagues (2010) are likely combustion products in cigarette smoke and these biomarkers are therefore less relevant for exposure to smokeless forms of tobacco, but could, in studies where STPs are used for smoking cessation, be indicative of reduced exposure following smoking reduction:

- Urinary biomarkers of 1,3-butadiene (MHBMA[77]), benzene (SPMA[78]), ethylene oxide (HEMA [79])
- Hemoglobin adducts of ethylene oxide (hydroxyethylvaline), 4-aminobiphenyl (4-aminobiphenyl-globin), and acrylonitrile (cyanoethylvaline)
- Biomarkers of carbon monoxide (exhaled CO, carboxyhemoglobin)

In addition to the above listed, other frequently measured biomarkers of tobacco exposure include cotinine in plasma or serum for exposure to nicotine; anatabine and anabasine, which

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[71] Nicotine equivalents: The sum of nicotine, cotinine, 3′-hydroxycotinine, and their glucuronides
[72] Total NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides
[73] Total NNN, *N*′-nitrosonornicotine and its glucuronides
[74] 1-HOP: 1-hydroxypyrene and its glucuronides/sulfates
[75] HPMA: 3-hydroxypropyl mercapturic acid
[76] HBMA: 4-hydroxybut-2-yl mercapturic acid
[77] MHBMA: The sum of 1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene and 1-(*N*-acetylcysteinyl)-2-hydroxy-3-butene
[78] SPMA: *S*-phenyl mercapturic acid
[79] HEMA: 2-hydroxyethyl mercapturic acid

are used to distinguish nicotine exposure from tobacco products from that of nicotine-replacement products, which contain only trace levels, if any, of these components; and urinary metabolites of B[*a*]P, naphthalene, phenanthrene, and fluorene (Hatsukami et al. 2003; Hecht 2002). Unchanged NDMA, NPYR as well as several nitrosamino acids have been measured in the urine of smokers, but correlation with tobacco use has been mixed due to endogenous formation of these nitrosamino compounds so they have not been frequently measured (Hecht 2002; USDHHS 2010).

To date, the available literature provides information on nicotine, TSNAs, cadmium, and selenium biomarkers investigated in traditional Swedish snus users. The data is presented in the following sections. The outline follows the same order for tobacco components as established in Section 2 (Product Chemistry) and includes a brief introduction to provide relevant available information on the formation, significance, and limitations of the discussed biomarker.

In Appendix III, the available data on biomarkers of exposure for traditional Swedish snus users, supplemented with available data for users of new products marketed as snus, is discussed in comparison with data for smokers and Nicotine Replacement Therapy (NRT) users. Where no data was identified for users of snus or new products marketed as snus, select studies of traditional US STPs users are discussed. Study details are provided in Table A III-7.

3.1 Biomarkers of Exposure to Tobacco Alkaloids: Nicotine

No studies were identified in which biomarkers of exposure to other alkaloids were measured in snus users. Therefore, this section focuses on biomarkers of nicotine exposure.

The uptake and fate of nicotine in the body are important determinants in the evaluation of its biomarkers of exposure. In addition, since nicotine is thought to be the primary addictive component of tobacco, its pharmacokinetic parameters are relevant for the assessment of the abuse liability of a tobacco product. Parameters associated with a greater likelihood of abuse are faster speed of drug delivery, clearance, and greater amount of drug absorption (Carter et al. 2009). The IOM (2012) stated that "In particular, acute blood nicotine absorption profiles in response to both single and repeated use of products is a relevant component in assessing the addictive potential of MRTPs." Acute dose effect studies that measure respective parameters (e.g., time to and maximum nicotine blood level (t_{max} and C_{max}) and the area under the curve (AUC)) often together with other physiological, psychomotor, and subjective effects are part of suggested study types for abuse liability assessments (Carter et al. 2009). Also, the IOM (2012) noted "A standard with regards to human abuse liability drug testing are acute dose-effect comparison studies, because of the correspondence between subjective ratings of drug effects and real-world abuse potential."

3.1.1 Nicotine Pharmacokinetics

During use of oral smokeless tobacco products as well as NRT products, nicotine is absorbed mainly in the oral cavity via the buccal mucosa and in part from swallowed tobacco juices in the gastro-intestinal tract (Benowitz 2009; Ebbert et al. 2004). This is in contrast to nicotine absorption from smoking, where inhaled nicotine is mostly absorbed through the alveoli in the lung into the blood stream.

As described in Section 2.3.3, nicotine is a weak base and in its ionized form does not easily cross biological membranes (as reviewed in Benowitz et al. 2009). Hence, absorption of nicotine is dependent on pH and is more rapid from alkaline tobacco products or in a more alkaline body environment. The absorption of nicotine through the lung is thought to be rapid and comprehensive due to the large surface area of the alveoli and small airways and dissolution of nicotine in lung fluid of pH 7.4; by comparison, absorption of nicotine from oral products is a slower process. For oral tobacco products, the extent and speed of oral absorption into the systemic circulation is largely dependent on product pH, e.g., the buffering capacity of moist snuffs were shown to be 10 to 20 times higher than the buffering capacity of human saliva (Ciolino et al. 2001), excluding the potential influence of foods and drinks that influence acidity in the mouth. Though oral absorption is rapid for more alkaline tobacco products, the rise in brain nicotine level is slower than with smoking, where high levels of nicotine reach the brain in 10 to 20 seconds (faster than with intravenous administration) (as reviewed in Benowitz et al. 2009; Hukkanen et al. 2005). A slower, more gradual increase in nicotine levels is thought to result in lower abuse liability (as reviewed in Benowitz et al. 2009). The fraction of swallowed nicotine from oral products can be well absorbed in the small intestines due to its alkaline pH and large surface area, but its bioavailability is low since it undergoes first-pass metabolism in the liver to cotinine and other metabolites before reaching the systemic circulation (as reviewed in Benowitz et al. 2009; Hukkanen et al. 2005; USDHHS 2010).

Nicotine is primarily and extensively metabolized in the liver to a variety of different substances. About 70 to 80% of nicotine in humans is converted to cotinine via a cytochrome P450 (CYP)-catalyzed pathway (as reviewed in Benowitz et al. 2009; Hukkanen et al. 2005). The main enzymes involved in this step are CYP 2A6 and 2A13 (Murphy et al. 2011). While nicotine has a short half-life in blood (~2 hours after intravenous administration or smoking), cotinine's blood half-life is much longer (~16 hours) (as reviewed in Hukkanen et al. 2005).

It should be noted that a considerable inter-individual variability in the elimination rate of nicotine and cotinine exists, which is due to genetic polymorphisms, a variety of other physiological influences (such as diet, age, time of day, gender, pregnancy), other influences (such as pathological conditions, medications, racial and ethnic differences), and finally, smoking itself. For example, the clearance of nicotine in smokers is lower compared to those in nonsmokers, which may be due to other components in tobacco products. There is indication that long-term STP use may also decrease cotinine levels as shown in a study of STP users where cotinine in saliva was measured (Mushtaq et al. 2011). It is thought that this effect is due to increased cotinine metabolism and elimination, similar to what has been observed with smokers (Mushtaq et al. 2011). Menthol, a flavorant in foods, personal care products, and tobacco, has also been shown to inhibit CYP 2A6-mediated nicotine metabolism and increase systemic nicotine exposure (as reviewed in Benowitz et al. 2009; Hukkanen et al. 2005).

3.1.1.1 Nicotine Pharmacokinetics in Snus Users

Pharmacokinetic parameters of nicotine in blood absorbed from snus have been investigated in several studies by Swedish researchers, researchers from British American Tobacco, and Swedish Match (Digard et al. 2012; Holm et al. 1992; Lunell and Curvall 2011; Lunell and Lunell 2005). Three of these studies were conducted with regular snus users and one study was

conducted among smokers who switched to snus for the purpose of the experiment (Lunell and Curvall 2011). In all studies, the experiment started after a minimum overnight (12-hour) period of abstinence. Nicotine parameters as measured in these studies are provided in Table A III-1.

Rise of Nicotine Blood Concentration and Time to Maximum Concentration (t_{max})

The time to maximum plasma nicotine concentration in snus users appears to be dependent on the snus usage time, but not nicotine content or portion size. In a recent study, Digard and colleagues (2012), reported that though different portion sizes of a loose snus product (*Granit*) were used (i.e., nicotine exposures were different depending on the portion size) each for 60 minutes, the median t_{max} was the same - 60 minutes (range, 45-90 minutes). This was similar to the finding for two pouched snus products (*Lucky Strike Original Brown and Bold*), with different nicotine contents (median t_{max} 60 min (range, 20-90 min and 45-90 min, respectively). By contrast, previous studies used experimental times of 30 minutes snus use and the reported mean or median t_{max} values were between 30 and 37 minutes (Holm et al. 1992; Lunell and Curvall 2011; Lunell and Lunell 2005).

Maximum and Total Nicotine Blood Concentration (C_{max} and AUC)

The mean maximum plasma concentrations (C_{max}) for snus users varied between studies and ranged from 10.8 to 29 ng/mL. The highest mean C_{max} values were measured in users of *General* and *Catch* snus brands under continuous use conditions with 12 administrations of 30 minutes each (Lunell and Lunell 2005). In the three other studies, the experimental design included only a single administration. The lowest C_{max} (10.8 ng/mL) was measured in snus users of a loose snus (*Granit*) and a pouched snus (*Lucky Strike Original Brown*) that had slightly higher nicotine content (10.8 mg and 10.7 mg per 1-g portions), but also had a slightly lower pH than the *General* and *Catch* brands (pH 8.0-8.3 vs. 8.4-8.7) tested by Lunell and colleagues (Digard et al. 2012; Lunell and Curvall 2011; Lunell and Lunell 2005).

Within each study, a correlation of the C_{max} with the total nicotine content of a product could be observed: Increasing the portion size of the loose snus from a 1-g to 2.5-g portion (nicotine content 27.1 mg/2.5 g) resulted in a respective increase of the geometric mean C_{max} (10.8 to 17.9 ng/mL) (Digard et al. 2012). In the same study, similar but smaller effects were seen with two pouched products of different nicotine content (difference of 4 mg nicotine per 1-g pouch). Under the continuous use conditions, *General* snus with a nicotine content of 8.84 mg/1-g portion resulted in a mean C_{max} of 29 ng/mL compared to a C_{max} of 20.95 ng/mL resulting from *Catch Mini* snus with a nicotine content of 4.53 mg/0.5-g portion (Lunell and Lunell 2005). In the same study, use of *Catch Dry Mini*, a novel brand of traditional Swedish snus, with similar nicotine content *as Catch Mini* (4.82 mg/0.3-g portion), but lower moisture and pH (pH 7.3), resulted in halving of the C_{max} (10.85 ng/mL).

A single use of *General* snus brands (*Onyx* and *White Large*) with nicotine contents of 8.65 or 9.92 mg/1-g portion by smokers naïve to snus use, resulted in mean C_{max} values of 13.7 to 14.8 ng/mL (Lunell and Curvall 2011).

A single use of a 2-g portion of *Ettan* snus resulted in a mean C_{max} of 17 ng/mL (at 35.5 min; the plasma cotinine level at 60 minutes was 279 ng/mL) (Holm et al. 1992).

The average plasma concentrations for nicotine after a single use of 2.5 g unspecified Swedish snuff during supine rest increased slowly from 0.3 ng/mL at zero minutes after 24 hours of abstinence to a plateau of 20.9 ng/mL nicotine at 110 min (plasma cotinine at time 0 was 117.1 ng/mL, the maximum 126.3 ng/mL at 140 min). The sampling period was 140 minutes (Hirsch et al. 1992).

In study 1 by Gray and colleagues (2008) in which habitual traditional STP users were given a 2-g portion of loose *General* snus, plasma nicotine increased from approximately 2 ng/mL at baseline after overnight abstinence to 8.7 ng/mL immediately after the 30-minute consumption of the snus. This study used a cross-over design (Latin square) where subjects used four different products, including snus, separated by 48 hours. Each condition was four hours and consisted of 30 minute product use and 30 minute rest period.

Area under the curve (AUC) values are difficult to compare between these studies since all were determined using different time periods. The lowest mean AUC was reported in the study with 2-g portions of *Ettan* snus and for a time period of 0-60 minutes (747.4 ng*min/mL) (Holm et al. 1992). The geometric mean AUCs for the time period of 0-120 minutes were calculated to be 960 and 1,614 ng*min/mL for the two different portion sizes of loose *Granit* snus (nicotine content, 10.8 and 27.1 mg, respectively) (Digard et al. 2012). In the same study and consistent with their different nicotine contents (10.7 vs. 14.7 mg/1-g portion) geometric mean AUCs for the two pouched snus products differed (*Lucky Strike Original Brown* and *Bold*, 1,008 vs. 1,224 ng*min/mL). Mean AUCs for a time period of 0-720 min (12 hours) were reported in the experiment with multiple uses to range from 1,141 to 1,570 ng*min/mL (19.02-26.16 ng*hrs/mL) for *General* and *Catch* brands with nicotine contents of 4.53 to 8.84 mg/portion (Lunell and Lunell 2005). Similar to what was observed with the C_{max}, the AUC value for *Catch Dry Mini* was approximately half of what was measured for the other two *Catch* brands in the same study (589 ng*min/mL or 9.81 ng*hrs/mL). The highest mean AUC values reported, 2,829 and 3,062 ng*min/mL, were for the time period zero to infinity for two *General* snus brands with nicotine content of 8.65 or 9.92 mg/portion (Lunell and Curvall 2011).

Summary of Nicotine Pharmacokinetic Parameters in Snus Users

In summary, the time to maximum plasma nicotine concentrations in snus users appears to be dependent on the usage time, but not on nicotine content or portion size. On the other hand, C_{max} and AUC appear mostly dependent on total nicotine content (per pouch or portion size) as well as pH of the product. Whether the snus was loose or pouched had no influence on these parameters.

3.1.2 Nicotine Biomarkers

Nicotine and its metabolites have been measured in blood, saliva, urine, hair, nails, and other bodily fluids. Cotinine in serum or plasma is a commonly measured biomarker of internal nicotine exposure.

While exposure estimates to tobacco are also often based on external tobacco use measures (e.g., in cigs/day), Benowitz and colleagues (2011) concluded that “CPD [cigs/day] does not provide an accurate estimate of nicotine and carcinogen exposure”. In their study, they observed that the reliability of this measure varies by race and it was particularly poorly

correlated in black smokers. These authors noted that both urine nicotine equivalents and plasma cotinine are useful for estimating carcinogen exposure. However, Zhu and colleagues (2013b) found that plasma cotinine levels and tobacco carcinogen exposure were different in subjects with different CYP2A6 activity and were therefore not a good quantitative marker to compare between CYP2A6 genotypes, sexes, and races. These parameters should therefore be accounted for in studies that use these measurements to compare nicotine exposures from any tobacco product.

Due to its relatively short half-life, nicotine levels are variable and blood levels fluctuate significantly throughout the day. Cotinine with its longer half-life is considered a more stable indicator of nicotine exposure. A high correlation among cotinine concentrations in plasma, saliva, and urine has been noted (as reviewed in Benowitz et al. 2009).

However, in addition to the factors contributing to inter-individual variability in nicotine and cotinine elimination described above, cotinine levels may not be representative of nicotine uptake and brain levels when comparing different uptake routes. While nicotine plasma levels were shown to be similar in smokeless tobacco users (including snus users) and smokers, cotinine plasma and urinary levels tend to be higher than in smokers (Benowitz et al. 1989; Hecht et al. 2007; Holm et al. 1992) (Appendix III Section A III 3.1.2). This is due to the extended first-pass metabolism of swallowed nicotine after gastro-intestinal uptake. In accordance with that, frequency of swallowing tobacco juice was an independent predictor of higher serum cotinine levels, whereas no correlation was found for serum nicotine levels (Ebbert et al. 2004).

Urinary nicotine or cotinine are frequently measured. Total nicotine or 'nicotine equivalents' is the sum of nicotine and its metabolites in urine: cotinine, and 3' hydroxycotinine, and their respective glucuronides, nicotine-GlcA, Cotinine-GlcA, 3' hydroxycotinine-GlcA; occasionally, nicotine-N'-oxide and cotinine-N-oxide are also included. Nicotine equivalents measured under steady-state conditions account for 73 to 96% of the daily nicotine dose received by a tobacco user and are therefore considered a valuable biomarker (Hecht et al. 2010).

3.1.2.1 Biomarkers of Nicotine Exposure in Snus Users

This section describes nicotine biomarkers after traditional Swedish snus use as analyzed in a clinical or interventional study, or in cross-sectional, population-based studies. Nicotine biomarker of exposure data as measured in these studies are provided in Table A III-2.

Nicotine and Cotinine in Plasma/Serum

In Swedish studies of regular snus users (N=21-92) with an average daily snus consumption between 21 and 32 g[80] mean nicotine plasma levels ranged from 3.2 to 15.5 ng/mL, but the time

[80] This indicates a main use of loose snus based on average loose snus of 29 g/day and average pouched snus use of 12 g/day (Digard et al. 2009)

of blood sampling was not specified (Bolinder et al. 1997b; Bolinder 1997; Bolinder et al. 1997a; Bolinder and de Faire 1998; Eliasson et al. 1991; Eliasson et al. 1995). In these same studies of Swedish firemen and individuals from the general Swedish population, the mean cotinine plasma levels were between 326 and 359 ng/mL. In one study of 27 regular snus users with an average snus consumption of 22 g/d where blood was sampled immediately after a use, the mean nicotine plasma level was 36.7 ng/mL (standard deviation (SD), 14.3), while the mean plasma cotinine level was 399.3 ng/mL (SD, 160.5) (Holm et al. 1992). In a study of 11 snus users in a Norwegian industrial worker cohort with an average snus consumption of 11 g/d (range, 0.3-29 g/d), the respective geometric mean (GM) serum cotinine level was 137 ng/mL (range, not detected-1312 ng/mL) (Ellingsen et al. 2009).

A study in Serbia was conducted to test the efficacy of Swedish snus as an aid to smoking cessation (Joksic et al. 2011). Smokers willing to quit (N= 319; average cigarette consumption 26-28 cigs/day) were offered snus or placebo and by the end of the study at week 48, the target date for complete smoking cessation, self-reported cigarette consumption had decreased to less than 10 cigarettes/day in both groups. The serum cotinine levels in snus and placebo users were decreased to 66.1 and 69.1 ng/mL, respectively, approximately 68% of baseline levels. The mean exhaled breath carbon monoxide levels were also similar (approximately 12 ppm) in both groups, and since cut-off values to define abstinence range from 4 to 10 ppm (Raiff et al. 2010), these levels reflect the continuing nicotine intake from smoking.

Nicotine and Cotinine Levels in Urine

In the available studies, biomarkers of nicotine exposure are presented in four ways: nicotine itself, cotinine, total cotinine, and nicotine equivalents.

In the study by Ellingsen and colleagues (2009), urinary nicotine and cotinine were also analyzed and were 26 (0.4-560) and 159 (8.2-428) μg/mmol creatinine; if corrected for the median urinary creatinine in men[81], the corresponding nicotine and cotinine concentrations were approximately 348 and 1908 ng/mL, respectively. In a Swedish study of snus users who consumed an average of 25 g/day, the mean urinary cotinine level was 1210 ng/mL (Wennmalm et al. 1991).

Two independent studies where STP users were switched from their own brands of STP to *General* snus were conducted in which urinary cotinine as well as total cotinine levels were measured (Gray et al. 2008; Hatsukami et al. 2004).

In study 2 by Gray and colleagues (2008), a Latin Square design was used to test two different potentially reduced exposure products (PREPs), one of which was loose *General* snus, in a group of 19 regular STP users. Each treatment period was five days, with wash-out periods

[81] 26 μg/mmol creatinine x 12 mmol creatinine/L = 348 μg/L = 348 ng/mL, with a median urinary creatinine concentration of 12 mmol creatinine per L urine in men (Cocker et al. 2011)

over the weekends during which participants were allowed to use their own STPs. Each participant completed four conditions (placebo, own STP, 2 PREPs). Users were given 45 g of snus on each of days 1-4 to use *ad libitum* over the next 24 hours. On day 5 of the switch to snus, the average urinary cotinine level was with approximately 1000 ng/mL not different from day 1.

In the study by Hatsukami and colleagues (2004), STP users were followed for four weeks after switching to reduced exposure products or medicinal nicotine patches, with 19 STP users switching to snus. At week 4 after the switch to snus, the mean snus consumption was 3.7 tins/week (approximately 13 g/day[82]) and the mean urinary total cotinine (cotinine and its glucuronide) level was 5926 ng/mL (range, 4415-7437 ng/mL). This was similar to those measured at week 1, although there was a significant "overall visit effect", because the mean cotinine level was decreased at the week-2 visit and increased again at the week-4 visit.

Nicotine Equivalents in Urine and Cotinine in Saliva

Two studies in Swedish snus users measured nicotine equivalents (nicotine and seven metabolites) in urine, as well as cotinine levels in saliva (Andersson et al. 1994; Andersson et al. 1995), and one study measured only saliva cotinine (Post et al. 2005).

The first study compared nicotine extraction (see Section 2.3.3) and uptake in 23 portion-bag users and 22 loose snus users (Andersson et al. 1994). Portion bags had a slightly higher nicotine content, but lower pH than loose snus (pH 7.9-8.2 vs. 8.5-8.6, respectively) and users consumed on average 14.4 g/d of portion bags versus 20.8 g/d of loose snus. The tobacco was kept in the mouth for about the same number of hours a day by both groups (averages, 12.3-13.1 hrs). The degree of extraction from pouched snus was significantly lower than from loose snus. Together with the lower overall daily consumption of pouched snus, the total nicotine extracted per 24 hours from portion bags was approximately half of what was extracted from loose snus. Despite these differences in extraction and consumption however, there was no statistically significant difference between the portion-bag and loose snus users for either the systemic nicotine dose, measured as nicotine equivalents in urine (34.5 ±23.1 mg/24 hrs and 35.6 ±18.6 mg/24 hrs, respectively), or saliva cotinine concentrations (342.9 ±180.8 and 326.6 ±135.6 ng/mL, respectively). The authors speculated that "This 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". In addition, Andersson and colleagues (1994) also evaluated changes in the oral mucosa of the subjects (see Section 5.2.2 for details).

The second study by the same researchers, conducted to evaluate short- and long-term effects of switching to a reduced-nicotine snus, compared biomarker levels in 24 snus users that

[82] One tin of General snus is assumed to contain 24 1-g portions. 3.7 tins/7 d x 24 g/tin /7 d = 13 g/d

switched for 10 weeks from their regular high nicotine content brand A to a brand B with approximately half the nicotine content (Andersson et al. 1995). Brand B snus also had a lower product pH (pH 7.9-8.2 vs. 8.2-8.5, respectively; Study 1). Both were pouched products. In a second part of the study (Study 2), 18 regular brand B users were investigated. While tobacco consumption increased slightly in users that switched from brand A to B from 16.4 g/day before the switch to 18.6 g/day at the end of the study, urinary nicotine equivalents and saliva cotinine decreased to similar levels as those measured in the regular brand B users, even though the consumption in brand B users was approximately 3 g lower (15-15.2 g/day). 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".

Despite their increased intake, biomarkers levels of internal nicotine exposure decreased in brand A users to approximately half of baseline to similar levels as those measured in regular brand B users: Nicotine equivalents and saliva cotinine level averages were 25.2 mg/24 hrs (range, 4-65 mg/24 hrs) and 336 ng/mL (range, 70.4-731 ng/ml), respectively, the week before the switch compared to 14.4 mg/24 hrs and 153 ng/mL, respectively, at the end of the study. By comparison, the average levels in regular brand B users was 14.3 mg/24 hrs (range, 2-41 mg/24 hrs) and 159 ng/mL (range, 31-335 ng/ml), respectively. Andersson and colleagues (1995) also investigated the subjects for oral mucosal soft tissue changes (see Section 5.2.2 for details).

Saliva cotinine levels were also measured in adolescent Swedish tobacco users (Post et al. 2005). In this cross-sectional study, conducted to assess the reliability of self-reported tobacco use based on internal biomarkers of nicotine exposure, the median[83] cotinine level measured in 28 snus users was approximately 80 ng/mL with a mean snus consumption of 31 pinches/week.

Summary of Nicotine Biomarkers Identified in Snus Users

A number of studies in regular snus users show that mean or median cotinine levels in plasma or serum range from 137 to 399 µg/L depending on the amount of snus consumed (average 11-32 g/day). In other studies, that included adolescent snus users (who consumed less snus), average saliva levels ranged from 80 to 343 ng/mL. Fewer studies in regular snus users measured urinary biomarkers of nicotine. Results were as follows: nicotine itself (based on one study), 29 µg/mmol creatinine; cotinine (as measured, based on two studies), approximately 1000 to 1210 µg/L; total cotinine (based on one study), 5926 µg/L; and nicotine equivalents (based on two studies), 14.3 to 35.6 mg/24 hrs.

[83] Data was provided in a box-plot. Although the legend implies that means were indicated, it appears to be a typo and is likely a median.

3.2 Biomarkers of Exposure to Trace Level Components

As noted in the Introduction, the available scientific literature provides information for some biomarkers of exposure to TSNAs, cadmium, and selenium in snus users.

3.2.1 *N*-Nitroso Compounds: TSNAs Biomarkers

Biomarkers of TSNAs are the main biomarkers measured and reported in the published literature; there is little information on biomarkers of other non-tobacco specific *N*-nitroso compounds for tobacco users. Formation, significance and limitations of the main TSNA biomarkers are briefly discussed below.

TSNAs and their metabolites have been determined in various human bodily fluids, including saliva, blood, and urine, as well as in toenails (IARC 2007; Shah and Karnes 2010). Furthermore, DNA adducts in leukocytes, lung and liver tissue as well as hemoglobin adducts have been measured in humans (Hecht 2008; Nilsson 2011). To date, the most commonly measured TSNA biomarkers are urinary metabolites of NNK.

3.2.1.1 Urinary NNAL and Total NNAL (Biomarkers of NNK)

In humans, primates and rodents, NNK is converted largely to NNAL. Subsequent major metabolic pathways are the same for NNK and NNAL. Both compounds can be activated via cytochrome P450-catalyzed α-hydroxylation, a pathway considered to be major with respect to NNK's ultimate carcinogenic potential. Recent studies suggest that NNK and NNAL levels are not directly impacted by CYP2A6 enzyme polymorphisms (Zhu et al. 2013a). NNAL, but not NNK, can be detoxified via glucuronidation (Hecht 2008; Stepanov et al. 2008b). NNAL and its glucuronides (N- and O- isomers: NNAL-*N*-Gluc and NNAL-*O*-Gluc), which together are referred to as "total NNAL" can be measured in urine, while unchanged NNK has not been detected in urine (Hecht 2008; Stepanov et al. 2008b). Urinary NNAL and its glucuronides are the most frequently quantified biomarkers of exposure to NNK (Shah and Karnes 2010).

Note that quantification of total NNAL does reflect the activation pathway. The activation pathway is thought to be the major route of NNK metabolism in both smokers and smokeless tobacco users based on experiments by Hecht and colleagues: To investigate the extent of α-hydroxylation, urinary metabolites attributed to different NNK metabolization pathways were quantified under regular use conditions for smokers smoking cigarettes spiked with [pyridine-D_4]NNK (Stepanov et al. 2008b). In this study, the metabolites from NNK α-hydroxylation accounted for 86% and total NNAL accounted for 12% of all identified urinary compounds in smokers. To determine the fraction of the NNK dose excreted as total NNAL in STP users, the amount of NNK extracted from tobacco after a single administration of a US STP after three weeks of abstinence was compared with the amount of excreted total NNAL (Hecht et al. 2008b). An average of 59% NNK was extracted from the moist snuff product and the amount of urinary total NNAL was calculated to be 14 to 17% of the NNK dose. Considering the very different study designs of these two studies, it appears difficult to conclude the extent of potential differences in NNK metabolism between STP users and smokers and how those might impact the percentage of NNK dose reflected in urinary total NNAL. Citing these studies described above, Hecht and colleagues (2010) stated that total NNAL captures approximately 12 to 17% of the NNK dose.

Because of the limitations listed above, Hecht and colleagues (2010) cautioned that a decrease in urinary total NNAL could also hypothetically mean that activation increases. This limitation should be considered when evaluating the meaning of a decrease in urinary NNAL levels for risk, although in general a decrease in exposure to NNK is likely. An ideal risk marker would be related to pathways that provide information about the activation to ultimately critical reactive metabolites or reaction products, such as adducts (Shah and Karnes 2010). However, urinary metabolites from the α-hydroxylation pathway are not specific to NNK and the same compounds can also be formed with nicotine.

Despite these limitations, in studies of smokers, urinary levels of total NNAL were strongly associated with risk for lung cancer (Church et al. 2009, Yuan et al. 2009, both cited in USDHHS 2010). Further, the ratio of NNAL glucuronide to free NNAL as a marker of NNK detoxification has been suggested to be correlated to an individual's risk of developing some tobacco-smoke induced cancers (Chung et al. 2011; Derby et al. 2009). With respect to head and neck cancer, a new matched case-control study with smokers did not observe increased urinary NNAL levels in cases, but levels of NNN and 1-hydroxypyrene, a metabolite of pyrene, were significantly increased (Khariwala et al. 2012). The same has not been established for any potential cancer risks in STP users (of any kind). In his recent review and analysis of published data of DNA and hemoglobin adducts in human and animal tissues, Nilsson (2011) concluded that "[w]hereas smoking and use of snuff [Swedish snus] result in similar exposures to the systemic carcinogens NNK and NNN, only smoking is associated with human lung cancer. This observation gives further support to the notion that TSNA probably play a minor role in the induction of smoking-related cancers." For more details on this study see Appendix III, Section A III 3.2.1.3.

In general, urinary NNAL levels were well correlated with serum or urinary cotinine levels, numbers of cigarettes smoked, or environmental tobacco smoke exposure in non-smokers (as reviewed in CDC 2012[84]). However, in both smokers and STP users, it has been observed that urinary total NNAL levels do not increase linearly at higher nicotine intakes that are measured by urinary cotinine (Hecht et al. 2008a; Lubin et al. 2007). The reason for these findings has not yet been established and the authors hypothesized that alternate pathways of NNK metabolism could be induced at higher nicotine and other tobacco constituents doses (Hecht et al. 2008a), but this is not known, and no biomarker measures for these possible alternate pathways are currently available.

Unlike cotinine, NNAL and its glucuronides are much more slowly eliminated in urine and hence total NNAL has a long terminal half-life: averages for smokers have been reported to be between 10 to 18 days (Carmella et al. 2009; Goniewicz et al. 2009); in studies that compared smokers and STP users, averages were 45 and 26 days, respectively, but this difference was

[84] CDC 2012. http://www.cdc.gov/biomonitoring/NNAL_BiomonitoringSummary.html, accessed April 2013.

not statistically significant due to large interindividual variations (Hecht 1999; Hecht 2002). It was hypothesized that depending on the study settings, exposure to environmental tobacco smoke might account for the large differences in reported half-lives (Goniewicz et al. 2009). The same authors also speculated that the half-life of NNAL in smokeless tobacco users might be similar to that in smokers (Goniewicz et al. 2009). NNAL could still be detected in urine 6 to 12 weeks after smoking cessation. Based on these findings, Goniewicz et al. (2009) concluded that in "testing of novel [tobacco] products, it will take 6-12 weeks for NNAL levels to reach a new steady state."

Some differences between oral and inhalation exposure have been identified for parts of the NNK metabolism, e.g., *N*-glucuronidation was significantly greater in smokers than in STP users, however, there was no significant difference in the percentage of free NNAL to total NNAL (41.4% vs. 36.6%, respectively) (Carmella et al. 2002). No studies were identified that provided information to establish how potential differences in NNK absorption, metabolism, distribution and excretion for the different routes of uptakes in humans may impact interpretation of urinary NNAL levels with respect to cancer risk.

Based on studies of predominantly US STP users, the CDC stated that the similar or slightly higher total NNAL levels in users of STPs compared to active smokers are "indicative of the higher levels of TSNA and NNK that may be present in smokeless tobacco" (CDC 2012). It should be noted that NNK concentrations in both conventional STPs as well as traditional Swedish snus have been declining over the past decades (see Section 2.3.6.1), although concentrations were formerly consistently higher in US conventional STPs than those detected in Swedish snus, with only few exceptions (Nilsson 2011).

3.2.1.2 Urinary NNN and Total NNN

Similar to NNK, NNN can be α-hydroxylated, a reaction thought to be primarily catalyzed by CYP2A6 (as reviewed in Zhu et al. 2013a). Different from NNK, both NNN itself and its glucuronides can be detected in urine and are often measured as total NNN. Total NNN is estimated to reflect approximately 1% of the NNN dose taken in (as reviewed in Hecht et al. 2010).

Higher urinary NNN levels in smokers have been associated with increased esophageal and head and neck cancer risk (as reviewed in Hecht et al. 2010; Khariwala et al. 2012; Yuan et al. 2011). Khariwala and colleagues (2012) also reported higher risk of head and neck cancers associated with 1-hydroxypyrene, a metabolite of pyrene. It should be noted however, that certain polymorphisms impact metabolism of NNN, and may therefore contribute to differences in NNN levels. For example, higher urinary NNN levels were also observed in smokers with lower CYP2A6 activity, indicating lower activation via the α-hydroxylation pathway (Zhu et al. 2013a). NNN has also been detected in some users of nicotine replacement therapy demonstrating its endogenous formation (Stepanov et al. 2009b; Stepanov et al. 2009a). In an *in vitro* study with saliva, researchers from the same group recently showed that NNN could be formed in detectable amounts from nornicotine without any addition of other substances, while incubation of saliva with nicotine and sodium nitrite resulted in only trace amounts of NNN (Knezevich et al. 2013). This indicates that there is a potential for endogenous formation of NNN from nornicotine that is already present in NRT products or metabolized from nicotine.

However to date, the extent of NNN's endogenous formation in other tobacco users has not been thoroughly investigated.

3.2.1.3 Adducts of NNK and NNN

As described above, NNK and NNN can be activated via cytochrome P450-catalyzed α-hydroxylation and form DNA and hemoglobin adducts, such as 7-methylguanine, O^6-methylguanine, O^4-methylthymidine and/or pyridyloxobutyl (POB; also called HPB-releasing[85]) adducts (Nilsson 2011). The activation pathway is considered to be important with respect to the ultimate carcinogenic potential of NNK. Studies of DNA and hemoglobin adducts of NNK and NNN were recently reviewed (Nilsson 2011). Similar to what was observed for HPB-releasing hemoglobin adducts, a new study did not find any correlation between HPB-releasing DNA adducts in oral cells of smokers with urinary total NNN or total NNAL (Stepanov et al. 2013).

3.2.1.4 Biomarkers of TSNA Exposure Identified in Snus Users

Studies of biomarkers of TSNA exposure from traditional Swedish snus are limited. Only two studies were conducted with regular snus users (Heling et al. 2008; Österdahl and Slorach 1988), while two others investigated changes in US STP users after they switched to snus (Gray et al. 2008; Hatsukami et al. 2004). TSNA biomarker of exposure data as measured in these studies is provided in Table A III-3.

Urinary Total NNAL in Snus Users

No studies of urinary NNAL or total NNAL measured in regular users of traditional Swedish snus were identified. Two clinical studies were available in which total NNAL was measured in conventional STP users who were switched to potentially reduced exposure products (PREPs), including traditional Swedish snus (*General*) (Gray et al. 2008; Hatsukami et al. 2004).

In the study by Hatsukami and colleagues (2004), 41 adult male conventional STP users were switched from traditional STPs to *General* snus. These researchers reported a decline in total NNAL levels by more than half in most users after two weeks with not much additional decline after four weeks (1.5 and 1.4 pmol/mg creatinine, respectively) compared to baseline levels (3.2 pmol/mg creatinine), measured during the two weeks prior to the switch. The average consumption in tins per week increased slightly during snus use compared to baseline STP consumption, and at week 4 the urinary total cotinine levels were similar to those at baseline. The week-4 snus consumption was 3.7 tins per week (~12.7 g/day). It should be noted that tins of pouched STPs often contain less total tobacco than those with loose STPs, e.g., one tin of pouched *General* snus contains 24 1-g portions while one tin of loose *General* snus contains 45 g. The authors concluded that, "[u]sing Swedish smokeless tobacco products marketed in

[85] Unstable POB adducts can be measured as released 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB).

the United States may not only reduce carcinogen exposure but also may decrease cancer risk."

In the study by Gray and colleagues (2008), described previously, a Latin Square design was used to test four different potentially reduced exposure products (PREPs) in a group of 19 regular STP users for five days each, with wash-out periods over the weekends during which participants were allowed to use their own STPs. Each participant completed four conditions, one of which was snus use. Users were given 45 g of snus on each of days 1 to 4 to use *ad libitum*. Gray and colleagues (2008) did not observe a significant difference in total NNAL levels of conventional STP users five days after switching to loose *General* snus compared to levels on day 1 (~600 pg/mL versus ~700 pg/mL, respectively). Limitations of this study, compared to that of Hatsukami and colleagues (2004), include a smaller sample size (only 19 STP users were investigated) and the shorter duration of snus use (lasting only 5 days). Given the long half-life of NNAL (10-45 days), it is possible that the duration of use (5 days) was insufficient to reveal differences in product NNK concentrations. Another limitation was that actual snus consumption was not reported in this study. Cotinine levels on day 5 were comparable to those on day 1, similar to the unchanged NNAL levels.

Urinary Total NNN in Snus Users

No studies of urinary NNN or total NNN measured in users of traditional Swedish snus were identified.

TSNAs in Saliva of Snus Users

One study conducted by researchers of the Swedish National Food Administration investigated the TSNA levels in the saliva of four habitual snuff dippers (3 pouched snus users, 1 loose snus user) before, during and after 30-minute use of a single dose of snus (Österdahl and Slorach 1988). Saliva samples were taken on two different days. The TSNA concentrations as well as the extraction of the TSNAs from the pouched products were determined by analyzing the snus before and after consumption (see Section 2.3.6.1). TSNA levels in saliva samples taken before and 20 minutes after the end of use were undetectable or trace amounts, which is in agreement with other studies that analyzed saliva samples of moist snuff users and smokers after the product was removed from the mouth (as reviewed in Caraway and Chen 2012). Saliva levels in samples taken during the dipping process varied strongly between users and experimental day: NNK, NNN, and NAT levels ranged from not detected to 16 ng/g, 3 to 140 ng/g, and trace levels to 85 ng/g saliva, respectively. The average total TSNA concentration during dipping was calculated to be between 15 to 125 ng/g saliva. Loose snus use resulted in higher maximum saliva levels compared to pouched snus use. The investigators calculated that with a saliva production of approximately 60 mL per hour the snus users were exposed to 0.9 to 7.5 µg TSNAs per hour of snuff dipping. It should be noted that the TSNA concentrations in the snus products used in this study were considerably higher than TSNA concentrations detected in snus in recent years (see Section 2.3.6.1 for more details on how TSNA concentrations in snus have decreased over time).

TSNA Adducts in Snus Users

Results from analyses of adduct levels extrapolated based on animal data and estimated intake of Swedish snus are discussed in Appendix III, Section A III 3.2.1.4 (Nilsson 2011). Nilsson

(2011) also cited a study abstract that reported POB-DNA adduct levels detected in oral mucosa samples of snus users (Richter et al. 2009b, as cited in Nilsson 2011). Another abstract could also be located that appears to refer to the same study or samples (Heling et al. 2008), but no full publication was located. POB-DNA adducts levels detected in oral mucosa of 33 Swedish snus users were 5280 ±372 adducts/10^9 total normal nucleotides (TN) (Richter et al. 2009b, as cited in Nilsson 2011) or 17.61 ±7.1 pmol HPB/mg DNA (Heling et al. 2008). These adduct levels were approximately nine times higher than those detected in tissue samples of 45 nonsmokers (600 ±102 adducts/10^9 TN (Richter et al. 2009b, as cited in Nilsson 2011) or 2.00 ±2.31.1 pmol HPB/mg DNA (Heling et al. 2008). POB-DNA adducts levels were also reported for smokers (Appendix III, Section A III 3.2.1.3). Considering this comparison, and the results from epidemiological studies, Nilsson (2011) concluded that the POB-DNA adduct study "results cast doubt on the involvement of POB-DNA adducts in causing oral cancer, especially from Swedish "snuff" […]".

Summary of TSNA Biomarkers Identified in Snus Users

In summary, there were four studies investigating TSNA biomarkers in regular snus users identified. Of those, one older publication from 1988 measured TSNA levels in saliva during snus use. TSNA concentrations in the snus products used were considerably higher than those reported in recent analyses of Swedish snus. Urinary total NNAL was measured in two clinical studies where conventional US STP users were switched to snus use, however only one study had an observation period of sufficient duration to examine for and detect differences in levels before and after the switch (Hatsukami et al. 2004). In this study, total NNAL levels decreased significantly (to half the concentration measured at baseline) by week 4 of *General* snus use. It is not known if the study was of sufficient duration (6-12 weeks) to reach NNAL steady-state levels after the switch (Goniewicz et al. 2009). Importantly, urinary total cotinine levels in this study did not change significantly, indicating the decreased toxicant exposure could not be explained by a decrease of product use (nicotine intake). No studies measuring biomarkers of NNN in traditional Swedish snus users were identified. POB-DNA adducts were significantly higher in oral mucosa of Swedish snus based on a study abstract; however, the importance of these adducts in oral cancer development has been questioned.

3.2.2 PAHs Biomarkers

No studies were identified in which biomarkers of exposure to PAHs were measured in snus users. More information on biomarkers of exposure to PAHs is provided in Appendix III, Section A III 3.2.2, where available data for use of new products marketed as snus and US STPs in comparison with smoking is discussed.

3.2.3 Aldehydes Biomarkers

No studies were identified in which biomarkers of exposure to aldehydes were measured in users of snus, new products marketed as snus, or STPs.

3.2.4 Metals and Metalloids Biomarkers

No studies that analyzed arsenic, beryllium, chromium, cobalt, lead, mercury, nickel, and barium levels in blood or urine in snus users were identified. More information on biomarkers of exposure to metals is provided in Appendix III, Section A III 3.2.4, where available data for use

of snus, new products marketed as snus and/or US STPs in comparison with smoking is discussed. The data is provided in Table A III-4.

3.2.4.1 Biomarkers of Exposure to Cadmium

Due to its long half-life in the body, cadmium levels in the blood reflect both recent as well as cumulative exposures, whereas cadmium levels in the urine reflect both cumulative exposure and the concentration of cadmium in the kidney (CDC 2009). Urinary levels thus reflect primarily total body burden of cadmium, and can be used as a marker of long-term exposure (ATSDR Draft 2008, Nordberg et al. 2007, as cited in Sand and Becker 2012).

Smoking is a significant source of cadmium exposure, and smokers have been shown to have increased biomarker levels of cadmium (ATSDR 2012). A recent analysis of National Health and Nutrition Examination Survey (NHANES) data concluded that urinary cadmium concentrations decreased markedly between 1988 and 2008 and the authors attributed this to declining smoking rates and changes in exposure to tobacco smoke (Tellez-Plaza et al. 2012). In this study, the geometric mean urinary cadmium concentrations declined for both smokers and nonsmokers, but the ratio between current smokers and never-smokers stayed approximately the same over the years. The concentrations in smokers were approximately twice as high as those in never-smokers. It should be noted that cadmium uptake via inhalation is significantly higher than via the oral route (ATSDR 2012).

Cadmium blood levels have been reported to be in the range of 0.4 to 1 μg/L in nonsmokers and the unadjusted geometric mean in non-tobacco users based on NHANES data from 1999-2008 was 0.30 μg/L (as reviewed in IARC 2012b; Naufal et al. 2011). The geometric mean in the US population 20 years and older in 2003-2004 was reported to be 0.378 μg/L (CDC 2009).

Cadmium levels in 24-hr urine of non-smokers were 1.34-8.04 nmol (0.15-0.904 μg) (Institute of Medicine 2012). The unadjusted geometric mean levels in urine from non-tobacco users based on NHANES data from 1999-2008 was 0.24 μg/g creatinine (Naufal et al. 2011). Never-smokers in 2003-2008 were reported to have geometric mean urinary cadmium levels of 0.19 μg/g creatinine (Tellez-Plaza et al. 2012). The geometric means in the US population 20 years and older in 2003-2004 were 0.260 μg/L and corrected for creatinine was 0.268 μg/g creatinine (CDC 2009).

Cadmium Biomarkers in Users of Snus

Two studies have investigated cadmium levels in snus users (Table A III-4). Ellingsen and colleagues (2009) measured blood cadmium levels in 11 Norwegian snuff users from a former chlor-alkali worker cohort. Their levels were similar to those of 49 non-smoking controls (mean, 2.9 nmol/L or 0.33 μg/L versus 3.3 nmol/L or 0.37 μg/L, respectively. The control cadmium blood levels in this study are in the range of those reported in the US population (CDC 2009).

In a Swedish study that measured time trends in burdens of several metals in the population in Northern Sweden, the authors noted that the use of moist snuff had no influence on cadmium concentrations in erythrocytes among never-smoking men: 28 snuff users had median erythrocyte cadmium concentrations of 0.24 μg/L versus 0.26 μg/L as measured in 110 non-

smoking non-snuff users (Wennberg et al. 2006). While this study also analyzed lead and mercury erythrocyte concentrations, no distinctions for snuff users were reported.

In summary, levels of cadmium biomarkers in snus users were similar to those detected in non-tobacco users.

3.2.4.2 Biomarkers of Exposure to Selenium

Blood and urinary levels are most often used to detect recent exposures to high levels of selenium (ATSDR 2003). The geometric mean serum selenium concentration reported for the adult US population ages 20 to 59 years, based on NHANES data from 1988-1994, was 124.17 μg/L (ATSDR 2003). Further, erythrocyte and blood glutathione peroxidase (GPX, a seleno-protein that protects from oxidative damage) activity is thought to be a biomarker for selenium deficiency, but not overexposure (ATSDR 2003). GPX activity has been shown to be decreased in smokers. While the precise mechanism of this effect is unknown it has been speculated that inflammatory processes caused by smoking might lead to an increased need for antioxidant protection, including by the seleno-protein GPX (ATSDR 2003, as cited in Ellingsen et al. 2009).

Selenium Biomarkers in Users of Snus

In the Norwegian study, mean blood and serum selenium levels in 11 snuff users from a former chlor-alkali worker cohort were similar to those of 49 nonsmoking controls: 1.50 μmol/L in blood or 1.55 μmol/L in serum (122.4 μg/L in serum) versus 1.52 μmol/L in blood or 1.54 μmol/L in serum (121.6 μg/L in serum) (Ellingsen et al. 2009). The control selenium levels in this study were in the range of those reported for the US population (ATSDR 2003). Further, the geometric mean of selenium serum levels in non-users of tobacco, reported for an NHANES population-based sample in 1999-2008 was in the same range, although slightly higher (unadjusted geometric mean, 137 μg/L) (Naufal et al. 2011).

Mean GPX activity in the snuff users was 140 U/L (range, 106-182 U/L) and not statistically significantly different from non-smoking controls (146 U/L (range, 105-203 U/L)) (Ellingsen et al. 2009).

In summary, levels of selenium biomarkers in snus users were similar to those detected in non-tobacco users.

3.2.5 Radionuclides Biomarkers

No studies were identified in which biomarkers of exposure to radionuclides were measured in users of snus, new products marketed as snus, or other STPs.

3.2.6 Biomarkers of Other Trace Levels Components

No studies were identified in which biomarkers of exposure to other trace level components were measured in snus users. More information on biomarkers of exposure to other trace level components is provided in Appendix III, Section A III 3.2.6, where available data for new products marketed as snus and/or US STPs in comparison with smoking is discussed.

3.3 Summary and Discussion of Biomarkers of Exposure to Snus

Biomarkers of exposure may be used to assess the actual internal dose of a tobacco component to which a tobacco user might be exposed. While limitations to the available biomarkers of exposure exist, they can be used to supplement information from product analyses as they reflect total exposure, bypassing differences in routes of exposure and product use behavior. In addition, biomarker levels on a population basis may give an indication of general trends in internal exposure to certain components of a well-characterized product. With respect to harm reduction, conclusions from these studies should be interpreted carefully and in the context of additional data from clinical and/or epidemiological studies.

A panel of biomarkers of exposure to components in tobacco products has been recently proposed for the use in product regulations. Many biomarkers of exposure are less relevant for non-combusted tobacco products such as snus; however, the panel does include the potentially relevant biomarkers of exposure to nicotine, TSNAs, PAHs, aldehydes, cadmium, and acrylamide.

To date, published studies are available that have investigated biomarkers of exposure to nicotine, TSNAs, cadmium, and selenium in regular users of traditional Swedish snus. .

Commonly measured biomarkers of nicotine exposure are cotinine in plasma or serum. However, their levels may be impacted by the route of exposure, i.e., first pass metabolism of nicotine to cotinine via the oral route may result in higher blood concentrations of cotinine that do not necessarily reflect increased exposure to the parent compound, nicotine. This metabolic pathway does not occur following exposure to nicotine via the inhalation route. Total nicotine equivalents in urine are considered to better represent the total nicotine dose absorbed. Information from nicotine pharmacokinetic parameters is relevant for nicotine delivery, total dose, and abuse liability assessments. The time to maximum plasma nicotine concentrations in snus users appears to be dependent on the usage time, but not on nicotine content or portion size. On the other hand, C_{max} and AUC appear mostly dependent on total nicotine content (per pouch or portion size) as well as pH of the product. Whether the snus was loose or pouched, had no influence on these parameters.

A number of studies in regular snus users show that mean or median cotinine levels in plasma or serum range from 137 to 399 ng/mL depending on the amount of snus consumed (average 11-32 g/day). In the saliva, average levels ranged from 80 to 343 ng/mL. Urinary biomarkers of nicotine measured in regular users of snus were as follows: for nicotine itself, 29 µg/mmol creatinine; for cotinine, approximately 1000-1210 µg/L; for total cotinine, 5926 µg/L; and for nicotine equivalents, 14.3-35.6 mg/24 hrs.

TSNAs and their metabolites have been determined in various human bodily fluids, including saliva, blood, and urine, as well as in toenails. Urinary NNAL is the most commonly measured biomarker of TSNA exposure, and is considered to reflect 12 to 17% of the NNK dose.

Four studies of TSNA biomarkers in users of Swedish snus were identified. Of those, one publication from 1988 measured TSNA levels in saliva during snus use; snus in the 1980s contained considerably higher TSNA concentrations than more contemporary snus products.

More recently, urinary total NNAL was measured in users of conventional US STPs that were switched to *General* snus use. Of the two clinical studies available, only one appears to have a sufficient duration to examine for and detect differences in levels before and after the switch. In this study, total NNAL levels decreased significantly (to half the concentration measured at baseline) by week 4. Importantly, urinary total cotinine levels in this study did not change significantly, indicating the decreased toxicant exposure could not be explained by a decrease in tobacco intake and mean product use was similar to that reported for regular snus users. No studies measuring biomarkers of NNN in snus users were identified. POB-DNA adducts were significantly increased in oral mucosa of Swedish snus based on information provided in a study abstract; however, the importance of these adducts in oral cancer development has been questioned.

With respect to the available studies of biomarkers of metals/metalloids, both levels of cadmium and selenium biomarkers in regular users of traditional Swedish snus were similar to those detected in non-tobacco users.

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4 Non-Clinical Toxicological Studies with Snus

The many and diverse components that have been detected in STPs (Section 2), have the potential to cause or contribute to a variety of adverse health effects if exposure to them is sufficiently high. The components most frequently analyzed in STPs, including Swedish snus, are TSNAs and nicotine. TSNA concentrations in Swedish snus have declined steadily since the 1980s, but together with nicotine they are the main known components that snus users are exposed to at higher levels than non-tobacco users.

The TSNAs NNK and NNN are considered human carcinogens based on studies in experimental animals and mechanistic evidence stemming from STP and/or tobacco users, including TSNA uptake, metabolic activation and adduct formation, as well as oral tumor induction (IARC 2007; IARC 2012d). Studies in the scientific literature have found associations between nicotine with a variety of different health effects, including cardiovascular effects, impairment of wound healing, diabetes, developmental and reproductive effects, neurological effects, and cancer (while nicotine itself is not a carcinogen, it may affect several mechanisms that are involved in tumor promotion), effects on the immune system, acute renal ischemic injury, and gastrointestinal tract effects (as reviewed in Benowitz 2009).

The purpose of *in vitro* and animal toxicity studies are to observe and inform the identity and continuum of adverse events, from molecular precursor to frank phenotypic toxicity, in a setting controlling for confounding. The limitations of extrapolating those data to human health risks are well known and include correcting for pharmacokinetic and dynamic differences between species (for animal studies) and interpreting molecular effects in a cell or tissue system that occur in isolation of the rest of the organism (for *in vitro* studies). Nevertheless, the animal and *in vitro* toxicity data provide information on adverse events and their underlying mechanisms that may be compared to the human experience and, with careful consideration of the data limitations, inform on accurately attributing weight-of-evidence to epidemiology and clinical studies and case reports.

In the past, toxicological studies of STPs were focused mainly on endpoints related to cancer, while other endpoints were seldom investigated. A recent review of the toxicology of smokeless tobacco emphasized the considerable gaps in knowledge of immune-related toxicity, and cardiovascular and reproductive systems effects (Willis et al. 2012).

With respect to the carcinogenicity of STPs, IARC (2007) concluded that “There is *sufficient evidence* in experimental animals for the carcinogenicity of moist snuff”. In 2012, IARC concluded “There is *sufficient evidence* in experimental animals for the carcinogenicity of smokeless tobacco” (IARC 2012d). IARC cancer classifications address the inherent hazard of an agent, but do not speak at all to likelihood of disease development in humans at plausible exposure. Further, the IARC assessment for STPs did not discriminate between snus and other moist snuff products or STPs. As described in Section 2, snus is a particular type of oral moist snuff product traditionally used and manufactured in Sweden and its production method differs from the US-type oral moist snuff products resulting in unique product characteristics.

In the following sections, the available studies of the toxicity of Swedish snus in cell culture and experimental animals are discussed. Most of these studies include endpoints to detect potential

carcinogenicity, in particular concerning oral tissue. Details of study designs and results are provided in Appendix IV, Tables A and B.

4.1 In Vitro Toxicity and Genotoxicity Assays of Swedish Snus

In vitro toxicology methods were originally developed to provide a tool for screening chemicals for their potential to cause disease and to develop and inform hypotheses about potential modes of action in particular organ or cell systems (as reviewed in Johnson et al. 2009). Most assays were developed to detect potential toxic signals with high sensitivity but low specificity. They also test chemicals in isolation of the other tissues, removing the effects of toxicokinetics, immune responses, and adaptive intercellular interactions present in the intact organism. For these reasons, results from these studies alone generally do not allow conclusions to be drawn about toxic potency of test substances in humans (as reviewed in Johnson et al. 2009).

Johnson and colleagues (2009) recently reviewed *in vitro* studies used to investigate tobacco products. In addition to the above described limitations, they noted: "Over the years, a panoply of tests have been used to asses tobacco toxicants; however, the interpretation of the data generated is not trivial for any tobacco product and particularly if the goal is to compare modified products. 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, […]."

With respect to STPs, Johnson and colleagues (2009) noted that "smokeless tobacco products perform poorly in these assays". Reasons for inconsistent responses seen with STPs in the available *in vitro* assays include disturbances of the test systems due to humectants and salt content in the products. Johnson and colleagues (2009) further stated "Although reliable as a screening tool for qualitative assessments, the available *in vitro* assays have been poorly validated for quantitative comparisons of different tobacco products". In particular, there is little standardization with respect to the preparation of extracts, which makes comparing results between different studies difficult to impossible.

Despite all of these limitations, in their report on Scientific Standards for Studies on Modified Risk Tobacco Products, the Institute of Medicine (2012) stated "Although all *in vitro* tests have limitations, the collective results can nevertheless provide potentially useful information". They further noted that, aside from endpoints relevant to cancer development, assays should also "address loss of normal cell physiology as reflected […] in regards to […] infection, inflammation, respiratory, or cardiovascular processes", disease endpoints suspected to be relevant for STP-derived substances.

Altogether, 13 publications containing data on experiments of snus in cell culture systems were located (Table A of Appendix IV) and are discussed in this section, except for a study that investigated the effect of snus extracts on the Herpes simplex virus. Results of one *in vivo* (mice) experiment are also included in this Section under Genotoxicity Testing of Snus.

4.1.1 Genotoxicity Testing of Snus

Five studies of snus, including one with a new product marketed as snus, were identified that measured endpoints related to genotoxicity or DNA repair (Coggins et al. 2012; Curvall et al. 1987; Jansson et al. 1991; Liu et al. 1997; Rickert et al. 2009).

4.1.1.1 Genotoxicity Studies

Jansson and colleagues (1991) investigated the genotoxic potential of Swedish snus. A salt-free snus was also evaluated to assess if effects might be related to the relatively high salt concentration in regular snus. Extracts were prepared in distilled water or methylene chloride (regular snus only). The extracts were tested *in vitro* using the Ames test to detect point mutations in the TA98, TA100, TA1535, and TA1537 strains of *Salmonella typhimurium,* as well as in the V79 Chinese hamster lung fibroblast cell line to detect induction of chromosome aberrations (CAs) and hypoxanthine-guanine phosphoribosyl transferase (*HPRT*) gene mutations, all in the presence and absence of S9 metabolic activation. Further, the extracts were tested for induction of sister chromatid exchanges (SCEs) in human lymphocytes. Micronuclei formation was studied in mouse bone marrow cells after *in vivo* exposure of mice via the oral route. Additionally, the methylene chloride extract was tested for the induction of sex-linked recessive lethal mutations in the fruit fly, *Drosophila melanogaster*. The aqueous salt-free snus extract was only examined for its potential to induce CAs.

The study authors concluded that CAs seen following exposure to the aqueous extract without metabolic activation were likely induced by the high salt content in snus (Jansson et al. 1991). *In vitro*, both extracts induced SCEs in human lymphocytes and induced CA after metabolic activation in mammalian cells, but neither caused micronuclei formation *in vivo* in mice. The methylene chloride extract was also unequivocally mutagenic after metabolic activation in *S. typhimurium* TA98 and TA100, but neither extract induced gene mutations at the *HPRT* locus in mammalian cells. All other results were considered negative for either extract. The study 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 recent study by Coggins and colleagues (2012) was conducted to follow up on the previous results and establish whether Swedish snus products “are active in *in vitro* assays classically used to predict carcinogenicity in humans”. Aqueous extracts of *General* PSOL[86], *Catch* Licorice PSWL[87], *Catch Dry Mini* PSW[88], *Catch Dry Mini 2* PSW (experimental flavoring), and the 2S3 reference US-type moist snuff were tested. The experimental snus *Catch Dry Mini 2* containing experimental flavoring was also extracted with dimethyl sulfoxide (DMSO). The extracts were tested *in vitro* in the Ames test in the TA98, TA100, TA102, TA1535, and TA1537

[86] PSOL: Portion Snus Original Large
[87] PSWL: Portion Snus White Large
[88] PSW: Portion Snus White

strains of *S. typhimurium,* in mouse lymphoma cells for gene mutations at the thymidine kinase (tk) locus, and in the V79 Chinese fibroblast cell line for the induction of micronuclei, all in the presence and absence of S9 metabolic activation. The extracts were also tested for potential cytotoxicity (cell viability via neutral red uptake) in a Balb/c 3T3 mouse fibroblast cell line.

Both water extracts of *Catch Dry Mini* samples were strongly cytotoxic at the highest extract concentrations tested (50 mg/mL), while the other products tested did not show more than approximately 20% loss in cell viability. Positive results of *Catch Dry Mini* in the Ames test, mouse lymphoma assay, and micronucleus assay seen at the higher concentration might be explained by overt cytotoxicity. In the Ames test, only the highest concentrations of extracts of *General* and *Catch Licorice* that were not metabolically activated induced two to three times higher mutagenic effects in TA1535 compared to solvent controls. *General* also increased responses significantly at the two highest concentrations in TA102. This product also increased responses at the highest concentration only, but without indication for a dose-response at lower doses, in TA1537 with and without activation and in TA1535 after metabolic activation. In both mouse lymphoma and micronucleus assays, *General* and *Catch Licorice* were mostly negative, except and only at the highest concentrations tested significantly positive with metabolic activation in the micronuclei assay. Similarly, *General* without metabolic activation after 24-hour treatment in the mouse lymphoma assay caused a significantly increased mutation frequency only at the highest concentration tested. There was no or no clear dose-response relationship at lower concentrations. As a whole, the genotoxicity tests indicated that *General* and *Catch Licorice* induced weak mutagenic responses in bacteria, while there was little to weak indication for gene mutagenicity and clastogenicity in mammalian cells. On the other hand, 2S3 responses after metabolic activation in the micronucleus assay were significantly and dose-related increased at the two highest concentrations tested. The study authors concluded that "These broadly negative findings in a controlled laboratory setting add to the large amount of epidemiological data from Scandinavia […] showing that SWS [Swedish snus] are associated with considerably lower carcinogenic potential when compared with tobacco products involving combustion of tobacco".

A study that did not investigate traditional Swedish snus, but, among other products, two new products marketed as snus, is included here to supplement the above presented data. Rickert and colleagues (2009) conducted a study to characterize several types of STPs, including two new products marketed as snus (*Du Maurier Original* and *Du Maurier Freshmint)*, available on the Canadian market. The concentrations of chemical components in these products are discussed in Appendix II to Chapter 2. The study, funded by Health Canada, also compared the cytotoxicity, genotoxicity, and mutagenicity of extracts of the diverse STPs. Extracts of 11 sample brands, including *Du Maurier Original* snus were prepared. While *Du Maurier* snus is not considered a traditional Swedish snus, a brief discussion of the results of this study is included here to strengthen the evidence. The researchers used artificial saliva, DMSO and dichloromethane to extract some samples, but *Du Maurier Original* snus was only extracted in DMSO. The extracts were subjected to the Ames test using the TA98, TA100, TA102, TA1535, and TA1537 strains of *S. typhimurium* and in the Chinese hamster ovary (CHO) micronuclei test, all in the presence and absence of S9 metabolic activation. The extracts were also tested for potential cytotoxicity (cell viability via neutral red uptake) in the CHO cell line.

Du Maurier Original snus DMSO extract caused less than 50% cytotoxicity at the highest sample concentration tested (20 µg/mL dry weight basis), which was similar to other US-type moist snuff tobacco products. In the Ames assay responses were weak and variable with no significant dose-response with most of the DMSO STP extracts, including the *Du Maurier* snus extract. None of the responses were increased more than two times over background. In the micronuclei assay with and without S9 metabolic activation, all of the DMSO extracts of the STP samples tested, including of *Du Maurier* snus, were weakly clastogenic with a flat dose-response relationship, but none of the samples induced more than 1.4% micronuclei, based on a target cytotoxicity of 50%. *Du Maurier* snus did not exceed 45% cytotoxicity at the highest extract concentration tested. The study authors noted that, while the concentrations of certain analytes, such as NNK, appeared to correlate with the results of the micronuclei assay, the high salt concentration in moist snuff could have contributed to their higher clastogenicity, compared to chewing tobaccos. The authors concluded that "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 interference in the products."

4.1.1.2 Urinary Mutagenicity Studies

Urinary mutagenicity testing provides a rapid screening method to indicate mutagen exposure *in vivo*. Curvall and colleagues (1987) investigated whether or not urine from snus users exhibited similar mutagenicity as smokers' urine. Twenty four-hour urine samples from Swedish snuff users (N=8), 1-week abstinent snuff users (N=6 of the same Swedish snuff users), smokers (N=8), and non-tobacco users (N=6) were tested in the Ames assay using *S. typhimurium* strain TA98 with or without S9 activation. Mean urinary nicotine and cotinine concentrations of the active snuff users were 1.39 mg/L (2 mg/24 hours) and 1.46 mg/L (2.12 mg/24 hours), respectively. These levels were similar to those measured in smokers' urine samples, but were significantly higher than those detected in controls and abstinent snuff users. While mutagenicity was significantly increased in smokers' urine, there was no difference between mutagenicity of urine from snuff users, abstinent snuff users, and controls. Mutagenic activity of the urine samples was detected only in the presence of S9 metabolic activation. Therefore, snuff use did not elevate the concentration of potential mutagens in urine, even though nicotine uptake was similar in snuff users and smokers based on urinary nicotine and cotinine concentrations.

4.1.1.3 DNA Repair Studies

Liu and colleagues (1997) investigated the activity of O^6-methylguanine-DNA methyl-transferase (MGMT) in human buccal fibroblasts exposed to aqueous and methylene chloride snuff extracts (prepared as in (Jansson et al. 1991). MGMT is an enzyme that repairs premutagenic O^6-methylguanine lesions induced in DNA by alkylating agents. In addition to snuff and tobacco extracts, the investigators also tested various other products (bidi smoke condensate, betel leaf and areca nut). Aqueous and organic snuff extracts as well as organic tobacco extract decreased MGMT activity at concentrations that also caused cytotoxicity (MTT assay), but the aqueous extracts had effects at lower concentrations (100 µg/mL) than the organic extracts (>700 µg/mL).

4.1.1.4 Summary of Genotoxicity Studies

Water extracts of traditional Swedish snus brand extracts and DMSO extracts of a new product marketed as snus (*Du Maurier*) caused weak and variable mutagenic responses in the Ames test (Coggins et al. 2012; Jansson et al. 1991; Rickert et al. 2009). By contrast, the methylene chloride extract of Swedish snuff (unspecified brand) was unequivocally positive in two strains only after metabolic activation (Jansson et al. 1991). Where cytotoxicity was tested separately, traditional snus brands and *Du Maurier* caused only mild cytotoxic effects at the highest concentrations tested (Coggins et al. 2012; Rickert et al. 2009). Water extracts of *Catch Dry Mini* (new product) did test positive in some *S. typhimurium* strains, but were also strongly cytotoxic. Unlike the snus extracts, urine from snus users did not produce any increased mutagenicity in one of those *S. typhimurium* strains (Curvall et al. 1987). Further, the water and organic extracts did not cause gene mutations (*HPRT*) in mammalian cells (Jansson et al. 1991) and a significant increase in response in the mouse lymphoma assay (*TK*) was only seen for the water extract of *General* snus after prolonged exposure and only at the highest concentration tested without metabolic activation (Coggins et al. 2012). Cytotoxic extracts of *Catch Dry Mini* also caused positive responses in this assay, as well as in the micronucleus test *in vitro*. All water and methylene chloride extracts with metabolic activation were positive in tests that indicate chromosome changes and DNA breakage (CAs, micronuclei, and SCE assays), at least at the highest concentration tested (Coggins et al. 2012; Jansson et al. 1991; Rickert et al. 2009). It should be noted that high salt concentrations as present in snus and apoptotic events, leading to DNA fragmentation (one of the hallmarks of apoptosis), can cause false positive results in clastogenicity assays. Finally, one study that tested micronuclei in mice did not indicate clastogenicity *in vivo* (Jansson et al. 1991).

4.1.2 Effects of Snus on Cells Relevant in Oral Tissue

Four studies tested *Ettan* snus extracts in cells related to the oral cavity (Andersson et al. 2006; Costea et al. 2009; Laytragoon-Lewin et al. 2011; 2004). For a summary see Table 4-1.

To investigate the effects of snuff extract on the growth and differentiation of oral epithelial tissues, Merne and colleagues (2004) used a three-dimensional epithelial cell culture system consisting of a co-culture of HaCaT cells, an immortalized human keratinocyte cell line, and fibroblasts from primary buccal mucosa in a collagen gel. Snus extracts were prepared in cell culture medium and diluted to a 1% concentration, reportedly containing 0.6 mg/mL nicotine. The cell culture was treated with this extract for 6 to 18 days. Cell morphology and by immunohistochemical staining markers of cell cycle (p53), proliferation (Ki-67), and differentiation (cytokeratins, involucrin, and filaggrin) were measured. Treatment for more than 12 days resulted in morphologic changes such as cellular damage (intercellular dyskeratosis, cellular vacuolization, lack of basal cell layer, apoptotic cells with nuclear fragmentation and other nuclear abnormalities), and impaired cellular adhesions. At the end of treatment, the thickened epithelium showed signs of severe degeneration, including necrotic cells. However, no consistent changes in matrix components, i.e., collagen and the fibroblasts, were detected. Cell proliferation was not increased by the snus extract. By contrast, Ki-67-positive cells were transiently but significantly decreased in treated cells. Expression of p53 was decreased in several snus extract-exposed HaCaT cells compared to control cells (HaCaT cells have a mutation in *TP53* gene resulting in increased p53 positive cells). The epithelial differentiation process also appeared to be disturbed, based on a decrease of cytokeratin 10 (CK 10), one of

the differentiation markers analyzed. The study authors noted that some of the cell changes were similar to those observed in snuff users' lesions and the lack of stimulation of cell proliferation detected *in vitro* was in agreement with findings from a previous study of those lesions by the same authors (Merne et al. 2002). The study authors also noted that low concentrations of snuff extract and nicotine seemed to stimulate cell proliferation (Ki-67 staining), whereas higher concentrations did not, possibly due to cytotoxic effects. This seems to be in disagreement of observations in another study that did find an increase in Ki-67 staining in lesions of heavy snuff users (Wedenberg et al. 1996). For a more detailed discussion of p53 and findings in snuffers' lesions, see Section 5.2.2.5.

Andersson and colleagues (2006) conducted a study to examine the effect of Swedish snuff in comparison with US moist snuff (Kentucky reference snuff) on the growth of periodontal ligament fibroblast cells isolated from three healthy volunteers and grown in culture. Snuff extracts were prepared in distilled water and diluted to 0.3%, 1%, and 3%. After 24 hours, the cells were evaluated for alkaline phosphatase levels and changes in growth and morphology. In pre-experiments, 10% of snuff extracts caused 100% cell death after 9 hours of treatment. In the main study3% extract concentration, both snus and US moist snuff extracts decreased both cell number and the production of alkaline phosphatase. These effects were not seen at less than 3% extract concentration. Based on the reported concentrations in the respective original extracts, a 3% dilution of the snus extract contained 318 µg/mL nicotine and 0.009 µg/mL TSNAs, while the same dilution of the Kentucky moist snuff extract contained 474 µg/mL nicotine and 0.045 µg/mL TSNAs. No differences in responses between Swedish and Kentucky moist snuff were observed. The authors concluded that smokeless tobacco has biological effects on periodontal tissues, in terms of the two markers measured.

Costea and colleagues (2009) conducted a study to compare the biological effects of toombak, a Sudanese moist snuff, with a Swedish snuff. The cells used were primary normal human oral keratinocyte (NOK) and fibroblast (NOF) cells isolated from superfluous tissues of clinically healthy buccal mucosa from adult patients undergoing surgical removal of wisdom teeth. In addition, a commercially available dysplastic oral keratinocyte cell line was used (DOK). Aqueous extracts of the snuffs were prepared with phosphate buffer and dilutions of 1/10 and higher were used to treat cells for up to six days. A number of endpoints, including cell viability (enzyme-linked immunosorbent assay (ELISA)), morphology and growth, cell cycle (flow cytometry), DNA double-strand breaks, apoptosis, and membrane permeability were assessed after various exposure time periods.

At a snus extract dilution of 1/100, a significant increase in number of cells in the G2 phase (G2/M block) was seen in both NOKs and NOFs after 48 hours of treatment (Costea et al. 2009). Twenty-four hours of treatment resulted in slight morphological changes in NOKs, while the cumulative adverse effect seen after six days of treatment was a significantly decreased number of NOKs. Interestingly, a highly diluted snus extract (10^{-6}) actually increased the number of NOFs. Based on the reported concentrations in the original extract, a $^{1}/_{100}$ dilution contained 170 µg/mL nicotine, 0.063 µg/mL NNK and 0.042 µg/mL NNN. In general, the $^{1}/_{10}$ dilution of the snus extract resulted in changes in the other parameters measured, such as an increase in DNA double strand breaks in NOKs, an increase in apoptosis, and a decreased cell viability in all cell types (>70%). The LD_{50} values in all three cell types were extrapolated to be

between dilutions of $^1/_{10}$ and $^1/_{100}$. In general, the NOKs were the most sensitive cell type. While the study authors considered the $^1/_{10}$ dilution of the snus extract a clinically nonrelevant dilution, it should be noted that nicotine and TSNA levels detected in the $^1/_{100}$ dilution of the snus extract are on the very low end of levels found in saliva in snus users (as reviewed in Costea et al. 2009), while those in the $^1/_{10}$ dilution were on the very high end. Compared to *Ettan* snus extract, toombak extracts diluted 10 to 100 times more caused similar or more pronounced changes in most measured endpoints. Based on the reported concentrations in the extracts, the 1/100 extract of toombak contained 300 µg/mL nicotine, 8.3 µg/mL NNK and 4.9 µg/mL NNN. These concentrations were in the mid-range of levels measured in toombak users. The investigators concluded that this study indicates a greater potential for toombak to induce adverse effects on normal oral mucosa than Swedish snuff.

Laytragoon-Lewin and colleagues (2011) compared the direct effects of snus with those of nicotine or alcohol (0.2% ethanol) alone or in combination with alcohol. A combination of alcohol with tobacco smoke from cigarettes of an American blend tobacco was also included. Cell types used were normal human fibroblasts (F19, a non-immortalized cell line) from the oral cavity, and normal human endothelial cells (see under Section 4.1.3 below). Snus extracts were prepared in the respective cell culture medium and diluted to nicotine concentrations ranging from 12.5 to 100 µM. Nicotine alone was tested up to concentrations of 400 µM. The cells were exposed up to 24 hours and cell proliferation (DNA synthesis), morphology, viability, and gene expression profiles were measured. Snus extract containing 100 µM nicotine caused cytoplasmic vacuolization, changes in gene expression patterns, and had a tendency to decrease cell proliferation of fibroblasts. However, at lower concentrations, cell proliferation was actually slightly increased. Similarly, a solution of 100 µM nicotine caused vacuolization, changed gene expression to a lesser extent, and had a tendency to increase proliferation, while 400 µM nicotine had a tendency to decrease cell proliferation of fibroblasts. For both snus extract and nicotine treatment, addition of ethanol enhanced cell proliferation. By contrast, smoke extract (prepared by extracting the collected smoke particulate phase with ethanol) at the same nicotine (100 µM)[89] and alcohol concentrations as the snus extract/alcohol combination induced strong cell abnormalities, increased cell death and decreased cell proliferation. Gene expression patterns in fibroblasts were changed to a similar extent as seen with snus. It should be noted that the study authors did not provide a statistical analysis of their results.

Summary

Ettan snus extract with nicotine concentrations between 16 µg/mL (100 µM) and 600 µg/mL caused a variety of changes in oral keratinocytes and fibroblasts (see also Table 4- 1): Morphological changes at the lowest extract nicotine concentration included cytoplasmic vacuolization; at higher concentration intercellular dyskeratosis, lack of basal cell layer, and

[89] The authors noted that by smoking 25 cigarettes per day, a smoker accumulates similar nicotine concentrations in the saliva.

impaired cell adhesions were also detected (Laytragoon-Lewin et al. 2011; Merne et al. 2004). Vacuolization was also observed with nicotine alone (Laytragoon-Lewin et al. 2011). In addition, apoptosis, decreased cell numbers, and indication of disturbances in cell differentiation were seen (Andersson et al. 2006; Costea et al. 2009; Merne et al. 2004). Cell proliferation was not significantly impacted at different extract nicotine concentration equal to or greater than 16 µg/mL (Laytragoon-Lewin et al. 2011; Merne et al. 2004). However, extracts at or below nicotine concentration of 4 µg/mL (25 µM) tended to increase cell proliferation. This was also observed with diluted nicotine solutions at or below 16 µg/mL (Laytragoon-Lewin et al. 2011).

Ettan snus extracts with nicotine concentrations at or above 1,700 µg/mL caused a marked increase in cell death (Andersson et al. 2006; Costea et al. 2009) as well as DNA double strand breaks in NOKs (Costea et al. 2009).

In general, keratinocytes seemed to be more sensitive than fibroblasts (Costea et al. 2009).

Table 4-1: Summary of *In Vitro* Studies of Swedish Snus in Cells Relevant in Oral Tissue				
Citation	**Snus**	**Cells Exposed to Snus Extract Alone**	**Effective Concentrations**	**Endpoints Affected**
Merne et al. (2004)	*Ettan*	Three-dimensional epithelial cell culture system of HaCaT cells (keratinocyte cell line), primary oral mucosa fibroblasts in a collagen gel	1% extract (10 mg/mL), containing 600 µg/mL nicotine	Cellular damage (intercellular dyskeratosis, cellular vacuolization, lack of basal cell layer, apoptotic cells with nuclear fragmentation and other nuclear abnormalities), impaired cellular adhesions, ↓ CK 10, no significant change in cell proliferation (Ki-67); no changes in matrix components incl. fibroblasts
Andersson et al. (2006)	*Ettan*	Periodontal ligament fibroblast cells	3% extract (10 mg/mL), containing 318 µg/mL nicotine and 0.009 µg/mL TSNAs[#] 10% extract	↓ Cell number and production of alkaline phosphatase Complete cell death after 9 hrs
Costea et al. (2009)	*Ettan*	Primary normal human oral keratinocytes (NOK) & fibroblasts (NOF), dysplastic oral keratinocytes cell line	1/100 extract (3 mg/mL) containing 170 µg/mL nicotine, 0.063 µg/mL NNK and 0.042 µg/mL NNN[#] 1/10 extract	↑ Number of cells in G2/M block in both NOKs and NOFs after 48 hours, ↓ number of NOKs after 6 days ↑ DNA double strand breaks in NOKs, ↑ apoptosis and ↓ cell viability in all cell types
Laytragoon-Lewin et al. (2011)	*Ettan*	Normal human oral fibroblasts (F19)	Extract* containing 100 µM** (16.2 µg/mL) nicotine	cytoplasmic vacuolization, changes in gene expression; no significant change in cell proliferation (DNA synthesis)

* Extraction volume not provided; **nicotine molecular weight: 162.12 g/mol

[#] The cause for the large difference in the nicotine to TSNA concentration ratio of snus extracts between the studies by Andersson and colleagues (2006) and Costea and colleagues (2009) is unclear. A combination of differences in extraction conditions (100 g snus/300 mL distilled water for 1 hr versus 100 g snus/300 mL phosphate buffer pH 7.2-4 at 37°C for 1 hr) and analytical methods might in part be responsible. It could also be speculated that cross-contamination from toombak could have caused the relatively higher TSNA concentration in the snus extract analyzed by Costea and colleagues (2009).

4.1.3 Effect of Snus on Cells and Tissues Relevant to the Cardiovascular System

Two studies tested snus extracts in cells relevant to the cardiovascular system (Laytragoon-Lewin et al. 2011; Sandhu et al. 2011).

Laytragoon-Lewin and colleagues (2011) tested *Ettan* snus extracts in adult normal human endothelial cells (HSAVEC). For details of this study see Section 4.1.2. Snus extract containing 100 µM (16 µg/mL) nicotine caused cytoplasmic vacuolization, changes in gene expression patterns, and decreased cell proliferation of endothelial cells. However, at lower concentrations

cell proliferation was slightly increased. Addition of ethanol only slightly influenced these outcomes. Similarly, a solution of 100 µM nicotine caused vacuolization and changed gene expression, and increased cell proliferation, while 400 µM nicotine decreased cell proliferation. As seen with snus alone, addition of ethanol only slightly influenced these outcomes, but enhanced the proliferative effect. By contrast, smoke extract with the same nicotine (100 µM) and additional alcohol concentrations as those in the snus extract/alcohol combination induced marked cell abnormalities, increased cell death and decreased cell proliferation. Gene expression patterns in endothelial cells were changed less than seen with snus and nicotine. It should be noted that the authors did not provide a statistical analysis of their results.

Sandhu and colleagues (2011) examined the effects of *General* snus extracts (water or DMSO-soluble fractions) on the expression and function of vasocontractile G-protein-coupled receptors (GPCRs), because vascular smooth muscle contraction, proliferation and apoptosis mediated by GPCRs are considered to be important in cerebral and cardiovascular disease pathogenesis (Hansen-Schwartz et al. 2003b, as cited in Sandhu et al. 2011). Cultured rat cerebral arteries were used as test system. Snus bags were dissolved in either water or DMSO and final extracts contained 250 µg/mL nicotine. Test concentrations in culture were 25 and 250 ng/mL nicotine as extract or pure nicotine solution. These concentrations were chosen because initial studies with nicotine concentrations seen in plasma of snus users (15 ng/mL[90]) showed no effect. Therefore, levels as seen in smokers (25 ng/mL[91]) were studied. After 24 hour exposure, cerebral artery contractions (myographic) in response to the respective target GPC receptor agonists and receptor expression (mRNA levels by real-time polymerase chain reaction (PCR); protein levels immunohistochemically) were measured. Three different target receptors were investigated: Endothelin ET_B (ET) receptor, Serotonin 5-HT_{1B} (5-HT) receptor, and Prostanoid TP (TP) receptor. Cerebral arteries exposed to all snus extracts exhibited altered G-protein-coupled receptor-mediated contractions; with significant impacts only on 5-HT receptor mediated contractions. Only DMSO snus extracts showed significant increase on receptor expression at the transcriptional level (mRNA level), with the lower concentration impacting the ET receptor and the higher concentration the 5-HT receptor. By comparison, nicotine impacted ET and 5-HT receptor mediated contractions only at its higher concentration, while the lower concentration decreased mRNA levels of all three receptors. Neither snus extracts nor nicotine solutions had any influence on receptor protein levels. The study authors suggested that both transcriptional and post-translational mechanisms are responsible for some of the receptor alterations. They concluded that “snus and nicotine may have potential impact on cerebral vasculature and on the development of cardiovascular disease”.

[90] As cited to be reported in Foulds et al. 2003 after one dose of snus use
[91] As cited to be reported in Benowitz et al. 1994 and Foulds et al. 2003

Summary

Snus extracts at nicotine concentrations of as low as 16 µg/mL (100 µM) impacted cell morphology, cell proliferation and gene expression pattern in human endothelial cells. Similar effects were seen with nicotine (Laytragoon-Lewin et al. 2011). Snus extracts at a nicotine concentration of 25 ng/mL altered GPC-receptor-mediated contractions in rat cerebral arteries and receptor expression on the mRNA level (Sandhu et al. 2011). Similar effects but with a somewhat different pattern were also seen with nicotine.

4.1.4 Effect of Snus on Immunological Parameters or Cells of the Immune System

Two studies tested snus extracts in cells relevant to the immune system (Cederblad et al. 2012; Hasseus et al. 1997).

Hasseus and colleagues (1997) investigated the effect of snus and some of its components on the local immune response. Lewis rat spleen cells, T-cells, and oral mucosa epithelial cells were used. T-cell proliferation was induced with concanavalin A (Con A). The extract of *Röda Lacket* snus was prepared in cell culture medium and diluted. After 72 hours treatment, cell proliferation (DNA synthesis) was measured. Snus extracts of 0.8% and 12.5% significantly decreased cell proliferation in spleen cells and oral epithelial cells, including Langerhans cells and T-cells. To evaluate cellular recovery from the toxic effects, cells were pretreated for 4 hour and cell viability (Trypan blue exclusion) assessed. Viable spleen cells were then incubated for 72 hours. Viable endothelial cells and T-cells were incubated according to a cross-over design, either pretreated endothelial cells together with untreated T cells or vice versa. In all three systems, there was no recovery after a treatment with an extract of more than 50%, whereas at an extract concentration of less than 6%, cells did recover. Anabasine, NAB, NNN, NNK, and NDMA were administered at similar concentrations as those detected in snus. None of them had significant impact on cell proliferation of either cell system, although NNN had a tendency to be stimulatory, while NAB had a tendency to be inhibitory in epithelial/T cells. None of the components were mitogenic. Thus, snus extracts had cytotoxic effects, as shown in the pretreatment experiments. The extracts inhibited cell proliferation in both spleen cells and epithelial cells together with T-cells, while the single components tested at similar concentrations as present in snus did not. A limitation of this study was that nicotine was tested only as a highly diluted solution ($^{1}/_{10000}$ of snus concentrations) due to issues with pH adjustment, a concentration that did not have any impact on cell proliferation. The study authors concluded that snus exposure to the oral mucosa may result in local immunosuppression from effects on both Langerhans cells as well as T-cells, but a specific component responsible for this effect could not be identified.

While a recent study by Cederblad and colleagues (2012) was conducted in peripheral blood mononuclear cells (PBMCs), which are a critical component of the immune system, the primary purpose of this study was to evaluate the effect of single nucleotide polymorphisms (SNPs) in combination with tobacco, nicotine, and alcohol exposure. The PBMCs from 54 healthy donors were analyzed for SNPs in 30 candidate genes. *Ettan* snus extract (solvent was not specified) was normalized to 100 µM nicotine (16 µg/mL). After an exposure duration of three days, cell cycle progression and cell death were measured. Snus treatment had no impact on cell cycle or cell death, compared to controls. Addition of alcohol did not change these findings. By

contrast, nicotine alone and at the same concentration significantly decreased the percentage of cells in the G0/G1-phase and increased those in the S- and G2-phase. Similarly, alcohol alone at a concentration of 0.2% significantly decreased the percentage of cells in the G0/G1-phase and increased those in the S-phase. While smoke extract (prepared by extracting the collected smoke particulate phase with ethanol) in combination with alcohol at the same nicotine and ethanol concentrations did not have any impact on the cell cycle, it significantly increased cell death. The study authors suggested that long-term exposure could provoke chronic inflammation and initiation of disease. While overall no significant increase in cell death with snus exposure was seen, the study authors noted that cell death following snus treatment (in individual PBMCs) was correlated to a SNP in the *ABCA1* gene, a gene that, in humans, encodes for the cholesterol efflux regulatory protein (CERP), a transporter protein that regulates cellular cholesterol and phospholipid homeostasis. Altogether, SNPS in 10 out of 30 candidate genes investigated correlated with cell cycling behavior induced by ethanol and/or tobacco products. The study authors concluded that certain SNPs might predict the individual risk of developing diseases and cancer induced by exposure to cigarette smoke and alcohol.

Similarly, a recent study that tested several tobacco product extracts[92] in different types of human hematopoietic cell types, including PBMCs, showed that cytotoxicity, DNA damage, and inflammation (as measured by interleukin-8 secretion) were induced at very low (less than 10 µg/mL) equi-nicotine units of a smoke total particulate matter extract in DMSO and whole smoke-conditioned medium. By comparison an extract of 23S reference moist snuff in artificial saliva and nicotine alone showed effects at concentrations at least 200 times higher, with nicotine being the least toxic (Arimilli et al. 2012). The 23S extract at the highest concentrations tested did not cause significant DNA damage in the tested cells, while nicotine itself did only at very high concentrations (2000 µg/mL).

Summary

In cells responsible for immune responses isolated from rat oral mucosa and spleen, snus extracts inhibited cell proliferation indicating a potential for immunosuppressive consequences (Hasseus et al. 1997). Nicotine extract concentrations were not provided. In human PBMCs, snus extracts with nicotine concentrations of 16 µg/mL had no effect on cell cycle or death, while nicotine alone at the same concentration moved cells from resting phase into synthesis and G2 phases (Cederblad et al. 2012). By comparison, a smoke extract at the same nicotine concentration in combination with alcohol had strong cytotoxic effects. Similarly, results from another study in human immune-relevant cells demonstrated that cytotoxicity, DNA damage,

[92] The study compared total particulate matter (TPM) dissolved in DMSO with a whole smoke conditioned medium (both from smoking 3R4F reference cigarettes by standard ISO method) as well as an extract of 23S reference moist snuff in artificial saliva. The chemical analysis showed that at similar concentrations of nicotine in TPM and 23S extract, TPM contained three times higher concentrations of NNK and two times higher concentrations of each NAT and NAB, while NNN concentration was slightly higher in the 23S extract. On the other hand, B[a]P was not detected in the latter while it was present in TPM. Concentrations of all measured components were generally more than 100 times lower in the whole smoke conditioned medium.

and increased inflammation markers were seen with smoke extracts at even lower nicotine concentrations (<10 µg/mL) while a reference STP or nicotine alone caused effects at least 200 times higher nicotine concentrations (Arimilli et al. 2012).

4.2 Studies of Swedish Snus in Experimental Animals (*In Vivo*)

The most informative animal models "mimic human tissue responses" and employ "a concentration and use pattern consistent with human exposure to smokeless tobacco products" (Institute of Medicine 2012). The IOM also stated that "attention needs to be focused upon direct contact of pathology sites in the oral cavity, gingiva/periodontum, and in nondirect contact disease tissues in respiratory and cardiovascular sites, which have been reported to be under the oral tissue's influence" (Institute of Medicine 2012).

Therefore, animal models that create a prolonged, direct oral exposure situation to the product, as well as gastric and potential systemic exposure from swallowed tobacco juices (representing the use patterns of STPs and snus) might provide useful information.

To date, STPs have been evaluated in several different animal models, including the Syrian hamster buccal pouch, the rat lip canal, and via dietary exposure of different strains of rats or transgenic mice (as reviewed in Institute of Medicine 2012). However, none of these models is physiologically representative of human exposure to snus, and some of the available models include invasive surgery. Therefore, and in addition to other limitations of animal studies, extrapolability of results from these studies to humans is limited.

Swedish snus has been investigated in a number of animal studies designed to investigate its potential carcinogenic effects (Hirsch et al. 1984a; Hirsch et al. 1986; Hirsch and Johansson 1983; Hirsch and Thilander 1981; Larsson et al. 1989; Sand et al. 2002; Schwartz et al. 2010; Song et al. 2010; Stenstrom et al. 2007). Seven of these studies examined the potential of snus to cause the development of oral tumors in the rat lip canal model. Two dietary studies in transgenic mice investigated snus' impact on tumor formation in the stomach and pancreas (Song et al. 2010; Stenstrom et al. 2007). See also Table B of Appendix IV.

4.2.1 Studies of Snus in the Surgical Lip Canal Rat Model

In a series of studies, Hirsch and colleagues developed and tested a rat model to examine the long-term effect of snuff exposure on the oral mucosa. These researchers aimed to create an environment similar to the buccal cavity in snuff users (Hirsch and Thilander 1981). To mimic the way users hold the product under the lip, a lip canal was created surgically by everting the lower lip to form it into a tube. The resulting canal is lined with oral mucosa and saliva can pass through it (Hirsch and Thilander 1981; Schwartz et al. 2010). After a post-surgical healing period, snuff was placed in the canal, where it remained for several hours at a time. Similar to the human situation, snuff is mixed with saliva, which is thought to aid in the extraction of components from snuff. It should be noted that snus users place the snus under the upper lip where less saliva accumulates compared to the lower lip (personal communication, Swedish Match). The majority of the experiments used Sprague Dawley rats.

4.2.1.1 Snus Products and Exposure

In each study, 200 mg of snus was filled into the test canal twice daily by injection and remained there until manually removed, with an average exposure time of 12 hours per day. The animals were treated five days per week for up to 30 months. Hirsch and colleagues estimated that the resulting snuff exposure of approximately 1 g/kg body weight/day is five times greater than the expected from human use[93] (Hirsch and Thilander 1981). In their first study, they also measured the nicotine blood concentration in two of the four snus-treated rats, which were 83 and 250 ng/mL[94]. Because only one dose was administered, no information for a potential dose-response relationship is provided.

The first four studies (Hirsch et al. 1984a; Hirsch et al. 1986; Hirsch and Johansson 1983; Hirsch and Thilander 1981) used *Röda Lacket* snus *(*Svenska Tobaks AB, Sweden), a snus with relatively high TSNA concentrations compared to other snus products on the market at the time (Hirsch et al. 1984b). Two later studies by the same researchers did not specify the type of Swedish snuff tested (Larsson et al. 1989; Sand et al. 2002). The latest study, conducted by American researchers, compared *Ettan* snus, two traditional US moist snuff products (*Copenhagen, Skoal*), and a new dissolvable STP in tablet form (*Stonewall*) (Schwartz et al. 2010).

In all studies by Hirsch and colleagues, the controls did not appear to receive any sham-treatment inside the lip canal, while the control rats in the study by Schwartz and colleagues (2010) received cotton inside the lip canal. Because of the lack of adequate control treatment in all but one study, the extent of which physical irritation contributed to the lesions observed in treated animals cannot be fully assessed. Another limitation of these studies is that only one dose was administered, providing no information for a potential dose-response relationship. It should also be noted that the TSNA concentrations have continuously been decreasing since the 1980s when most of the rat lip canal studies were conducted.

4.2.1.2 Snus-Induced Oral Lesions

Histopathological Description of Non-Malignant Lesions

Control Groups: At the end of the experimental periods, the tissue in the lip canal of controls exhibited slightly hyperplastic epithelium with thickening of both the stratum granulosum and spinosum, the surface covered with a thickened orthokeratin layer, and no to mild subepithelial connective tissue inflammation (Hirsch et al. 1984a; Hirsch et al. 1986; Hirsch and Johansson 1983; Hirsch and Thilander 1981). One study also noted slight-moderate fibrosis (Hirsch et al.

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[93] Human snus consumption has been reported to be on average 11 to 12 g/day for pouched snus and 29 to 32 g/day for loose snus (Digard et al. 2009), corresponding to 0.16 to 0.46 g/kg body weight/day for a 70-kg person.

[94] Compared with mean C_{max} nicotine concentrations in blood detected in snus users (11-29 ng/mL, see Section 3.1), the nicotine blood concentrations measured in the two rats were between 3 to 10 times higher.

1984a). Similar to the previous findings, Schwartz and colleagues (2010) reported slight to extensive hyperplasia and hyperkeratosis in the lip canal oral mucosa of the control rats treated with cotton.

Snus-Treated Groups: Lesions in the lip canal and/or oral mucosa after Röda Lacket snus treatment were generally marked by mild to moderate squamous epithelium hyperplasia, focal severe hyperplasia, hyperorthokeratosis with vacuolated cells, focal acanthotic proliferation of the epithelium (increase in thickness of the stratum spinosum) accompanied with the development of rete pegs (Hirsch et al. 1984a; Hirsch et al. 1986; Hirsch and Johansson 1983; Hirsch and Thilander 1981). The squamous epithelium also showed mild focal atypia and focal ulcerations. Mild to severe inflammation of the underlying connective tissue as well as fibrosis was seen.

Changes in the gingival sulcus epithelium (crevicular epithelium[95]) included moderate to severe hyperplasia, increased keratinization, atrophy, and focal ulcerations (Hirsch et al. 1986). Minor to occasional severe hyperplasia of the lip and crevicular epithelium was described by Larsson and colleagues (1989) after treatment with unspecified Swedish snuff.

With increased duration of exposure, lesions included hyperplastic and atrophic squamous epithelium and inflamed and prominently fibrotic connective tissue (Hirsch and Johansson 1983). With highly alkaline snuff (pH 9.3) the epithelial lining was more atrophic and ulcerated with less frequent vacuolization and fibrosis compared to snuff with regular pH (Hirsch and Johansson 1983).

In rats treated with *Ettan* snus, histological changes in the lip canal included hyperplasia, hyperkeratosis, and varying degrees of acute, sub-acute and chronic inflammation in the stroma (connective tissue) (Schwartz et al. 2010). These researchers noted that only in rare instances, the inflammatory infiltrate extended into the epithelium from the stratum basalis to the stratum corneum. While these authors also noted that they did not see any correlation between the inflammation and dysplastic changes (see below), they speculated that "it is reasonable to assume that ST induced inflammation may contribute to the original development of the dysplasia and abnormal epithelial extensions". Long-term inflammation is considered to predispose to the development of dysplasia (as reviewed in Rakoff-Nahoum 2006).

Reversibility

In snus-treated rats allowed to recover for one or four months after cessation of the 13-months treatment, the incidence and/or severity of atypical squamous epithelium, acanthosis, inflammation, and ulcerations in the lip canal lesions decreased compared to those seen immediately after treatment end (Hirsch et al. 1986). Instead, the lesions became more atrophic

[95] Crevicular epithelium: The stratified squamous epithelium lining the inner aspect of the soft tissue wall of the gingival sulcus. Synonyms: sulcular epithelium.

and the subepithelial connective tissue was marked by severe fibrosis. A few ulcerations persisted. Similarly, hyperplasia, keratinization, and ulcerations decreased in the gingival sulcus epithelial lesions. The study authors concluded that "Snuff exposure results in the development of a hyperplastic, reactive, reversible lesion of the oral mucosa, suggesting that snuff has predominantly promoting activity, when administered for a relatively short period of time." Schwartz and colleagues (2010) reported that a 3-months follow-up examination did not show reversibility of the observed effects in their 12-months study, but the data was not presented.

Dysplasia[96]

Dysplastic changes in a lesion indicate a greater probability that the lesion will undergo malignant transformation, compared to normal tissues, and is considered a premalignant change. Dysplasia is, however, reversible, if the stimulating factors are removed (Purkait 2011).

In their first study, Hirsch and colleagues (Hirsch and Thilander 1981) noted that the type of histological lesions seen in four rats exposed for nine months correlated with those reported in human studies. However, the animals showed a higher incidence of "hyperkeratosis, hyperkeratotic lesions and slight dysplastic lesions", possibly due to the higher snus exposure level, retention time, and potential species differences. No information on incidence of dysplasia in the test animals was provided.

In the second study by Hirsch and colleagues (1983), 2/16 animals that were exposed for 22 months to snuff with regular pH showed severe dysplastic changes in the crevicular epithelium. The remaining 36 rats exposed for 9 to 12 months to regular pH snuff or for 18 to 22 months to high pH snuff did not exhibit oral epithelial dysplasia.

In their third study, Hirsch and colleagues (1984a) reported mild dysplasia in the lip mucosa of 3/10 rats exposed for 18 months. In addition, the authors reported dysplasia of the crevicular epithelium of the lower incisor in 10% of the rats.

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[96] Dysplasia refers to an alteration in size, shape, and organization of cells, a disorderly but non-neoplastic proliferation. Histomorphologic changes of epithelial dysplasia: Loss of basal cell polarity, parabasilar hyperplasia, increased nuclear:cytoplasmic ratio, drop-shaped rete ridges, abnormal epithelial maturation, increased mitotic activity, mitoses in the superficial half of the surface epithelium, cellular pleomorphism, nuclear hyperchromaticity, enlarged nucleoli, loss of cellular cohesiveness, individual cell keratinization in the spinous cell layer (WHO 1978 as cited in Oral Cancer Foundation, Chapter IV Premalignant Lesions of CDC Oral Cancer Background Papers, accessed 12/2012, http://www.oralcancerfoundation.org/cdc/cdc_chapter4.htm). Dysplasia is graded based on lesions showing combinations and degrees of cytologic atypia: "Atypia confined to basilar and parabasilar keratinocytes constitutes mild dysplasia, whereas atypia extending into the midspinous layer is termed moderate dysplasia. When cellular atypia extends to the surface layer, the terms severe dysplasia and carcinoma in situ (complete top-to-bottom cytologic atypia) are applied." (Oral Cancer Foundation, Chapter II State of the Science of CDC Oral Cancer Background Papers, accessed 12/2012, http://www.oralcancerfoundation.org/cdc/cdc_chapter4.htm)

In the fourth study, none of the lesions observed in 30 rats that were snus-treated for 13 months were described as dysplastic (Hirsch et al. 1986).

Similarly, none of the lesions in the lip or crevicular epithelium seen in 13 rats exposed to Swedish snuff for 17 to 22 months were reported to be dysplastic in the researchers' first of two studies using unspecified Swedish snuff (Larsson et al. 1989).

By contrast, in a later report[97] by Sand and colleagues (2002) dysplasia of the squamous epithelium on the lip and crevicular epithelium was detected in 2/13 rats exposed to unspecified Swedish snuff for 22 months.

Schwartz and colleagues (2010) described changes in *Ettan* snus-treated rats to be mostly mild dysplasia, marked by low levels of pleomorphism, hyperchromatism, and dyskeratosis at the stratum basalis and adjacent layers, and limited growth (rete pegs) into stroma (connective tissue). While histopathological changes were increased for all treated animals compared to controls, the degree of reported dysplasia in tissue of *Ettan*-treated rats was similar to that of Stonewall-treated and significantly lower than in rats treated with two traditional US-type moist snuff brands. The greatest dysplastic changes were seen with *Skoal*.

To strengthen their histopathological assessment and rating of dysplasia of the lip canal tissue, Schwartz and colleagues (2010) also analyzed specific marker proteins (proliferating cell nuclear antigen (PCNA) and p16) by immunohistochemical staining, in particular in regions where histopathology indicated abnormal tissue. Mitotic figures were also evaluated.

PCNA is considered a marker of cell proliferation, because it is involved in DNA replication, chromatin remodeling, DNA repair, sister chromatid cohesions, and cell cycle control. It is associated with dysplasia and oral squamous cell carcinomas (SCCs). The number of PCNA-positive cells was significantly higher in rat lip canal oral mucosa of *Ettan*-treated rats compared to controls or *Stonewall*-treated rats (Schwartz et al. 2010). Compared to *Ettan*-treated animals, PCNA staining was slightly higher in tissues of Copenhagen-treated rats and higher yet in Skoal-treated animals. Significance of the differences between treatment groups was not provided.

Mitotic figures are the microscopic appearances of cells undergoing mitosis, i.e. actively dividing. As with other measures of cell proliferation, mitotic figures are often associated with malignancies. The number of cells undergoing mitosis was similar in *Ettan*- and *Stonewall*-treated rats and compared to controls, but significantly higher in *Copenhagen* and *Skoal* exposed rats.

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[97] The study design and animal count were identical to the study by Larsson and colleagues (1989), which suggest that lesions in the same animals might have been reevaluated, but this could not be confirmed and time of sacrifice was given as after approximately 23 months (2002) while it was 16-30 months (sacrificed when moribund) in the study by Larsson and colleagues (1989). The studies are therefore discussed separately.

P16 is a protein present in normal as well as hyperplastic oral keratinocytes. Different from cell proliferation markers, which are not tumor specific, decreased p16 levels are associated with moderate to severe dysplasia and full malignancy of the oral epithelium in rodents, consistent with P16's role as tumor suppressor gene in this type of tissue. Schwartz and colleagues (2010) examined p16 expression in areas of moderate to severe dysplasia and/or abnormal epithelial extensions (rete pegs). When these areas were not available a number of random fields were analyzed. The amount of p16-positive cells in abnormal areas was slightly decreased in *Ettan*-treated rats compared to *Stonewall*-treated rats and controls, but this difference was not significant. Significantly decreased p16 levels were seen in tissue from *Copenhagen*- and *Skoal*-treated rats.

Thus, in the study by Schwartz and colleagues (2010), *Ettan* snus-treated rats displayed mild dysplasia in their lip canal tissue together with increased PCNA staining, a marker for cell proliferation, compared to cotton-treated controls (Schwartz et al. 2010). However, the number of cells undergoing mitosis was not increased, and there was no significant decrease of the number of p16-positive cells, both markers of increased malignancy risk. Schwartz and colleagues (2010) concluded, "While all ST products caused dysplasia, the products with lower levels of TSNAs and unprotonated nicotine caused less, consistent with the model that tobacco with low levels of nitrosamines might potentially induce fewer carcinomas in humans". In this study, *Skoal* had the highest TSNA concentration of all STPs tested, almost 13 times higher than *Ettan* snus and 229 times higher than *Stonewall*. More specifically, NNK and NNN concentrations in *Skoal* were reported to be 1.5 and 19 times higher, respectively, than in *Ettan* snus and 108 and 347 times higher than in *Stonewall* snus. Nicotine concentrations in *Skoal* on the other hand differed only by 1.5 and 2.3 as compared to *Ettan* and *Stonewall*, respectively (more details are reported in Appendix VI, Table B). It should be noted that nicotine alone has been demonstrated to stimulate cell proliferation in vitro (see Section 4.1). This study confirms that there are differences in oral mucosal responses to different types of STPs that are associated with their different physical and chemical properties.

Summary

When lesions seen in the mucosa of the lip canal and/or inside the oral cavity in snus-treated rats were described as dysplasia, the dysplasia was scored to be mostly mild in four studies (Hirsch et al. 1984a; Hirsch and Johansson 1983; Hirsch and Thilander 1981; Schwartz et al. 2010) and further specified in one study (Sand et al. 2002). In addition, in one early study severe dysplasia was observed in two animals (Hirsch and Johansson 1983) (See Table 4- 2). In two studies, the observed lesions were not considered dysplastic (Hirsch et al. 1986; Larsson et al. 1989). While tissue in snus-treated rats showed increased cell proliferation (PCNA), when the mild dysplastic lesions in the same animals were tested for p16 expression, a more tumor-specific marker, no significant difference to control animals was detected (Schwartz et al. 2010).

Table 4-2: Dysplasia Observed Oral Lesions in Rat Lip Canal Model After Snus Exposure				
Citation	**Snus**	**Exposure Duration**	**Number of Rats exposed to Snus alone**	**Dysplasia Incidence**
Hirsch et al. (1981)	*Röda Lacket*	9 months	4	slight dysplastic lesions
Hirsch et al. (1983)	*Röda Lacket*	9-22 months	52	11/52 (slight) in lip mucosa; 2/16 (severe) at 22 months in crevicular epithelium
Hirsch et al. (1984a)	*Röda Lacket*	18 months	10	3/10 (mild) in lip; 10% in crevicular epithelium
Hirsch et al. (1986)	*Röda Lacket*	13 months	30	none
Larsson et al. (1989)	Swedish snuff	17-22 months	13	none
Sand et al. (2002)	Swedish snuff	23 months	13	2/13 in lip and crevicular epithelium
Schwartz et al. (2010)	*Ettan*	12 months	15	All (mostly mild) in lip canal

Tumors

No tumors were detected in four rats exposed to snus for nine months (Hirsch and Thilander 1981). In 52 snus-treated animals, one single oral squamous cell carcinoma (SCC) was detected after 8.5 months (Hirsch and Johansson 1983). It was described as ulcerated and located in the left side of the oral cavity, extending from the incisor, involving both upper and lower jaws, and invading the bone. The authors noted that spontaneous tumors of the oral mucosa are extremely rare in Sprague Dawley rats and that "the possibility that the tumor was induced by snuff therefore cannot be completely ruled out". Hirsch and colleagues (1984a; 1986) reported no oral SCC in 30 or 10 rats treated with snus for 13 or 18 months, respectively, in their third and fourth studies, but detected one oral SCC in the crevicular epithelium close to the orifice of the lip canal in 1/13 rats treated with snus for 17 to 22 months (Larsson et al. 1989). Similarly, the same research group reported cancer of the head and neck in 1/13 rats treated with snus for 22 months, but did not specify the location[98] (Sand et al. 2002). Schwartz and colleagues (2010) reported no tumors in 15 rats exposed for 12 months to *Ettan* snus or any of the other STPs tested.

Summary

Assuming that the studies by Larsson and colleagues (Larsson et al. 1989) and Sand and colleagues (Sand et al. 2002) were separate studies and did not re-evaluate the same animals, there were seven studies involving a total of 137 Sprague Dawley rats exposed to

[98] In this study, nine tumors in eight rats were observed in the head and neck region, six of which were oral SCCs: three of those SCCs were located in close proximity to the entrance of, but not inside the test canal, and three in the crevicular epithelium close to the orifice of the lip canal; there were three extra-oral cancers of the head and neck region (Sand et al. 2002).

approximately 1 g/kg/day snus via the surgical lip canal and 68 controls. In the snus-treated animals a total of two confirmed oral SCCs and one unspecified head/neck tumor were detected (see Table 4-3). No spontaneous SCCs were detected in any of the controls. The tumor incidences were not statistically significantly different between exposed and controls, either in any of the individual studies or if all studies were combined.

Table 4-3: Oral or Head/Neck Tumors Observed in Rat Lip Canal Model After Snus Exposure

Citation	Snus	Exposure Duration (months)	Number of Rats		Tumor Incidence in Snus Groups*
			Controls	Exposed to Snus Only	
Hirsch et al. (1981)	*Röda Lacket*	9	2	4	0
Hirsch et al. (1983)	*Röda Lacket*	9-22	15	52	1 oral SCC at 8.5 months
Hirsch et al. (1984a)	*Röda Lacket*	18	10	10	0
Hirsch et al. (1986)	*Röda Lacket*	13	10	30	0
Larsson et al. (1989)	Swedish snuff	17-22	8	13	1 oral cavity SCC**
Sand et al. (2002)	Swedish snuff	23	8	13	1 unspecified**
Schwartz et al. (2010)	*Ettan*	12	15	15	0

* There were no oral SCCs in controls; ** 1 nose SCC was also seen *** See footnote 98 for explanation

Impact of Other Factors on the Development of Oral Tumors and Lesions in Combination with Snus Use

Hirsch and colleagues (1984a) noted that the severity of mucosal abnormalities in snuff dippers was not associated with the amount of snuff used or duration of use, indicating that genetic variation or other environmental factors were contributing to the response. One co-factor discussed in the development of oral tumors with tobacco is a concomitant herpes simplex virus-1 (HSV-1) infection. HSV-1 infection has been associated with leukoplakia, epithelial dysplasias, and oral, head, and neck cancers in multiple reports (Larsson et al. 1989). HSV-1 is a ubiquitous human oral pathogen that causes recurrent herpes labialis (cold sores), with worldwide rates of herpes simplex virus (HSV) infection estimated to be between 65% and 90% (Chayavichitsilp et al. 2009; Larsson et al. 1989). HSV-1 has been demonstrated to be capable of transforming cells in previous in vitro studies (as reviewed in Larsson et al. 1989). In addition, Hirsch and colleagues (Hirsch et al. 1984b) demonstrated that snuff is capable of preventing HSV-1-induced cell lysis *in vitro*, increasing the number of cells that might become malignant instead of being destroyed.

Therefore, Hirsch and colleagues investigated the impact of Herpes simplex virus type I (HSV-1) infection alone or together with snus (HSV1+snus) on the development of oral lesions and tumor development (Hirsch et al. 1984a; Larsson et al. 1989; Sand et al. 2002).

The tumor-promoting potential of snus was also investigated in experiments with the known tumor initiator 4-nitroquinoline-N-oxide (4-NQO) (Larsson et al. 1989; Sand et al. 2002).

In the first study that tested snus in HSV-1 inoculated rats, oral SCCs developed in 2/7 animals of the HSVC1+snus group, compared to none in controls, HSV-1-only, and snus-only groups (Hirsch et al. 1984a). The authors concluded "[t]he results of this study indicate that HSV-1 in combination with snuff exposure may also be associated with the development of squamous cell carcinomas of the oral cavity" and hypothesized "It is possible that the snuff acts as a co-carcinogenic substance due to its restrictive effects on cytolytic HSV-infections […]".

In the second study, animals of the HSV1+snus group (N=15) did not develop SCCs of the oral cavity or lip, but the authors reported a cavernous hemangioma of the gingival mucosa. No tumors were seen in these regions in controls and the HSV-1-only group, while one oral SCC was observed in the snus-only group (Larsson et al. 1989). The authors concluded that "The incidence of squamous cell carcinomas of the head and neck region did not significantly differ between the different groups".

In the third study, one unspecified[99] tumor of the head and neck region developed in 1/15 HSV1+snus-treated animals, compared to none in controls, one in snus-only and two in HSV-1-only groups (Sand et al. 2002).

Two studies reported a higher rate of mild to moderate dysplastic oral lesions compared to snus-only treatment (>50% vs. 30% in the lip mucosa and 86% vs. 10% in the crevicular epithelium of lower incisor (Hirsch et al. 1984a); 2/15 (13%) vs. 0/13 in the crevicular epithelium (Larsson et al. 1989), while one study did not report a difference (1/15 (7%) vs. 2/13 (15%) in the squamous epithelium of the lip and crevicular epithelium (Sand et al. 2002)).

Summary

Assuming that the studies by Larsson and colleagues (Larsson et al. 1989) and Sand and colleagues (Sand et al. 2002) were separate studies and did not re-evaluate the same animals, there were three studies involving a total number 37 Sprague Dawley rats inoculated with HSV-1 and exposed to approximately 1 g/kg/day snus via the surgical lip canal and 31 HSV-1 controls. In the HSV-1+snus-treated animals a total of two confirmed oral SCCs (Hirsch et al. 1984a) and one unspecified head/neck tumor were detected (see Table 4-4). The HSV-1 controls had one lip SCC and two unspecified head/neck tumors. The tumor incidences were not statistically significantly different between exposed and controls, either in any of the individual studies or if all studies were combined.

[99] In this study, nine tumors in eight rats were observed in the head and neck region, six of which were oral SCCs: three of those SCCs were located in close proximity to the entrance of, but not inside the test canal, and three in the crevicular epithelium close to the orifice of the lip canal; there were three extra-oral cancers of the head and neck region.

Table 4-4: Oral or Head/Neck Tumors Observed in Rat Lip Canal Model After Snus+HSV-1 Exposure

Citation	Snus	Exposure Duration (months)		Number of Rats Exposed to Snus+HSV-1	Tumor Incidence in Snus+HSV-1 Group [HSV-1 Controls]
Hirsch et al. (1984a)	*Röda Lacket*	18	7	7	2 [0] oral SCC
Larsson et al. (1989)	Swedish snuff	18-24	12	15	0 [1 lip SCC]*
Sand et al. (2002)	Swedish snuff	23	12	15	1 [2] unspecified**

* 1 [1] ear duct SCC were also seen. ** See footnote 99 for explanation

No significant differences in tumor incidence were observed between rats treated with 4-NQO alone or 4-NQO+snus-treated (Larsson et al. 1989; Sand et al. 2002). One of the studies reported a higher incidence of dysplasia in the 4-NQO+snus-treated rats compared to 4-NQO-only-treatment in the lip and crevicular epithelium (4/12 vs. 1/12) (Larsson et al. 1989).

Oral Tumor Location

None of the tumors reported in the above studies were located directly inside the lip canal. They were located in the left side of oral cavity (extending from the incisor and involving both upper and lower jaws), in the palatal side of right molar region of upper jaw, or in the lingual side in the molar region and invaded the bone (Hirsch et al. 1984a; Hirsch and Johansson 1983). Hirsch and colleagues (Hirsch et al. 1984a) noted that the tumors detected in their study were in close contact with the crevicular epithelium, an area that was reported to "have a weak protective capacity against chemical substances". The researchers later stated that all 3 tumors found in the 1983 and 1984 studies likely originated from the gingival sulcus epithelium and not from the squamous epithelium of the test canal in the lip (Hirsch et al. 1986). They noted that the gingival sulcus or crevicular epithelium appeared more sensitive to snuff exposure than the tongue and buccal mucosa. These authors speculated that the close distance from the test canal to the incisors together with a constant retaining of the snuff in the gingival sulcus results in a longer exposure time. Additionally the area is covered with thin unkeratinized epithelium that might be more sensitive to chemicals (Hirsch et al. 1986). In their subsequent studies, the researchers described the tumors to be either in close proximity to the entrance or in the crevicular epithelium close to the orifice of the lip canal (Larsson et al. 1989; Sand et al. 2002).

Other Endpoints Measured

Mast cells have a primary role in response to inflammation and subsequent repair processes and can aid tumor development but may also contribute to the body's defense against tumors (Sand et al. 2002; Theoharides and Conti 2004). Some studies have shown that animals deficient in mast cells show increased tumor incidence after exposure to carcinogens. Therefore, Sand and colleagues (2002) investigated the effect of the different treatments on the amount of subepithelial mast cells in the oral mucosa. No changes in mast cell counts in the test lip canals were seen for HSV1+snus or snus-only treated animals, compared to controls,

while HSV-1 treatment alone lead to a slight reduction in mast cell count. Only 4-NQO caused a significant decline in the mast cell population. The authors concluded that Swedish snuff (either alone or with HSV-1) has only minimal effects on subepithelial oral mast cells.

4.2.1.3 General Health and Non-Oral Lesions and Snus Exposure

In three of their studies, Hirsch and colleagues (1984a; 1983; Larsson et al. 1989) reported on general health and treatment-related changes outside the oral cavity and lip of the test animals.

While there were no particular clinical signs or significant differences in body weight gain seen in snus-treated rats, physical activity declined after nine months, compared to 14 months in controls (Hirsch and Johansson 1983). On the other hand, snus-treated groups had slower body weight gain than the other groups in a later study (Larsson et al. 1989).

In three studies of HSV-1-inoculated rats, several rats (N=3, N=2, and N=2 in respective studies) were in poor condition and died from encephalitis (Hirsch et al. 1984a; Larsson et al. 1989; Sand et al. 2002). In both of the two later reports, the authors reported that 11 rats suffered from pronounced autolysis and had to be excluded. The researchers noted that rats subjected to repeated HSV-1 infection had signs of a generalized infection and speculated that this might make eukaryotic cells more susceptible to carcinogens in snuff, suppress immune response, and increase risk for tumor development (Larsson et al. 1989). They suggested this as a possible explanation for tumor development distant to the administration sites.

In their first comprehensive study of snus in the lip canal model, Hirsch and colleagues (1983) reported an increased incidence (6/26) of squamous papillary hyperplasia of the forestomach in rats treated with snus for 18 to 22 months, compared to controls sacrificed at the same time (0/5). Snus-treated rats also developed two adenomatous polyps, one squamous cell papilloma and one neurofibroma of the skin, lesion that were not observed in the controls. The authors concluded that “pathological findings outside the oral mucosa were rather rare”.

In their subsequent study, tumors detected in the snus-only-treated group, but not in controls or HSV-1-only groups, included one anal SCC, one sarcoma of the retroperitoneum, one cystic choliangioma of the liver, and one pheocytochroma of the adrenal gland (Hirsch et al. 1984a). These rats had also an increased incidence of squamous papillary hyperplasia of the forestomach (5/10). The seven HSV-1+snus rats had one sarcoma of the retroperitoneum, one cystic choliangioma of the liver, one ovary adenofibroma, and one desmoplastic fibroma of the skin. Squamous papillary hyperplasia of the forestomach was exhibited in 2/7 of these rats. The study authors concluded that “Snuff-exposed rats (snuff alone and snuff-HSV) had a significantly higher incidence of malignant tumors than control rats and rats exposed to HSV alone ($p<0.05$)”.

In a further study, Larsson and colleagues (1989) reported the following lesions in the snus-only-treated group that were not present in controls or HSV-1-only-inoculated animals: one nose SCC, one colon adenocarcinoma, one skin demoplastic fibroma. In 5/15 of these animals squamous papillary hyperplasia of the forestomach were detected. In contrast, a considerably higher number of different lesions were seen in the HSV-1+snus rats: two adenocarcinoma of the breast, one pheochromocytoma of the adrenal gland, one of each sarcoma of the stomach,

salivary gland, and scrotum, two adenomas of the breast, two adrenal cortex adenomas, and one fibrous histocytoma of the breast. In 2/13 of these animals squamous papillary hyperplasia of the forestomach were detected. Therefore, the total number of tumor-bearing animals and malignant tumors was increased in HSV-1+snus animals, compared to controls, HSV-1-only, and snus-only groups. The study authors concluded, "Even though snuff appeared as a general tumor promoter in combination with HSV-1 infection, it did not exert any specific promoting effects on the oral cavity".

Similar to what was seen for the oral lesions, there was no significant difference in lesions outside the oral cavity between in the 4-NQO and 4-NQO+snus groups. The study authors concluded "that we were not able to show that snuff functions as a tumor promoter in rats initiated with 4-NQO in the lip" (Larsson et al. 1989).

Summary

There were three studies that reported extra-oral lesions in Sprague Dawley rats exposed to approximately 1 g/kg/day snus via the surgical lip canal with or without HSV-1 infection.

One non-neoplastic extra-oral lesion consistently observed in all of the above studies was squamous papillary hyperplasia of the forestomach (1984a; 1983; Larsson et al. 1989), indicating stomach irritation from tobacco juices. Hyperplasia is not considered to be a premalignant lesion and consistent with this statement no tumors of the forestomach were observed in spite of a total exposure times of 18 to 24 months.

Sporadic incidences of different tumors outside the oral region were observed in snus-treated as well as in HSV-1+snus animals, but not in the respective controls in three studies (Hirsch et al. 1984a; Hirsch and Johansson 1983; Larsson et al. 1989). In two studies, the number of total extra-oral tumors were combined and was similar in snus and HSV-1+snus groups, but significantly higher than in controls (Hirsch et al. 1984a) or significantly higher in the HSV-1+snus group compared to snus-treatment alone and controls (Larsson et al. 1989). In the latter study, the authors concluded that snuff acted as a general tumor promoter in combination with HSV-1 infection. These authors noted a generalized infection in their HSV-1 inoculated animals. However, although there is some indication of interaction between HSV-1 and Swedish snuff, the two studies reporting this interaction with respect to tumors outside the oral/lip area did not yield consistent results. A difference in the chemical composition of the snus used (*Röda Lacket* snus vs. unspecified Swedish snuff) and small number of animals per groups might have contributed to the inconsistent findings. In addition, it should be noted that the National Toxicology Program (NTP) generally does not endorse combining tumors regardless of their tissue sites in the assessment of chemical substances in cancer bioassays (Huff 2002).

4.2.1.4 Summary of the Findings from the Studies of Snus in the Surgical Lip Canal

In seven studies in the surgical lip canal rat model (Hirsch et al. 1984a; Hirsch et al. 1986; Hirsch and Johansson 1983; Hirsch and Thilander 1981; Larsson et al. 1989; Sand et al. 2002; Schwartz et al. 2010), administration of snus for up to 23 months at a dose slightly higher than in human snus users caused lesions in the lip canal and/or oral cavity with histopathological

similarities to those seen in snus users. The incidence and severity of some characteristics of the lesions decreased upon cessation of treatment after 4 months in one study (Hirsch et al. 1986), while another study did not observe reversibility of the effects after 3 months (Schwartz et al. 2010). Dysplastic lesions were graded as mostly mild. Beyond histopathological assessments, one 12-month study also analyzed the lip canal mucosa for specific markers cell changes. Snus treatment significantly increased cell proliferation, but p16 (a more tumor specific marker) was not significantly decreased (Schwartz et al. 2010) indicating little potential for malignancy in the lesions. With the assumption that the studies by Larsson and colleagues and Sand and colleagues were separate studies and did not re-evaluate the same animals, two or three oral SCCs in a total of 137 Sprague Dawley rats were detected (Hirsch and Johansson 1983; Larsson et al. 1989; Sand et al. 2002). No spontaneous SCCs were detected in any of the 68 controls. In three studies where snus use was combined with HSV-1 infection (Hirsch et al. 1984a; Larsson et al. 1989; Sand et al. 2002), two confirmed oral SCCs and one unspecified tumor of the head and neck were reported in a total of 37 HSV-1+snus-treated animals and one or three lip/oral SCCs in HSV-1 controls were detected. The tumor incidences were not statistically significantly different between any of the snus-treated groups and their respective controls, either in any of the individual studies or if all studies were combined. Similarly, no differences were seen in tumor incidence between Lewis rats initiated with 4-NQO with or without snus treatment. Three studies also reported tumor sites outside the oral region, which were sporadically distributed in a variety of tissue sites with no obvious pattern. The National Toxicology Program (NTP) generally does not endorse combining tumors regardless of their tissue sites in the assessment of chemical substances in cancer bioassays. In the three studies that reported extra-oral lesions, squamous papillary hyperplasia of the forestomach was consistently observed with snus treatment, possibly indicating stomach irritation from tobacco juices. This type of lesions is not considered to be a premalignant lesion.

4.2.2 Studies of Snus in Transgenic Mice with Dietary Exposure

A group of investigators performed two studies investigating the impact of snus administered in the diet on potential targets for tumor formation outside the oral region (Song et al. 2010; Stenstrom et al. 2007). Both studies compared wild-type mice to transgenic mouse models, in which mice were predisposed to either chronic inflammation of the pancreas or the development of spontaneous stomach tumors. Transgenic animals are species sensitive to certain diseases or conditions that are for example seen in humans. They are informative for identifying particular mechanisms of and gene-specific sensitivities to toxic responses. However, care must be taken when interpreting results from transgenic animal models for the general human population.

4.2.2.1 Snus Products and Exposure

In both studies, mice were fed a *General* snus-containing diet for 6 and 15 months, respectively, with snus content gradually increasing from 5 to 9% over 2 to 3 months. This corresponds to an

estimated snus intake of approximately[100] 6 to 11 g/kg body weight/day (male mice) or 6.5 to 12 g/kg body weight/day (female mice). By comparison, snus users have an average daily snus consumption of 11 to 12 g for pouched snus and 29 to 32 g for loose snus (Digard et al. 2009), corresponding to 0.16 to 0.46 g/kg body weight/day for a 70-kg person. On a mg/kg/day-basis snus consumption in these experiments in mice is therefore at least 26 to 75 times higher[101] than human consumption, not considering that in snus users gastric exposure occurs from tobacco juices only, rather than the whole product.

The researchers measured cotinine levels either in the kidney or in urine to quantify snus intake. Average kidney cotinine levels were similar in all snus-treated mice (range of means, 665,700-838,800 ng/mL), with slightly lower levels in wild-type mice, compared to a strain of mice genetically predisposed to developing gastric cancer (INS-GAS mice) (Stenstrom et al. 2007). Mean urinary cotinine and trans-3´hydroxycotinine levels were also either not significantly or only slightly different between snus-treated wild-type and Elastase-IL-1-β (EL-IL-1-β) mice (36,275 and 114,064 ng/mL vs. 23,174 and 118,176 ng/mL, respectively) (Song et al. 2010). Compared to mean urinary cotinine levels detected in regular human snus users (see Section 3.1) levels in mice were 10 to 30 times higher.

Stenström and colleagues (2007) noted that snus exposure in their study was probably higher than that experienced by a daily snus user, while Song and colleagues (2010) stated that snus intake in their study was comparable with that of snus users.

Song and colleagues (2010) also exposed mice to an extract of tobacco smoke from the 2R4F reference cigarette, smoked using a modified Federal Trade Commission (FTC) standard protocol and administered via drinking water[102]. The corresponding average urinary cotinine and trans-3´hydroxycotinine levels were between 60 to 386 times lower than those detected in the snus extract-exposed mice in the same study and cotinine levels were approximately 10 times lower than in humans. This comports with Song and colleagues (2010) statement that the amount of tobacco smoke intake in the treated mice in this study may be lower than in human

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[100] Default values for food intake of mice: 3.25 g/day or 130 g/kg body weight/day (females) and 3.6 g/day or 120 g/kg body weight/day (males). Minimum intake: 3.25 g/day x 5% =0.1625 or 120 g/kg/day x 5% = 6 g/kg/day; maximum intake: 3.6 g/day x 9% = 0.324 g/day or 130 g/kg/day x 9% = 11.7 g/kg/day. For male mice only: minimum: 0.18 g/day or 6 g/kg/day; maximum: 0.32 g/day or 10.8 g/kg/day.

[101] Taking into account a scaling factor of 7 for mice and systemic exposure the difference would be approximately 4-10 times.

[102] The extract of 40 puffs per mL phosphate-buffered saline was diluted 1/100 into drinking water. This corresponds to approximately 2 puffs per day or 67 to 80 puffs/kg body weight/day. (Default values for drinking water intake of mice: 4 mL/day or 200 mL/kg body weight/day (females) and 167 mL/kg body weight/day (males). Intake: 5 mL/day x 1/100 x 40 puffs/mL = 2 puffs/day or 66.8-80 puffs/kg/day). By comparison, the average smoker takes about 10 puffs per cigarette, resulting in 200 puffs per day if 20 cigarettes are smoked, corresponding to 2.8 puffs/kg body weight/day for a 70-kg person. The respective average urinary cotinine and trans-3´hydroxycotinine levels were slightly lower in smoke-treated wild-type mice, compared to smoke-treated Elastase-IL-1-β mice (93.5 and 1103 ng/mL as well as 145.2 and 1946 ng/mL, respectively). In comparison, urinary cotinine levels in active smokers range from 4.05-10,788 ng/mL, with a geometric mean of 1,043 ng/mL urine (Goniewicz et al. 2011).

cigarette smokers. However, the very different routes of uptake via drinking water versus inhalation in addition to species differences in absorption, distribution, metabolism and excretion do not allow a direct comparison of exposure between the mice and human smokers.

4.2.2.2 Transgenic Mouse Model of Gastric Cancer

The objective of the study by Stenström and colleagues (2007) was to investigate the potential carcinogenicity of snus to the stomach, especially in hosts with a high risk for cancer development. The researchers used a gastrin transgenic mouse model, the INS-GAS mouse that expresses a human gastrin gene under insulin promoter control in pancreatic β-cells. This results in elevated levels of circulating amidated gastrin and development of spontaneous intestinal metaplasia, dysplasia, and carcinoma in situ. The majority of INS-GAS mice develop spontaneous stomach cancer by 20 months of age (as reviewed in Stenstrom et al. 2007). It is thought that the mechanism leading to cancer development in these mice involves gastrin-dependent increases in apoptosis and proliferation and subsequent progressive loss of parietal cells and achlorhydria (as reviewed in Stenstrom et al. 2007).

In addition, infection with *Helicobacter pylori*, a bacterial infection classified as Group 1 carcinogen (*carcinogenic to humans*) by IARC (2012c) and thus increasing the risk for developing gastric cancer in hosts was studied as a cofactor. Because the infection rate with *H. pylori* is approximately 40% in the young adult population in Sweden and Norway, this appeared to be a relevant concurrent infection for which a potential synergism with snus exposure had not been established.

In this study, six groups of 8 to 22 male wild-type or INS-GAS mice served as controls or were fed a *General* snus-containing diet for six months (Stenstrom et al. 2007). In addition, six weeks prior to snus treatment, one group of each, wild-type and INS-GAS mice, were inoculated with *H. pylori*.

General Histopathological Description of Stomach Wall Lesions

In wild-type mice (N=8), snus treatment alone resulted in mild morphologic changes in the stomach, but none of the changes were significantly different from the wild-type controls (N=11) based on pathological grading.

Wild-type mice infected with *H. pylori* and treated with snus (N=17) developed intestinal metaplasia, foveolar hyperplasia, oxyntic gland atrophy, epithelial defects, and inflammation severity similar to all groups of INS-GAS mice; there was a slight but not significant increase of severity in the order of wild-type snus+*H. pylori*, INS-GAS controls (N=8), INS-GAS snus (N=8), and INS-GAS snus+*H. pylori* (N=12), respectively.

Dysplasia

Uninfected wild-type mice did not exhibit significant dysplastic changes, compared to wild-type controls.

Wild-type snus+*H. pylori* mice and all INS-GAS mice had significantly higher dysplasia scores, compared to wild-type controls, with a slight increase in score in the same order as described above. In INS-GAS mice, snus treatment alone did not have any impact the dysplasia score

compared to INS-GAS controls, but additional *H. pylori* infection did increase the score significantly.

Carcinoma in Situ[103]

Snus treatment alone did not cause any carcinoma in situ in wild type mice.

Carcinoma *in situ* was observed in the order of INS-GAS controls (2/8), INS-GAS mice with snus treatment (4/8), wild-type snus+*H. pylori* mice (9/17), and INS-GAS snus+*H. pylori* mice (12/12). In INS-GAS mice, snus treatment alone significantly increased the incidence compared to INS-GAS controls according to the authors, but the number of animals (N=8) tested appears to be too small to allow this statement of statistical significance. Similarly, in both wild-type and INS-GAS mice, snus and additional *H. pylori* infection increased the incidence significantly compared to both controls and snus treatment alone.

Cell Proliferation

No difference in PCNA staining was detected between wild-type and INS-GAS controls and wild-type snus-treated mice.

Snus treatment in INS-GAS mice with or without *H. pylori* infection increased PCNA positive cells significantly, compared to both controls or snus treated mice alone. The highest numbers of positive cells were seen in the INS-GAS snus+*H. pylori* group and lower numbers in INS-GAS snus and wild-type snus+*H. pylori* groups.

Apoptosis

The only studied endpoint significantly impacted by snus treatment alone in wild-type mice was the number of caspase-3 positive cells, reflecting the number of apoptotic cells.

Snus treatment had no detectable impact on the number of apoptotic cells in INS-GAS mice, which exhibited higher numbers compared to wild-type controls. In the INS-GAS snus+*H. pylori* group the apoptotic rate was decreased compared to snus-treated mice or INS-GAS controls. In wild-type mice, the snus+*H. pylori* increased the number of apoptotic cells further. The authors did not offer any explanations for the different effects that the treatments had on the apoptotic rate.

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[103] Carcinoma in Situ: high grade dysplasia (see footnote xxx); tumor stage Tis for gastric cancer: intraepithelial tumor without invasion of the lamina propria (National Cancer Institute 2013, http://www.cancer.gov/cancertopics/pdq/treatment/gastric/HealthProfessional/page1/AllPages/Print, accessed June 2013); the study authors adopted defining characteristics for dysplasia and carcinoma in situ from consensus guidelines on mouse models of intestinal cancer (Boivin 2003, as cited in Stenstrom et al. 2007).

Cells of the Stomach Lining

The type of cells of the stomach lining that were impacted by the different treatments was determined by staining for specific cell products. Snus-treatment independently decreased the number of ECL (enterochromatin-like) cells[104] (based on pancreastatin-staining) in both wild-type and INS-GAS mice, but A-like cells (ghrelin-producing cells) and mucous cells were not impacted by any of the treatments.

Summary

Snus treatment of wild-type mice in the diet for six months did not cause any significant histopathological changes, dysplasia, or carcinoma *in situ,* or even increased cell proliferation in the stomach wall, compared to untreated wild-type mouse controls. The only significant change seen was an increased rate of apoptosis, based on caspase-3 positive cells.

By contrast, significant changes in several or all of the above endpoints were seen when snus treatment was combined with hypergastrinemia and/or *H. pylori* infection: In INS-GAS mice, snus increased cellular proliferation rate and incidence of carcinoma *in situ*, compared to controls. *H. pylori* infection in addition to snus treatment of wild-type mice increased all parameters compared to uninfected snus-treated or control wild-type mice. *H. pylori* infection in addition to snus treatment of INS-GAS mice increased all parameters (except it decreased apoptotic rate) compared to INS-GAS mice that were uninfected snus-treated or controls.

The authors noted that the observed carcinoma *in situ* were associated with increased rates of epithelial cell proliferation and apoptosis, both common features of gastric carcinogenesis. They concluded that the results of their study "support the hypothesis that snus exposure accelerates gastric cancer development in the setting of hypergastrinemia and/or *H.P.* [*H. pylori*] infection" and "illustrate the potential co-carcinogenic effect of snus in animal models, which may be relevant for a subset of patients". Based on their findings the authors suggested that "snus is a potential gastric carcinogen in mice".

A fundamental flaw of this study is the lack of groups of wild-type or INS-GAS mice that were *H. pylori* inoculated, but not snus-treated. Thus, the contribution of the infection or potential interaction with snus treatment is unknown. In addition, the small numbers of animals does not allow establishing significance in the carcinoma *in situ* incidence between INS-GAS mice with or without snus treatment.

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[104] ECL cells: Neuroendocrine cells in the gastric glands of the gastric mucosa, beneath the epithelium. These cells produce pancreastatin. The authors stated that, because carcinoma *in situ* development was accompanied by a decrease in ECL cells, this indicated minor, if any, role for these cells in the carcinogenic process, even though others have associated dedifferentiation of those cells as part of the process in development of gastric carcinoma.

4.2.2.3 Transgenic Mouse Model of Chronic Pancreatitis

Both smoking and chronic pancreatitis are known risk factor for pancreatic adenocarcinoma and smoking in combination with hereditary chronic pancreatitis has been shown to predispose to pancreatic cancer (as reviewed in Song et al. 2010). The objective of the study by Song and colleagues (2010) was to investigate the carcinogenic effects of snus and cigarette smoke in the pancreas and their interaction with chronic pancreatitis. Therefore, the researchers used a transgenic mouse model of chronic pancreatitis, the Elastase-IL-1-β (EL-IL-1-β) mouse. These mice moderately express the human interleukin-1-β (IL-1-β), a proinflammatory cytokine involved in pancreatic inflammation. Genetic expression of IL-1-β is associated with chronic pancreatitis. Chronic pancreatitis that the mice develop at an early age is considered to closely mimic that found in humans. In EL-IL-1-β mice, however, no preneoplastic or neoplastic lesions are observed after more than 24 months, suggesting that chronic pancreatitis alone may not be sufficient to induce pancreatic cancer in this animal model.

In this study, eight groups of 20 to 30 wild-type or EL-IL-1-β mice served as controls (receiving either phosphate-buffered saline in drinking water or a standard SDS diet), were given a *General* snus-containing diet, or the smoke extract-containing drinking water (described above) for 15 months (Song et al. 2010).

General Histopathological Description of Pancreatic Lesions

In wild-type mice treated with either tobacco product, epithelial cells in in the segmental and main pancreatic ducts had normal morphology and no other histopathological changes were detected.

All EL-IL-1-β mice, by four months of age (approximately 2 to 3 months after study begin) developed severe chronic pancreatitis characterized by chronic inflammation, acinar atrophy, tubular complexes, and fibrosis. The epithelial cells in the pancreatic ducts were columnar or slightly elongated cubic. These mice also developed glandular atrophy marked by fatty replacement of lost acinar cells. After 4 months, more than 90% of mice displayed moderate to marked (50-75%) acinar atrophy.

Snus treatment for 4 to 5 months in EL-IL-1-β mice caused the epithelium to flatten in a few main as well as segmental pancreatic ducts in 15/29 (52%) animals. In 7/29 (24%) of these mice, inspissated mucus was detected in main ducts with a dilated appearance. Further, glandular atrophy was detected in 10/29 (35%) animals. There was no difference in fibrosis and tubular complexes, compared to controls.

In EL-IL-1-β mice treated with smoke extract for 4 months, flattened epithelium in main pancreatic ducts and in more than 40% of segmental ducts was seen in 16/22 (73%) animals. In addition, severe (>75%) glandular atrophy was detected in 14/22 (64%) animals; this effect was more severe with earlier onset than seen in mice exposed to snus-containing diet. There was no difference in fibrosis and tubular complexes, compared to controls.

Although the observed changes resulting from exposure to either tobacco product were similar in nature, they were generally more severe in tobacco smoke extract-treated animals than in snus-treated animals, even though based on urinary metabolites, nicotine uptake seemed to be

considerably lower (see above, Section 4.2.2.1). There was no statistical difference between the incidences of flattened ductal epithelium, but the glandular atrophy incidences were significantly lower in snus-treated mice EL-IL-1-β, compared to smoke extract-treated EL-IL-1-β mice. It should however be noted that a quantitative comparison of the parameters measured without any information of at least a marker component such as nicotine concentration in the tobacco products themselves, appears not meaningful given the different nature and administration forms of the products (snus in diet vs. smoke extract in drinking water).

Cell Proliferation and Apoptosis

In all tobacco-treated wild-type mice, immunohistochemical staining for Ki-67 (a marker of cell proliferation) and the TUNEL assay (for the detection of apoptosis) were normal.

In tobacco-treated EL-IL-1-β mice, the flattened cells had a higher proliferation rate compared to EL-IL-1-β controls, but apoptosis was not detected.

Other Markers Measured

The expression of COX-2 was investigated, because the COX-2 pathway is a target of tobacco components such as NNK. There was little to no COX-2 expression in wild-type mice controls or tobacco treated wild-type mice.

In control EL-IL-1-β mice compared to control wild-type mice COX-2 expression was slightly increased. At 4 months of either snus or smoke extract treatment in EL-IL-1-β mice, COX-2 expression was increased more than two times compared with control EL-IL-1-β mice.

Expression of genes associated with chronic pancreatitis (TNF-α, IL-6, TGF-β1, and SDF-1) were measured as pancreatic mRNA by real-time PCR. IL-6 was also analyzed in serum. For TNF-α or IL-6 expression, there was no difference between wild-type control or tobacco-treated mice.

EL-IL-1-β mice treated with snus had transiently (at 7-9 months) significantly increased TNF-α expression, compared to EL-IL-1-β controls. mRNA levels of IL-6, TGF-β1, and SDF-1 were not significantly affected.

In contrast, smoke extract consistently and significantly increased TNF-α between 4 and 9 months compared to controls, but not at later time points. All other marker gene mRNAs were significantly higher expressed at the time points measured. Song and colleagues (2010) noted that TNF-α upregulation and the onset of severe glandular atrophy were correlated.

Summary

In wild-type mice, neither snus in the diet nor smoke extract in drinking water administered for 15 months caused any morphological changes, increases in cell proliferation or apoptosis in the pancreas, and did not increase COX-2 expression, or impact the expression of other markers of chronic pancreatitis.

On the other hand, treatment with snus in EL-IL-1-β mice caused flattening of the pancreatic duct epithelium and glandular atrophy, with increased proliferation in the flattened cell areas, but

no apoptosis. Further, expression of COX-2 and, transiently, TNF-α were increased, while the other three measured markers of chronic pancreatitis were not impacted.

Smoke extract- and snus-treated treated EL-IL-1-β mice exhibited similar morphological changes, but they were generally more severe, appeared earlier, and with higher incidence in the smoke extract-treated mice. Smoke extract also significantly increased expression of all measured markers of chronic pancreatitis and as well as of COX-2 compared to controls.

Song and colleagues (2010) concluded that the study showed "for the first time the importance of interactions between tobacco and chronic pancreatitis in altering the biology of the pancreas. The findings support the notion that both cigarette smoke and snus are potentially cytotoxic and carcinogenic to the pancreas." The authors also suggested that studies of individual constituents of cigarette smoke and snus should lead to a better understanding of both their similarities and differences.

Song and colleagues (2010) pointed out several methodical limitations. While snus intake in the mice was comparable with those of snus users, the administration of smoke extract via drinking water may have resulted in a lower tobacco intake in the treated mice than that expected for human cigarette smokers. The study authors also noted that the study and observation period was potentially too short for the development of pancreatic cancer, a lesion that in humans occurs after age 50. Finally, the number of mice surviving to the study end of 15 months was small.

In addition, quantitative comparison of the impact of the different treatments on endpoints measured or extrapolation to the human situation without any information of at least a marker component such as nicotine concentration in the tobacco products themselves, is not possible given the different nature and administration forms of the products (snus in diet versus smoke extract in drinking water) and respective exposure patterns of tobacco products in humans. Nevertheless, this is the only chronic feeding study of snus and pancreatic toxicity in experimental animals published in the scientific literature; the results may provide mechanistic information underlying the effects of these products on pancreatic tissue and certain immunological parameters.

4.2.2.4 General Health and Other Lesions

While Stenström and colleagues (2007) did not report on the general conditions of the mice, Song and colleagues (2010) noted that all mice tolerated the tobacco products without any significant clinical adverse effects, such as skin sensitivity, loss of hair or changes in body weight. Further, no changes in other organs, e.g., oral mucosa, lungs, bladder, stomach, colon, kidneys were observed as compared to controls.

4.2.2.5 Summary of the Findings in the Studies in Transgenic Mice with Dietary Exposure to Snus

Two studies in mice that were fed with snus in the diet for 6 to 15 months at doses that on a mg/kg/day basis are more than 20 times higher than those in human snus users were available. In the wild-type mice strains treated with only with snus, an increased rate of apoptosis was detected, but no significant changes in cell proliferation markers or histopathology of the

stomach wall were observed after 6 months (Stenstrom et al. 2007). In the pancreas, no changes in markers for cell proliferation, apoptosis, chronic pancreatitis or histopathology and not changes in any other organs were detected after 15 months (Song et al. 2010).

When snus treatment was combined with hypergastrinemia in a transgenic mouse model of stomach cancer and/or *H. pylori* infection in histopathological changes including an increase in carcinoma *in situ,* cell proliferation and apoptosis (except for INS-GAS+*H. pylori* snus-treated mice) was observed. Therefore, snus treatment together with hypergastrinemia appeared to have a co-carcinogenic effect. However, it should be noted even though the authors claimed statistical significance for this effect, the number of animals is too small to establish significance. With respect to the potential interaction of snus with *H. pylori* infection, conclusions are not possible because no group of *H. pylori*-infected controls was included.

When snus treatment was combined with chronic pancreatitis in a transgenic mouse model, histopathology included changes in the pancreatic duct epithelium and glandular atrophy, with increased proliferation in the flattened cell areas, but no apoptosis. COX-2 expression and one marker of pancreatitis were increased. No malignancies were observed.

4.3 Summary, Discussion, and Conclusions of Non-Clinical Toxicological Studies

Swedish snuff/snus has been investigated *in vitro* in a variety of cell types, in genotoxicity assays, and animal models, i.e. surgical lip canal rat model and dietary studies in transgenic mice in comparison with wild-type strains.

In Vitro Studies

The available *in vitro* studies in cell types relevant to oral tissue, the cardiovascular system and the immune system indicate that snus extracts can cause concentration-dependent changes in cell morphology, viability, and other endpoints, including markers of cell proliferation, gene expression, and expression and function of GPCR receptors. Effects observed were often similar to those seen with nicotine alone, when it was tested in the same studies alongside the snus extracts. While most studies did not use extraction conditions that might mimic the human use condition, some studies employed extract dilutions with nicotine concentrations that might be comparable to concentrations seen in saliva or plasma of tobacco users. However, it is unknown to what extent the effects seen *in vitro* are relevant for the highly complex *in vivo* situation.

Genotoxicity Testing

In three sets of genotoxicity assays, most snus extracts, at best, showed weak and variable mutagenicity in bacteria, except for a snus extract in methylene chloride that was positive. However, urine from snus users did not cause mutagenicity in bacteria in one study and snus extracts did not cause significant dose-related gene mutations in mammalian cells. All extracts after metabolic activation were positive in tests for chromosome changes and DNA breakage *in vitro*, but no clastogenicity was seen in the micronuclei assay *in vivo*. This pattern of responses is not indicative of genotoxicity relevant for human snus users.

Rat Lip Canal Studies

While of invasive nature, the seven experiments involving the surgical lip canal rat model appear to present a route of exposure sufficiently comparable to human use that they are considered informative for human risk assessment. The duration of the experiments was sufficient to assess chronic effects and the snus dose was higher than in human snus users. A limitation of the studies is that only one dose was administered, providing no information for a potential dose-response relationship. Another limitation is the lack of adequate control treatment in all but one study; therefore, the extent of which physical irritation contributed to the lesions observed in treated animals cannot be fully assessed. It should also be noted that the TSNA concentrations have continuously been decreasing since the 1980s when most of the rat lip canal studies were conducted.

Non-malignant oral lesions similar in histopathology to those seen in human snus users ("snus-induced lesions") were observed in snus-treated rats. Information from one study indicates that these lesions are at least in part reversible after cessation of exposure. While dysplasia (mostly mild) was seen frequently in the rat lesions in a total of seven studies, a marker of potential malignancy was not significantly altered in one study and the oral SCC incidence was low in all studies in snus-treated Sprague Dawley rats. The authors noted that oral SCCs are rare in Sprague Dawley rats, but the slightly increased incidence in the snus-treated groups did not achieve statistical significance compared to controls, either in any individual study or if all studies were combined to assess oral SCC outcome (2 or 3/137 snus-treated vs. 0/68 controls; 2 or 3/37 HSV-1+snus-treated vs. 1 or 3/31 HSV-1 controls; $p>0.25$, Fisher's Exact Test).

With respect to extra-oral tumors, no consistent pattern of tumor induction in any other organ was seen at statistically increased incidence for a particular tumor type or site. Squamous papillary hyperplasia of the forestomach was consistently increased with snus treatment, but is not considered a premalignant lesion. There was also no indication of a tumor-promoting effect of snus from studies that tested snus together with a tumor initiator in the surgical lip canal model in Lewis rats.

In conclusion, based on toxicological experiments in the surgical lip canal rat model and considering the limitations of these studies, there is little evidence that snus use could present a significant risk for the development of oral cancer or other tumors in humans.

Dietary Exposure Studies in Wild-Type and Transgenic Mice

Two studies in wild-type and transgenic mice strains may provide some mechanistic information, although the differences in exposure route, i.e., intake of the whole tobacco in the diet as opposed to human exposure of the oral cavity and of the gastrointestinal (GI) system via tobacco juices, make the data difficult to interpret. While the study durations were long enough to see chronic effects, they were not adequate to assess potential carcinogenicity in the wild-type mice. The snus dose was more than 20 times that in human users based on a mg/kg body weight/day basis, there was only one dose group, and reversibility was not tested. In the wild-type mouse strains, treatment with snus alone for 6 months did not cause any changes in the stomach wall except for an increased expression of an apoptosis marker and no changes in the pancreas were detected after 15 months.

Snus treatment for six months combined with hypergastrinemia in a transgenic mouse model of stomach cancer and/or *H. pylori* infection caused histopathological changes in the stomach wall, including an increase in carcinoma *in situ,* cell proliferation and alterations in apoptosis. While these changes in the stomach wall also occurred in wild-type mice that were infected with *H. pylori* and treated with snus, the contribution of snus cannot be established due to the lack of a *H. pylori*-infected control group. In addition, the number of mice was too small to establish statistical significance of snus treatment in the hypergastrinemic mouse model. When snus treatment for 15 months was combined with chronic pancreatitis in a transgenic mouse model, several changes in histopathology and markers indicated early preneoplastic lesions. However no malignancies were observed.

The dietary studies in mice were inadequate to evaluate the potential for snus to cause cancer in the stomach wall or pancreas in healthy humans with or without *H. pylori* infection. Based on the experiments in transgenic mice, due to the small number of animals investigated, there is little evidence that snus exposure in combination with hypergastrinemia may cause malignant lesions. There is some indication that snus treatment in combination with chronic pancreatitis may cause lesions described as preneoplastic in the pancreas in the transgenic mouse model. However, the relevance of all of these findings in transgenic mice for human health risk is unclear, in particular considering the other limitations of the study design, including the differences in exposure route.

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. Many of the health outcomes examined are those associated with smoking; 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 specific validity of each of these studies is beyond the scope of this document, but the general strengths and limitations are noted for cohorts, below, in section 5.1.1, and specifically for health outcomes for which the data are uncertain. 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, but this methodology was used to assess several short-term, so-called acute, health effects.

Evidence of the potential 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.

Cohort studies: Potential health effects of snus have been studied widely using Swedish and other cohorts from the Scandinavian countries. As part of the Sweden national health care system, health care and vital statistics records are linked in computerized databases, making the system useful for studying potential health effects, particularly where exposure information, in this instance, on tobacco use, available. Like all cohort studies, the specific cohorts have

their strengths and weaknesses; including varying size, participation rates, regional characteristics, and most importantly, characterization of tobacco use. In many of these cohort studies, changes in tobacco use status could have occurred at any time between initial enrollment in the cohort, when in most cohorts, the only information on tobacco use was obtained, and the follow-up period. The Swedish national trend in tobacco use, that is, decreasing rates of smoking, and increasing use of snus, are well documented, and discussed briefly, bellow, in Section 5.1.3.

One of the largest cohorts applicable to snus research is the Swedish Construction Industry's Organization for Working Environment Safety and Health study that collected data over a 24 year period (1969-1993). The primary strength of the cohort is its large size (up to over 340,000 men depending on exclusion criteria used in the individual studies), the high prevalence of snus use (28%), and the large number of never-smoking snus users. There are limitations when using data from this cohort, however, most notably ambiguities in the coding of smoking status in the early years of data collection, and lack of time-dependent characterization of tobacco use during follow up. Johansson et al. (2005) commented that the composition of snuff has changed substantially over the years since the Construction Worker cohort was first formed, and that smokeless tobacco bought on the Swedish market is now "practically free from nitrosamines." They further comment that this change in the composition of snuff could imply that the results from the construction worker study are no longer valid. To this point, Lee (2011) comments that there is a tendency for studies of the Construction Workers Cohort to report associations not found elsewhere (e.g., esophageal and stomach cancers (Zendehdel et al. 2008); and cardiovascular risk factors and events (Bolinder et al. 1994; Bolinder et al. 1992; Hergens et al. 2007; Hergens et al. 2008b; Hergens et al. 2008a). Lee also points out that none of the Construction Worker studies examine risk by job type within the cohort; he suggests that the preference for the use of snus over smoking may be related to certain job types.

Another cohort that has contributed to the understanding of potential health risks of snus use is the Northern Sweden Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Project. Participation in this study is limited to a sample of residents from the two most northern counties of Sweden, where data were collected on daily use of smokeless tobacco (among other things) among adults in over a 13-year period (Eliasson et al. 2004; Rodu et al. 2004). The strengths of the cohort include the accurate and consistent definitions of tobacco use, standardized data collection, and a high percentage of participants involved in a follow-up examination. A limitation of the study -and most cohort studies-- is that change in tobacco status could have occurred at any time during the study and follow-up period. For example, Eriksson and colleagues (2011) analyzed trends between 1986 and 2009 in major cardiovascular risk factors in this cohort, and found that the prevalence of smoking halved in that time period, such that 11% of women and 9% of men were smokers in 2009.

The Swedish Twins Registry cohort is the largest population-based twin registry in the world and has been the basis of several significant research studies including Hansson and colleagues (Hansson et al. 2009). The study population is considered representative of the general Swedish population, though is limited in the size of the cohort; and the available data for the cohort include many important potential confounders for study of tobacco-related disease (age, smoking status, diabetes, high blood pressure, high cholesterol).

Other key studies include the Malmo Diet and Cancer Cohort, two different Uppsala County Cohorts, Swedish Annual level-of-living survey and Swedish Survey of living Conditions, Swedish Birth Registry, and the Northern Swedish Cohort.

Use of smokeless tobacco is less prevalent in the United States, and only a few key US based cohorts that have formed the basis for key studies on US smokeless tobacco products. These include NHANES follow-up studies and the Cancer Prevention I and II Studies. As mentioned throughout this report, US smokeless tobacco differs from Swedish snus; nonetheless, studies from US STP users can provided some evidence to support the potential health effects of Swedish snus, and selected studies are discussed in this section of the report.

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. The listing in Appendix V provides the statements or conclusions by researchers or public health organizations related to smokeless tobacco (including snus) often in comparison to health risks from smoking. 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.

The following sections of this chapter review and discuss the major health outcomes studied in association with Swedish snus.

5.2 Non-Neoplastic Oral Effects

This section presents a review of studies conducted to evaluate non-neoplastic oral effects in individuals that use snus. This includes potential effects on anatomical sites such as the lips, buccal mucosa (i.e., the cheek membrane), and gums (the gingivae), and teeth. Studies that have been conducted to evaluate the potential for snus to cause oral cancer are not included in this discussion, as these studies are reviewed in the section on cancer.

Differences in physicochemical properties (e.g., pH, ingredient composition, particle size, humidity, and molality) of the various oral smokeless tobacco products, including snus, can affect the teeth and the oral mucosa (Andersson et al. 1995). The composition of snus was discussed in Chapter 2 of this report; properties of snus potentially related to effects on the oral cavity are presented in the discussion below. These potential effects of snus on the oral cavity can be divided into two general categories: dental effects including potential effects on teeth and gums (Section 5.2.1), and oral mucosal effects, such as snuff dipper's lesion and potential precancer effects (Section 5.2.2).

In examining any of the studies of potential noncancer oral effects, methodological considerations, such as study design, samples sizes, insufficient detail on product identification and exposure levels, lack of data control or comparison population (i.e., non-tobacco or non-snus users), varying definitions of the dental and oral conditions, and failure to control for important confounders (e.g., dietary and oral hygiene habits, and socioeconomic status), are important considerations in drawing conclusions. For example, in an investigation of individuals from Jönköping, Sweden, Hellqvist and colleagues (2009) reported that nonusers of snus visit the dentist more and brush their teeth more frequently than users, while Hirsch and colleagues (1991) reported that snus use is more common among groups with lower socioeconomic status. There are known associations between socioeconomic status and dietary and oral hygiene habits, or dental conditions such as periodontitis, as indicated by Julihn and colleagues (2008). Details of the available studies conducted to evaluate potential non-carcinogenic oral effects in snus users are provided below.

5.2.1 Dental Effects and Periodontal Disease

Several studies identified in the literature address the effects of snus on the teeth and the periodontal tissues. These effects can be generally divided into the following categories: (1) dental conditions (plaque, caries, tooth wear, and tooth loss); (2) gingivitis (inflammation of the gums); (3) gingival recession (receding gums); and (4) periodontal disease (periodontitis) (often preceded by gingivitis, an infection of the tissues surrounding and supporting the teeth and indicated by alveolar bone loss, pocket depth, attachment loss, bone height), though many outcomes are examined within the same study. These are summarized in Appendix A-1 (cross sectional studies) and Appendix A-2 (one case-control study).

5.2.1.1 Dental Conditions

Eight cross-sectional studies examined the association between various dental conditions and snus use (Bergstrom et al. 2006; Ekfeldt et al. 1990; Hirsch et al. 1991; Hugoson et al. 2012;

Hugoson and Rolandsson 2011; Monten et al. 2006; Rolandsson et al. 2005; Wickholm et al. 2004).

One study investigated the potential effects of snus use on tooth wear. The 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 years. 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), though use of snuff and saliva pH were found to be minor factors, accounting for less than 2% of the variance. The authors did not account for socioeconomic status, or dietary or oral hygiene habits.

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.

Wickholm and colleagues (2004) compared the prevalence of periodontal disease in four 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). Using standardized definitions, the authors examined the prevalence, across the tobacco groups, among participants with evidence of plaque, gingivitis, calculus, and gingival recession. The prevalence of having a higher score on the plaque index was not significantly different among the never tobacco users compared to any other tobacco group, including ever snuff users. For the calculus index, ever snuff users had a higher prevalence compared to never tobacco users, and was similar to the other tobacco-user groups. When comparing either the mean plaque index or calculus index among snus users and nonsnus users, the odds ratios were not statistically significant, as reanalyzed by Kallischnigg et al. (2008). The authors did not account for socioeconomic status, or dietary or oral hygiene habits.

Rolandsson et al. (2005) examined 80 adolescent males between 16-25 years of age, including 40 snuff users and 40 nonusers. 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. There were no statistical differences between snuff users and nonusers regarding restored tooth surfaces, number of teeth, and presence of plaque. Rolandsson and colleagues (2005) found no significant differences in oral hygiene habits between snus users and nonusers of tobacco.

Bergström and colleagues (2006) examined the relationship between use of Swedish moist snuff and several potential oral effects, including plaque index. Participants were healthy men who were current, former, or never-users of snuff. Using a 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) for any dental effect, including the mean plaque index. The authors did not account for socioeconomic status, or dietary or oral hygiene habits.

A study by Monten and colleagues (2006) examined use of snus and oral health 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. With respect to the specific dental conditions, there were no significant differences in the mean number of teeth or proportion of sites showing plaque between boys who used snus but did not smoke and boys who had never used tobacco. 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. Monten and colleagues (2006) found no significant differences in oral hygiene habits between snus users and nonusers of tobacco.

Hugoson and Rolandsson (2011) examined the relationship between current snus use and periodontal health compared with non-tobacco users among three study populations ascertained in 1983, 1993 and 2003 in the city of Jonkoping, Sweden. After adjusting for age, gender and sociodemographic variables, there was no significant association between snus users and number of teeth, or plaque index relative to non-tobacco users.

Hugoson and colleagues (2012) also investigated the relationship between tobacco use and dental caries among three study populations ascertained in 1983, 1993 and 2003 in the city of Jonkoping, Sweden. A stratified random sample was invited to take part in a dental health exam, which included 130 participants who turned 20, 30, 40, 50, 60 & 70 in these years. 550, 552 and 523 attended the 1983, 1993 & 2003 exams, respectively. The participants were examined clinically and radiographically and decayed and filled tooth surfaces were recorded. The prevalence of decayed and filled tooth surfaces among snus users was significantly lower compared to non-users of tobacco during the years 1983 and 1993. There was no statistically significant difference in the year 2003. In an analysis adjusted for age, gender, education, employment, and marital status, a significant association between snus use and decayed and filled surfaces was not observed.

5.2.1.2 Gingivitis

Gingivitis is an early stage of periodontal disease, and is defined as 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. Of six cross-sectional studies that examined the prevalence of gingivitis, gingival index, or gingival bleeding among snus users, none reported a significant association with this dental effect (Bergstrom et al. 2006; Hugoson and Rolandsson 2011; Monten et al. 2006; Rolandsson et al. 2005; Wickholm et al. 2004), with the exception of Modeer et al. (1980). The studies are described below.

Modeer and colleagues (1980) reported that 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. The mean gingival index of snus users was 1.10 compared to 0.89 among nonusers (a gingival index of 2 or 3 is considered gingivitis). Furthermore, the evidence to support an association of snuff with gingivitis is limited by the

inability to control for confounding variables in this study (the authors did not account for socioeconomic status, or dietary or oral hygiene habits).

Wickholm and colleagues (2004), discussed previously, compared the prevalence of periodontal disease in four groups of Swedish male and female adults and categorized tobacco groups based on exclusive tobacco use. When comparing the mean gingival index among snus users and nonsnus users, the odds ratio was not statistically significant, as reanalyzed by Kallischnigg et al. (2008). As stated earlier, the authors did not account for socioeconomic status, or dietary or oral hygiene habits.

In the Rolandsson et al. (2005) study, which examined 80 adolescent males between 16-25 years of age, including 40 snuff users and 40 nonusers with similar oral hygiene habits, there were no statistical differences in the gingival index between snuff users and nonusers.

The study by Monten and colleagues (2006) reported that there were no significant differences in the proportion of sites showing full mouth gingivitis or for the subgroup of maxillary anterior tooth region between boys who used snus but did not smoke and boys who had never used tobacco. Both groups of boys were found to have similar oral hygiene habits. 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.

Bergström and colleagues (2006) examined the relationship between use of Swedish moist snuff and several potential oral effects, including gingival bleeding on probing. Participants were healthy men who were current, former, or never-users of snuff. Using a 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) for any dental effect, including the gingival bleeding on probing. The authors did not account for socioeconomic status, or dietary or oral hygiene habits.

As described previously, Hugoson and Rolandsson (2011) examined the relationship between current snus use and periodontal health compared with non-tobacco users among three study populations ascertained in 1983, 1993 and 2003 in the city of Jonkoping, Sweden. After adjusting for age, gender and sociodemographic variables, there was no significant association between gingivitis relative to non-tobacco users.

5.2.1.3 Gingival Recession

There were four cross-sectional studies that specifically examined gingival recession (receding gums).

Andersson and Axéll (1989) compared the prevalence of gingival recession among users of loose and portion-bag snus. They observed gingival recessions 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. Loose snuff was significantly associated with gingival recession compared to the use of portion-bag snuff, while the authors provided no comparison of the effects of loose or portion-bag snuff use with non-use of tobacco.

Wickholm and colleagues (2004), discussed previously, compared the prevalence of periodontal disease in four groups of Swedish male and female adults and categorized tobacco groups based on exclusive tobacco use. When comparing the prevalence of gingival recessions among snus users and nonsnus users, the odds ratio was not statistically significant, as reanalyzed by Kallischnigg et al. (2008). As stated earlier, the authors did not account for socioeconomic status, or dietary or oral hygiene habits.

The 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). However, participants with gingival recessions had significantly increased odds of using snus (odds ratio (OR)=3.7; 95% confidence interval (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.

As described previously, Hugoson and Rolandsson (2011) examined the relationship between current snus use and periodontal health compared with non-tobacco users among three study populations ascertained in 1983, 1993 and 2003 in the city of Jonkoping, Sweden. Compared to nonusers of tobacco, snus users exhibited a significantly lower percentage of sites with gingival recession ≥1 mm after adjusting for age, gender and sociodemographic variables.

5.2.1.4 Periodontal Disease

Periodontal disease is often preceded by gingivitis, is described as an infection of the tissues surrounding and supporting the teeth and is indicated by alveolar bone loss, pocket depth, attachment loss, and bone height. However, not all gingivitis progresses to periodontitis; later stages of periodontal disease (known as periodontitis) are irreversible. 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.

Six cross-sectional studies (Bergström et al. 2006; Hugoson and Rolandsson 2011; Julihn et al. 2008; Monten et al. 2006; Rolandsson et al. 2005; 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 and A-2). None of these seven studies reported a significant relationship between the use of snus and periodontal disease or indicators of periodontal disease.

Wickholm and colleagues (2004), discussed previously, compared the prevalence of periodontal disease in four groups of Swedish male and female adults and categorized tobacco groups based on exclusive tobacco use. All groups of tobacco users had a higher prevalence of periodontal disease than never-users of tobacco, and there was a significant association between smoking and periodontal disease (compared to never-smoking). The OR for former snuff use (n=31) was elevated after adjusting for age, gender, education and smoking and/or plaque, although was not statistically significant (OR=2.55, 95% CI 0.80, 6.80). The OR for

periodontal disease among current snus users was not elevated (OR=0.66, 95% CI: 0.30-1.32), and there was no association with increasing can-years of snuff use was observed.

In the Rolandsson et al. (2005) study, which examined 80 adolescent males between 16-25 years of age, including 40 snuff users and 40 nonusers, there were no statistical differences between snuff users and nonusers regarding probing pocket depth. As stated previously, Rolandsson and colleagues (2005) found no significant differences in oral hygiene habits between snus users and nonusers of tobacco.

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). The authors did not account for socioeconomic status, or dietary or oral hygiene habits.

The study by Monten and colleagues (2006) reported that there were no significant differences in probing pocket depth, clinical attachment loss or alveolar bone level between boys who used snus but did not smoke and boys who had never used tobacco. 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. As stated previously, Monten and colleagues (2006) found no significant differences in oral hygiene habits between snus users and nonusers of tobacco.

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% CI: 0.7 – 1.89). The only risk factors that were statistically significantly correlated with incipient bone loss were subgingival calculus and proximal restoration ≥ 1. Odds ratios were adjusted for education level and occupational status of both parents of the participants.

Hugoson and Rolandsson (2011) examined the relationship between current snus use and periodontal health compared with non-tobacco users among three study populations ascertained in 1983, 1993 and 2003 in the city of Jonkoping, Sweden. After adjusting for age, gender and sociodemographic variables, there was no significant association between severity of periodontal disease, and frequency of probing pocket depth ≥ 4mm relative to non-tobacco

users. The authors concluded that using snus did not seem to be a risk factor for periodontal disease.

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. The authors did not account for socioeconomic status, or dietary or oral hygiene habits.

5.2.1.5 Summary and Discussion for Dental Effects and Periodontal Disease

- Dental Conditions: Of the eight cross-sectional studies of dental effects, two reported a significant association with the use of snus and dental caries and tooth loss (Hirsch et al. 1991) and tooth wear (Ekfeldt et al. 1990). Neither study accounted for the potential confounding effects of socioeconomic status, or dietary or oral hygiene habits. Several studies that did account for these potential confounding factors did not find a relationship between the use of snus and dental caries (Hugoson et al. 2012; Rolandsson et al. 2005) or for tooth loss (Hugoson and Rolandsson 2011; Monten et al. 2006; Rolandsson et al. 2005). None of the five studies that investigated the relationship between dental plaque and snus use reported a significant relationship between the two (Bergstrom et al. 2006; Hugoson and Rolandsson 2011; Monten et al. 2006; Rolandsson et al. 2005; Wickholm et al. 2004). Three out of those five studies accounted for socioeconomic status, or dietary or oral hygiene habits (Hugoson and Rolandsson 2011; Monten et al. 2006; Rolandsson et al. 2005).
- Gingivitis: Of six cross-sectional studies of gingivitis, gingival index, or gingival bleeding, one reported a significant association between a higher gingival index and the use of snus (Modeer et al. 1980). The authors of this study did not report whether oral hygiene habits or sociodemographic variables differed between snus users and nonusers of tobacco. The mean gingival index of snus users was 1.10 compared to 0.89 among nonusers (a gingival index of 2 or 3 is considered gingivitis). Among the five studies that reported no association with gingivitis or other endpoints associated with gingivitis (Bergstrom et al. 2006; Hugoson and Rolandsson 2011; Monten et al. 2006; Rolandsson et al. 2005; Wickholm et al. 2004), three of the five accounted for either oral hygiene habits and/or socioeconomic variables (Hugoson and Rolandsson 2011; Monten et al. 2006; Rolandsson et al. 2005).
- Gingival Recession: Of three cross-sectional studies that compared gingival recession among snus users and non-users of tobacco, one reported that participants with gingival recessions had significantly increased odds of using snus (Monten et al. 2006). The authors found no significant differences in oral hygiene habits between users and nonusers of snus. Of the two other studies, one found that the prevalence of gingival recession among snus users and nonusers was not significantly different (Wickholm et al. 2004), while the other reported a significantly lower percentage of sites with gingival recession ≥ 1 mm among snus users compared to nonusers (adjusted for sociodemographic variables) (Hugoson and Rolandsson 2011). A fourth study found that loose snuff was significantly associated with gingival recession compared to the use of portion-bag snuff, while the authors provided no comparison of the effects of loose or portion-bag snuff use with non-use of tobacco (Andersson and Axell 1989).

- Periodontal Disease: None of the six cross-sectional studies nor the one case-control study (Kallestal and Uhlin 1992) reported a significant association between the use of snus and periodontal disease, or individual indicators of periodontal disease. Most studies, with only two exceptions (Bergstrom et al. 2006; Kallestal and Uhlin 1992), adjusted, or accounted for, socioeconomic status or oral hygiene habits. The five remaining studies accounted for either socioeconomic factors (Hugoson and Rolandsson 2011; Julihn et al. 2008; Wickholm et al. 2004) or oral hygiene habits (Monten et al. 2006; Rolandsson et al. 2005).

Lee (2011) presented a review of the available studies that examined dental-related outcomes. He concluded that a relationship of snus to periodontal and gingival diseases is not clearly established. Further, he stated that a possible relationship with tooth loss and dental caries is not established. His conclusions are consistent with an earlier review conducted by Kallischnigg and colleagues (2008). In that review, the authors evaluated the relationship between smokeless tobacco products and non-cancerous oral diseases in both Europe and the U.S. The authors concluded that the results from the Swedish studies reveal no clear relationship between snuff use and periodontitis or gingivitis. The authors described the evidence of an association between snuff use and gingival recession as limited, where several studies failed to compare to nonsnuff users; they noted, however, that one controlled study did observe a significant increase in gingival recession among male adolescent snuff users, and another study observed a higher prevalence of gingival recession among loose snuff users compared to portion-bag users.

5.2.2 Oral Mucosal Effects

5.2.2.1 Snuff Dipper's Lesion

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 (Axell 1987). Histologic changes observed in snuff-induced lesions (SILs) include hyperplasia of the epithelium with large, vacuolated cells, and a chevron type of keratinization. Numerous studies have observed that snus use is associated with this characteristic reaction in the oral mucosa (Appendix B, Andersson et al. 1989; Andersson et al. 1990; Andersson 1991; Andersson et al. 1994; Andersson et al. 1995; Andersson and Axell 1989; Andersson and Warfvinge 2003; Axell 1976; Axell et al. 1976; Axell 1987; Axell and Hedin 1982; Axell and Henricsson 1985; Axell 1993; Frithiof et al. 1983; Hirsch et al. 1982; Larsson et al. 1991; Martensson 1978; Mornstad et al. 1989; Rolandsson et al. 2005; Roosaar et al. 2006; Rosenquist et al. 2005; Salonen et al. 1990; Wallstrom et al. 2011). This type of lesion has been referred to by various names, including snuff dipper's lesion, snuff-induced leukoplakia, or snus-induced lesions. The lesion generally appears at the location in the mouth where the snus is held.

Most of the studies summarized in Appendix B, graded clinical changes associated with oral mucosal lesions on a four-degree severity 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.

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.

The severity of oral mucosal lesions appears to be related to the daily duration, amount consumed, as well as the form of snuff used daily (i.e., loose snuff vs. portion-bag snuff). An association with characteristics of the snus product, such as higher pH and increased nicotine content, has also been suggested (Andersson and Warfvinge 2003; Mornstad et al. 1989; Wallstrom et al. 2011). The following section summarizes the findings related to these exposure factors and product characteristics.

Hirsch and colleagues (1982) found that patients with degree 3 (10.1 hours/day; 17.9 g/day on average) and 4 (10.6 hours/day; 22.3 g/day on average) lesions used snuff approximately twice as long per day as patients with degree 1 (5.2 hours/day; 6.8 g/day on average) and 2 (6.5 hours/day; 15.2 g/day on average) lesions. Statistically significant differences in consumption were only observed between degree 1 and degree 4 lesions. The study limitations include a relatively small sample size (50 participants), and potential confounding from alcohol use and smoking. Rolandsson et al. (2005), in a study of 40 male snuff users, ages 16-25 years old, also found that that the hours of daily snuff use had a statistically significant effect on the development of oral mucosal lesions. The mean daily duration of snuff use increased with severity among those with no (2.0 hours/day) lesions, degree 1 (7.2 hours/day), 2 (9.6 hours/day), and 3 (12.3 hours/day) lesions, with no degree 4 lesions observed. The amount of snuff used was not a significant predictor of snuff lesions. Mornstad and colleagues (1989) reported that the severity of the lesions among snuff users were positively correlated with age, years with the habit, amount of snuff consumed per day, and with the time with contact between snuff and the oral mucosa. Rosenquist and colleagues (2005) also reported that those who used snuff for more than 10 hours per day developed more pronounced lesions. However, Wallstrom and colleagues (2011), who conducted a small clinical follow-up study of 18 men without a history of smoking, did not find a significant correlation between the severity of the lesions and total exposure to loose snuff in terms of the years with the habit, daily hours of consumption and amount consumed on a daily basis. Participants had used snuff for an average duration of 14.7± 2.7 hours/day. Andersson and colleagues (1994) found no correlation between the degree of lesions with either total dose of nicotine or lifetime duration (the average duration of snus use was 14.5 years (loose) and 7.4 years (pouch)).

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. The 1989 study was based on 14 matched pairs of loose and portion-bag users analyzed for histological changes related to the package form from a total of 252 biopsies obtained from snuff users. In the 1994 study, a total of 45 habitual snus users (men) were selected: 22 loose snus users and 23 portion-bag users (45 total snuff users who had participated in the Andersson and Axell 1989 study). In the latter study, for example, Andersson and colleagues

(1994) observed less pronounced clinical changes in the oral mucosa in users of pouched snus compared with the changes in the mucosa of moist 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 authors reported that differences in severity of oral lesions among portion-bag and loose snuff users were not correlated to exposure and uptake of tobacco components such as nicotine, as measured in urine and saliva cotinine. The pH of the snus products was alkaline (7.9-8.6) and about 0.5 units higher in loose snus than in portion-bag snus. The authors suggested that the difference in tissue response between portion-bag users and loose snus users was probably due to the pH differences of the two types of products. The authors stated that this is further supported by the fact that users of chewing tobacco, which has considerably lower pH, exhibit only slight changes in the buccal mucosa.

Following that study, Andersson and colleagues (1995) then reported that they found no decisive pH differences between two different brands of snus, thus making the theory relating to the importance of pH value questionable. The only recorded difference between the brands was the nicotine content. Mornstad and colleagues (1989) noted that of three different brands of snus, more severe lesions were observed among the brand ("Ettan") with the highest pH (9.2). In a later study of subjects recruited from the same population as Andersson and colleagues (1989; 1994), Andersson and Warfvinge (2003) noted that even though snuff users had an alkaline salivary pH during and shortly after snuff use, mucosal changes were recorded only at the sites where the pinch of snuff was placed. The authors noted that the amount of epithelial vacuolization was unchanged when only pH was lowered but decreased significantly when nicotine content was also lowered, and suggest that nicotine and pH may act synergistically as partial causes of snuff induced lesions. Wallstrom and colleagues (2011) also reported some evidence that suggests the potential influence nicotine may have on the oral mucosa. They found that 71% of subjects with oral lesions remaining after six months of abstinence from loose snuff had continued to use nicotine replacement therapy (gum) during that time, whereas only 18% of subjects without oral lesions remaining after six months used nicotine replacement therapy.

Rolandsson and colleagues (2005) also found that product type (loose snuff vs. portion-bag snuff) had a statistically significant effect on the development of snuff lesions. Out of the 18 snuff users in this study using loose snuff, 16 showed degree 2-3 snuff lesions, while only 8 of 22 portion-bag users showed degree 2 lesions (none showed degree 3 lesions).

Natural history and reversibility

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.

With respect to 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.

With respect to 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. 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. 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.

Wallstrom and colleagues (2011), as described previously, also investigated the reversibility of SILs. They found that after six months of abstaining from snuff use, SILs did not resolve completely in 39% (n=7) of the 18 study participants. As mentioned previously, five of these seven subjects were still using nicotine replacement therapy on a daily basis (three chewing the gum and two placing it under the lip), while the two other participants were nicotine-free. However, the authors noted that the clinical changes among the participants who still exhibited

SILs at six months were less severe and the area of the affected mucosa had diminished in size.

5.2.2.2 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 human immunodeficiency virus (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).

5.2.2.3 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).

5.2.2.4 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.

5.2.2.5 Biological Markers Associated with Oral Cancer in Oral lesions from Swedish Snus Users

Oral carcinogenesis is considered a multi-stage process. Identification of biomarkers as reliable predictors for the progression of oral lesions into malignant tumors (particularly oral squamous cell carcinomas [SCC]) has been the topic of many investigations (Montebugnoli et al. 2008). In particular, proteins involved in cell cycle regulation and/or indicators of cell proliferation have been studied in this context.

One of the frequently measured proteins is p53, a key factor in cell cycle regulation. P53 is the expression product of tumor suppressor gene *TP53* which, if mutated to express dysfunctional p53, is predictive of tumor development. In SCC of the head and neck, mutations in the *TP53* gene are the most commonly observed genetic alterations (Somers et al. 1992, as cited in Schildt et al. 2003). Dysfunctional p53 protein is generally considered to lead to an increase in its half-life and hence intracellular accumulation compared to the wild-type protein. It can therefore be detected more readily (as reviewed in Schildt et al. 2003). In addition, due to its prominent regulatory function in cell cycle checkpoints, increased expression of functional wild-type p53 can be a response to genetically altered hyper-proliferating cells (as reviewed in Montebugnoli et al. 2008). Therefore, overexpression (and accumulation) of p53 protein in oral lesions is often considered a reliable predictor of progression to oral SCC. However, only 50% of oral SCCs are associated with p53 overexpression. This marker has, therefore, not been considered highly sensitive, and is thought to have low predictive value when used as a single marker (as reviewed in Montebugnoli et al. 2008). Several limitations for the use of p53 as a predictor of SCC progression lie in the methodology: Protein overexpression is measured via immunohistochemical staining methods using antibodies that cannot distinguish between the wild-type and mutant p53 protein. Increased expression of normal wild-type p53 also occurs within non-neoplastic cells during increased phases of cell proliferation, e.g. due to stimuli from inflammation, trauma, etc. (as reviewed in Montebugnoli et al. 2008). Further, binding of wild-type p53 protein to other proteins or a disturbance of degradation pathways may increase cellular p53 levels without predictive value for malignant transformation (as reviewed in Schildt et al. 2003). On the other hand, mutations in *TP53* may not necessarily result in increased stability of the mutant protein (as reviewed in Montebugnoli et al. 2008).

In addition to p53, protein markers of cell proliferation are frequently measured, such as proliferating cell nuclear antigen (PCNA), which is involved in DNA repair and replication, and Ki-67, a non-histone protein present during the non-resting phases of the cell cycle (as reviewed in Schildt et al. 2003).

Five studies from researchers in Sweden, Oklahoma City and Finland were identified that investigated several of these markers in tissue samples of oral lesions from snus users (Ibrahim et al. 1996; Merne et al. 2002; Schildt et al. 2003; Wedenberg et al. 1996; Wood et al. 1994).

Wedenberg and colleagues (1996) analyzed upper lip biopsy specimens from oral lesions of 15 Swedish non-smoking snuff dippers for p53 and Ki-67 protein expression. Both markers were considerably[105] increased in the lesions of snuff dippers compared to normal oral mucosa from four never-tobacco using controls, but there was no clear correlation between cells that exhibited higher levels of the markers and histomorphological changes. Lesions were characterized by increased epithelial thickness, hyperkeratinization, and chronic inflammation, but no signs of epithelial dysplasia or SCC were detected. The authors concluded that these findings "may indicate that overexpression of the p53 gene contributes to subsequent malignant cell transformation related to snuff-dipping."

In a study that was cited by Merne and colleagues (2002) and included samples from the same individuals investigated by Wedenberg and colleagues (1996), oral leukoplakia lesions of 12 snuff users were analyzed and compared with normal tissue specimens of the same individuals as well as from 12 healthy non-tobacco users (Wood et al. 1994). Wood and colleagues (1994) did not specify the origin of the control or snuffers' tissue samples or the type of snuff used by those individuals. In addition to the snuffer's lesions, in part 2 of this study, archived leukoplakia specimens collected between 1985 and 1992 from unspecified patients (available in the Oklahoma City University Hospital), were also analyzed. The authors positively correlated the degree of dysplasia in these samples with p53 protein expression. The leukoplakia lesions from snuff users were graded as mild epithelial dysplasia and p53 expression was slightly but significantly increased, compared to expression in healthy oral tissue from the same individuals. However, the increase was not statistically significant compared to normal tissue specimens from controls. The snuffer's lesions were comparable in rank to the mildest leukoplakia seen in the archived samples, both in histopathological changes and p53 expression. Of the 12 samples, 5 were p53 positive. While one of the antibodies used in this study could detect a mutant form of p53, it did not result in positive staining. Thus, it was not possible to verify whether the increased p53 expression in the lesions tested was a result from a *TP53* gene mutation.

Ibrahim and colleagues (1996), researchers from the same group as Wedenberg, analyzed tissue samples of oral lesions from patients diagnosed with premalignant or malignant lip or intra-oral lesions. The patients were snuff dippers from Sweden and Sudan (N = 15 and 22, respectively), and 137 non-snuff dipping individuals from Sweden, Norway, and Sudan. The 120 Scandinavian non-snuff dipping controls included 19 (16%) smokers. Healthy oral tissue specimens from five individuals with no history of tobacco or alcohol use were added as

[105] Mean numbers of positively stained cells were 255 and 28 times greater, respectively.

negative controls. None of the 15 lesions from Swedish snuff dippers were SCCs, and all were characterized as fibro-epithelial hyperplasia. By contrast, 64% of the lesions from Sudanese snuff dippers were SCCs, with the remaining eight lesions being either epithelial dysplasia or carcinoma *in situ*. On the other hand, more than 80% of lesions from Scandinavian non-snuff dipping controls (including several smokers) as well as from Sudanese non-tobacco users were SCCs (101/120 and 14/17, respectively). With respect to p53 expression detected in the samples, a similar low percentage (13-14%) of Swedish and all Sudanese snuff user lesions stained positive for the p53 protein (2/15 and 3/22 lesions, respectively). By contrast, 60% and 53% of all the lesions from Scandinavian non-snuff using and Sudanese non-tobacco using controls had increased p53 expression, respectively (72/120 and 9/17, respectively). Interestingly, SCCs from the Sudanese snuff dippers stained positive for p53 protein at a significantly lower frequency (21%) than those from controls (66%). In summary, lesions from Swedish snuff dippers had a low frequency of p53 lesions and the authors concluded that the overall relative frequency of p53 expression in all snuff dipper's lesions (Swedish and Sudanese) was lower than in oral lesions from non-snuff dippers.

In a study by Merne and colleagues (2002), biopsy samples of oral lesions from 14 Finnish moist snuff users were analyzed for markers of cell cycle regulation, proliferation, cell stress, as well as for various cytokeratins and collagen type IV. The lesions were compared with healthy oral mucosa samples from 12 never-tobacco users. As with other snus-induced lesions, the oral lesions were characterized by epithelial thickening and hyperkeratinization, and mild chronic inflammation; however, no dysplasia was observed. No significant difference in the number of p53- and p21- (a downstream target of p53) stained cells was seen between snuff users' lesions and healthy control tissue. Of all tissue samples, only 2 of the 14 lesions from snuff dippers stained positively for p53. These two lesions also showed strong p21 expression and were graded as clinical category 2. The number of cells expressing markers of cellular proliferation (PCNA and Ki-67), was lower in snuffers' lesions than in healthy tissue from controls, but staining intensity was described as higher. The authors noted that these findings indicate epithelial thickening may be caused by an increased life span of cells rather than by higher turnover/proliferation rate. Based on these results, the authors also concluded that oral lesions from snuff use are associated with suppressed cellular proliferation and infrequent p53 dysfunction, which may partially explain the low rate of malignancy in the snus-induced mucosal lesions.

Schildt and colleagues (2003) analyzed tumor samples (114 confirmed SCCs) from the oral cancer participants in their case-control study in Sweden to investigate correlations of various exposure factors (including smoking, oral snuff use, alcohol, infections, etc.) with biological markers for oral cancer by univariate analysis. Schildt et al. (1998b) did not observe an association between the use of oral snuff and the risk for oral cancer. The tumors were evaluated by immunohistochemistry for alteration in protein expression (p53, PCNA, Ki-67, and bcl-2), as well as for mutations in the tumor suppressor gene *TP53*. Of the 114 cases in this study, 12 were active snuff and 8 ex-snuff users. Of these active and ex- snuff users' SCC samples, 9/12 and 5/8 were positive for p53, respectively. Overall, 72/114 SCC cases (63%) were positive for p53. By contrast, only 36% of all cases and even less of the active or ex-snuff user cases were positive for a mutation in *TP53.* When the SCC characteristics of the cases was compared to matched (by age, sex, and county) healthy controls, which consisted of 20

active and 6 ex-snuff users, there was no clear relationship between any of the biological markers as examined in the cases and snuff use; the authors did note that the number of snuff users involved in the study was small. Alcohol consumption was a risk factor for increased biomarker levels in the cases, but the odds ratio was not significant. The only factor that was significantly associated with increased risk for all tumors, as well as for p53 protein positive tumors only, was oral infection (especially herpes simplex virus (HSV) infection).

In summary, three studies analyzed specific oral lesions found in Scandinavian snuff users. The lesions were described as fibroepithelial hyperplasia (Ibrahim et al. 1996) or increased epithelial thickness, hyperkeratinization, some vacuolization and chronic inflammation, but not epithelial dysplasia or SCC (Merne et al. 2002; Wedenberg et al. 1996). In one study, lesions (not confirmed to be from use of Swedish snuff) were described as mild epithelial dysplasia (Wood et al. 1994). Two of these studies detected significantly increased p53 expression in snuff-induced lesions, compared to healthy tissue (Wedenberg et al. 1996; Wood et al. 1994), while one did not (Merne et al. 2002). Two studies showed a low (13-14%) frequency of p53 expression in snuffers' lesions (Ibrahim et al. 1996; Merne et al. 2002), but histopathology did not indicate dysplastic changes. In the one study were lesions were considered mildly dysplastic, the frequency of positive p53 staining was increased among the snuff user's lesions (Wood et al. 1994), but the type of snuff used could not be confirmed to be snus. While the study by Schildt and colleagues (2003) indicated a higher overall frequency of positive p53 staining in SCCs, including in those from snuff users, there was no correlation with snuff use.

There are several limitations of these studies. All of these studies had small sample sizes of snus users. In four studies, no information on alcohol use or underlying oral infections was provided; both of these risk factors were found to be associated with SCC risk in the study by Schildt et al. (1998a; 1998b). In the single case control study accounting for alcohol consumption, information on overlap with tobacco use was not provided. All but one study used antibodies for detection of p53 that cannot be used to distinguish between mutant and wild-type. Only one study investigated the actual TP53 gene for mutations in certain gene locations, but no positive association with snus use was detected (Schildt et al. 2003). The one study that found increased p53 staining in snuff lesions that were also characterized as mildly dysplastic used altogether different antibodies, including one that specifically recognizes mutant forms of p53 (Wood et al. 1994). However, these authors did not detect any mutant p53. Therefore, the available studies do not indicate that snuff user's lesions were associated with an increase in biomarkers that indicate progression to malignancy.

Table 5-2: Summary of Histopathological Characterization and p53 Expression in Lesions from Snuff Users

Citation	Histopathological Characterization of Lesions from Snuff Users	Number of p53 Positive Samples/Total Number of Snuffer's Lesions' Samples	Comparison of p53 Staining With Healthy Control Tissue
Ibrahim et al. (1996)	fibroepithelial hyperplasia	2/15	Not provided
Merne et al. (2002)	increased epithelial thickness, hyperkeratinization, some vacuolization and chronic inflammation, but no signs of epithelial dysplasia or SCC	2/14	No significant difference
Wedenberg et al. (1996)	increased epithelial thickness, hyperkeratinization, some vacuolization and chronic inflammation, but no signs of epithelial dysplasia or SCC	Not provided	Increased
Wood et al. (1994)	Mild epithelial dysplasia	5/12	Increased
Schildt et al. (2003)	Squamous cell carcinoma	9/12 (active snuff) 5/8 (ex-snuff)	N/A

5.2.2.6 Summary and Discussion of Oral Mucosal Effects

- Swedish snus causes a characteristic type of oral mucosal lesion that regress following cessation of snus use. There is no evidence that they progress to cancer, even with long-term 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.
- A limitation in the available data is that the studies are largely descriptive in nature (e.g., cross-sectional), and some studies have important limitations including small sample sizes, and failure to control for important confounders.
- The available studies do not indicate that snuff user's lesions were associated with an increase in biomarkers that indicate progression to malignancy.

Lee (2011) presented a review of the available studies that examined snuff-induced lesions. He concluded that current snus users generally have "100% incidence, with severity clearly associated with daily time used and amount consumed." Further, he stated short-term quitting reduced severity, and that longer-term quitting results in the elimination of the lesion. His conclusions are consistent with an earlier review conducted by Kallischnigg and colleagues (2008). In that review, the authors evaluated the relationship between *smokeless tobacco* products and non-cancerous oral diseases in Europe and the U.S. The reviewers concluded that the available evidence confirms a strong association of current use of *smokeless tobacco*, particularly snuff, with prevalence of oral mucosal lesions. Among the 15 Scandinavian studies

described in the review, the severity of the snuff induced lesions was associated with the length of time snuff was used and with the amount consumed per day. The severity was lower in users of portion-bag snuff than in users of loose snuff.

5.2.3 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:

- No effects of snus use were on gingivitis, gingival recessions, and other dental conditions were consistently identified among studies that controlled for important confounders such as socioeconomic status (SES) and oral hygiene habits.
- The use of snus is not associated with periodontal disease or any individual indicators of periodontal disease based on the results of seven studies, five of which accounted for the potential confounding effects of SES or oral hygiene habits.
- Swedish snus causes a characteristic type of oral mucosal lesion that regress following cessation of snus use. There is no evidence that they progress to cancer, even with long-term 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.
- A limitation in the available data is that the studies are largely descriptive in nature (e.g., cross-sectional), and some studies have important limitations including small sample sizes, and failure to control for important confounders.
- The available studies do not indicate that snuff user's lesions were associated with an increase in biomarkers that indicate progression to malignancy.

5.3 Cancer

As previously discussed, snus contains low levels of several animal carcinogens, including TSNAs. The potential association between snus use and increased risk of cancer has been an area of active research for over 50 years. This section discusses the epidemiological studies that have been published to examine on the relationship between snus and various types of cancer.

5.3.1 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)[106]. 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.

5.3.1.1 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

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

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).[107]

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. This was discussed previously in Section 5.2.2.5. 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 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

[107] 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

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. The strengths of this cohort include 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. Despite the large sample size, however, this finding was based on only 10 exposed cases of oral cancer. Additionally, as noted repeatedly with this cohort, 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 equivalent to that of 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 large body of evidence finds that there is no consistent finding of an 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. Other meta-analyses (Lee 2011; 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."

5.3.1.2 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 Swedish Construction Worker 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 body mass index (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. Smoking is considered to be a well-established and stronger risk factor for squamous cell carcinoma of the esophagus compared to the adenocarcinoma subtype (Surgeon General 2004). 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, 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 more recent meta-analyses (Lee 2011; 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.

5.3.1.3 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, one underlying assumption is that a causal relationship between an exposure and outcome exists; this often has not been established (Hennekens and Buring 1987). In addition, if other important risks for the disease are not examined in the same study, the purported risk factor may be taken out of context of other important risk factors for the outcome of interest. 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, as discussed in the above sections of the report, 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.

5.3.2 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.

5.3.2.1 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, two additional meta-analyses that examined risk of pancreatic cancer risk among North American and European smokeless tobacco users (Lee and Hamling 2009b) or among snus users only (Lee 2011), reported no significantly elevated summary risk of pancreatic cancer among smokeless tobacco users using smoking adjusted risk estimates or those restricted to never smokers. Boffetta and colleagues (2008) selected one of each of the two relative risk estimates (either smoking-adjusted or restricted to never smokers) from each study: the smoking-adjusted estimate from Boffetta et al. (2005) and the never-smokers estimate from the Luo et al. (2007), both of which were the higher point estimate from each study.

Additional, related evidence comes from a recent pooled analysis, in which data from 11 case-control studies of pancreatic cancer throughout North America, Europe and Australia were pooled to examine tobacco use and risk of pancreatic cancer (Bertuccio et al. 2011). Data were available on smokeless tobacco (snuff, chewing tobacco, or both) from 6 of the 11 studies. Though it is unlikely that Swedish snuff was a major product used in any of the populations included in the analysis, these results are potentially relevant with respect to Swedish snus in that smokeless tobacco used in North America and other western countries are expected to contain more TSNAs than Swedish snus. TSNAs are thought to be the components of tobacco products that are likely associated with an increased risk of pancreatic cancer.

In the pooled analysis, odds ratios were estimated and adjusted for major potential confounders available from the individual studies, including age, sex, education, race/ethnicity, BMI, history of diabetes, and total alcohol consumption. No increased risk of pancreatic cancer was observed among ever (OR=0.98, 95% CI: 0.75-1.3) or exclusive (OR= 0.62, 95% CI: 0.37-1.04) smokeless tobacco users. The authors state that their "results on smokeless tobacco use are in broad agreement" with the recently published meta-analysis of all published data by Sponsiello-Wang et al. (2008), and conclude that "while based on small numbers, no significant association emerged for pipe smoking and smokeless tobacco use." Additional strengths of this pooled analysis include the availability and use of data from individual studies, adequate control of important potential confounders for pancreatic cancer, and the confirmed association with cigarette smoking. Note also that the odds ratio for the association between smoking and pancreatic cancer (OR=1.50, 95% CI: 1.39-1.62), was of the same magnitude observed in other studies of this risk factor for pancreatic cancer (Friedman et al. 1997; McLaughlin et al. 1995) which can be used as an indicator of the adequacy of the tobacco-related exposure assessment and other methodology of this study. Though not specific to Swedish snus, this pooled analysis contributes additional evidence that smokeless tobacco of any type is likely to confer less risk for pancreatic cancer than smoking, if an excess risk exists at all.

5.3.3 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 report 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.

5.3.4 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.

5.3.5 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) did not observe 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.

5.3.6 Other Cancers

Seven studies have examined the effect of snus use on risks of types of cancer other than those that have been discussed previously; 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, possibly 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[108] among approximately 10,000 Swedish men. With respect to smoke-related cancers, a significantly elevated risk was

[108] Smoke-related cancers, designated by the authors, include: oral & pharyngeal (ICD7 (International Classification of Diseases): 140-148), esophageal & gastric (ICD7: 150-151), pancreatic (ICD7: 157), laryngeal and pulmonary (ICD7: 161-162), kidney, bladder & other urinary organs (ICD7: 180-181)

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 alone was significantly associated with both the development of any cancer and smoke-related cancers in the analysis. For any cancer type, no excess risk was observed among ever-daily snus users among never-smokers and snus users that included some smokers. Residual confounding from smoking or misclassification of tobacco use are important concerns, nonetheless, the authors concluded 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 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.

Nordenvall and colleagues (2010) examined the impact of smoking and snus use on anal and colorectal cancer incidence among 336,381 males in the Swedish construction worker cohort. There was no excess risk of colon (RR = 1.08; 95% CI: 0.91-1.29), rectal (RR = 1.05; 95% CI: 0.85-1.31), or anal (RR = 0.61; 95% CI: 0.07-5.07) cancer among exclusive users of snus. No dose-response relationships were observed based on duration of snus use at inclusion, however a significantly elevated risk was observed for the left-sided colon sub-site among snus users with 35-44 years of total estimated snus use at inclusion and during follow-up. A significant excess was not observed among the group with at least 45 years of total estimated snus use. The authors commented that the results among the 35-44 year group were imprecise, that multiple significance testing may have generated borderline significant results by chance, and that larger studies were warranted.

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. The summary risk for overall cancer among snuff users as used in Scandinavia was also not significantly elevated.

5.3.7 Summary of Epidemiological Studies on Cancer Outcomes

The following conclusions can be drawn about the association between snus use and potential cancer risks:

- The available evidence suggests that use of Swedish snus is not associated with an increased risk of oral cancer. Results of high-quality epidemiology studies specifically examined the possibility that use of snus causes oral cancer, and found no relationship; only one study found a significant association with oral cancer. Several meta-analyses restricted to Swedish snus did not find a significantly increased risk of oral cancer, and other public health committees have agreed that snus does not increase the risk of oral cancer.
- Well controlled epidemiological evidence indicates that Swedish snus is not associated with lung cancer.
- Four analytic epidemiology studies have examined the relationship between snus use and esophageal cancer; one study, of the Swedish Construction Worker cohort, found evidence of a significant association with one type of esophageal cancer (squamous cell, the subtype most strongly associated with smoking), but not another type (esophageal adenocarcinoma). The meta-analysis that used this squamous cell finding result reported an increased summary risk estimate, whereas the meta-analyses that used the combined cell type risk estimates from the individual studies did not report an increased summary risk estimate for esophageal cancer. Overall, the epidemiology studies suggest no association between snus use and esophageal cancer, but limitations in the available studies, and inconsistent results of the meta-analyses indicate a need for additional study of this outcome.
- 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). As with esophageal cancer, the authors of one of the meta-analyses chose different risk estimates from other researchers, who combined like risk estimates and did not observe an increased risk of pancreatic cancer among snus users, or among smokeless tobacco users in the US and other Western populations. Combined with evidence from a recent pooled analysis of the risk of pancreatic cancer among smokeless tobacco users in other Western populations, the available evidence suggests that snus and other smokeless tobacco forms are not associated with pancreatic cancer.

- For stomach cancer, no studies found that use of snus was associated with any significant increase in risk of overall or cardia stomach cancer (cardia is the upper portion of the stomach), but one study found an elevated risk for the noncardia subtype of stomach cancer. These data suggest no association between snus use and stomach cancer overall, but additional research will help confirm whether the finding for the noncardia subtype is real.
- Several other cancer endpoints have been evaluated in a limited number of studies (kidney and bladder cancer, laryngeal cancer, hematopoietic cancers, skin cancers, anal cancer, colorectal cancer, all cancers combined). The only statistically significant increase in risk associated with the use of snus and a specific cancer was for Hodgkin's disease 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 any 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 concluded that relative risks are consistently lower for snus users than those associated with smoking cigarettes.

5.4 Cardiovascular Effects (Risk Factors and Disease)

The use of snus and its association with cardiovascular-related markers and endpoints has been investigated in a number of epidemiological studies. This section reviews studies of snus use and risk of the acute cardiovascular effects of increased heart rate and blood pressure, effects on biochemical markers such as lipid profiles and insulin resistance, longer-term risk factors for cardiovascular disease (CVD) such as high blood pressure and hypertension and obesity, and chronic cardiovascular diseases such as myocardial infarction, coronary heart disease (CHD), stroke, sudden cardiac death (SCD) and total cardiovascular death.

Snus contains nicotine, which is known to have effects on vasoregulation, cardiac control, and autonomic homeostasis (Benowitz 2008). The evidence suggests that smoking can alter biochemical risk factors of cardiovascular disease such as lipid and lipoprotein profiles, can contribute to insulin resistance in smokers, and is known to cause increased risk of CVD (Campbell et al. 2008; Pope et al. 2009), so it is of interest to understand the potential effects of snus use on the cardiovascular system as well. Other than the commonality of nicotine, however, smoke from cigarettes contains numerous additional cardiovascular system toxins that

are not found in snus, including carbon monoxide and fine particulate matter (PM), via inhalation exposure.

The body of published literature examining the relationship between use of snus and the various measures of CVD risk and disease outcomes includes four experimental/clinical studies, two cohort studies, two case-control study, and twelve cross-sectional studies. Specific outcomes studied include acute cardiovascular effects (e.g., elevated blood pressure and heart rate), long-term risk factors for CVD (e.g., fibrinolytic activity, hypertension, obesity/BMI, cholesterol levels), and chronic CVDs (e.g., myocardial infarction (MI), coronary heart disease (CHD), sudden cardiac death (SCD), and total cardiovascular death).

Of the cross-sectional 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). Two of the cross-sectional studies (Angman and Eliasson 2008; Eliasson et al. 1995) use data from the MONICA Study (Monitoring Trends and Determinants in Cardiovascular Disease). The same population of male Swedish construction workers was utilized for several cross-sectional and prospective analyses (Arefalk et al. 2012; Bolinder et al. 1994; Bolinder et al. 1992; Hergens et al. 2007; Hergens et al. 2008b). Additional studies on cardiovascular effects were reported using the Swedish Twin cohort (Hansson et al. 2009); the Swedish Survey of Living Conditions data (Haglund et al. 2007; Johansson et al. 2005); and the Malmö cohort (Janzon and Hedblad 2009).

All studies considered in this section are summarized in detail in Appendices V-J1-J7 (CVD parameters), and K1-K2 (CVD disease outcomes), grouped by study type (cross-sectional, case-control, cohort, and experimental studies). In addition, the results from all of these studies for the various short- and long-term cardiovascular-related parameters and outcomes are presented below, in Table 5- 3 and Table 5- 4, categorized by whether the findings of each study by CVD parameter, were statistically significant. With the exception of studies of acute effects on blood pressure and heart rate, the results presented in the tables are limited to those studies that control for current smoking among snus users either by restricting the analysis to exclusive snus users or through multivariate analyses; studies that do not control for current smoking were excluded and are listed in a table footnote.

In addition to the tables listing the findings that are statistically significant, Appendix I contains a listing, by the same CVD parameters, of the relative risk estimates, where available from the studies, including subanalyses by daily intake, age, and other subgroup analyses. Note that many studies did not provide this type of summary statistic.

In addition to these two results tables, the remainder of this section presents the discussion of the findings by the short- and long-term CVD effects.

5.4.1 Risk Factors for CVD

5.4.1.1 Blood Pressure and Heart Rate

Acute 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, it is expected that snus use would produce an increase in heart rate and blood pressure in users. 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). Benowitz (2008) reported that smokers may develop at least partial tolerance to the acceleration of heart rate produced by nicotine.

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). Four experimental (clinical) studies examined heart rate and blood pressure in a clinical setting after short term exposure to snus or analyzed data stratified by time since exposure; three of the studies were conducted in snus users (Bolinder et al. 1997b; Hirsch et al. 1992; Rohani and Agewall 2004), and the remaining study examined snus use in smokers (Lunell and Curvall 2011). In this latter study, Lunell and Curvall (2011) measured heart rate on several occasions among smokers who were given portioned snus following a 12-hour abstinence from smoking. After administration of snus for 30 minutes, participants' heart rates increased on average about 9 beats per minute, and reached a maximum after 20 minutes. After 30 minutes, heart rates had leveled out and did not continue to increase among study participants.

Rohani and Agewall (2004) conducted a randomized cross-over study in which 20 snuff users had brachial artery dilation, blood pressure and heart rate measured compared to their baseline, following administration of pouched moist snuff (assumed to be Swedish snus). Heart rate and blood pressure were significantly increased at 20 minutes, and heart rate was significantly increased at 35 minutes. Additionally, 10 participants were given placebo, and the same measurements were obtained; the authors reported that no significant changes in heart rate, blood pressure, or flow-mediated dilation were observed following use of the placebo (though the data were not presented).

In one of three studies among fire fighters ages 35-60 years old, Bolinder and colleagues (1997b) reported that after adjusting for confounders, heart rates of the 48 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. They noted, however, that smokeless tobacco does not appear to influence exercise capacity in healthy, physically trained participants.

Hirsch et al. (1992) conducted a clinical study among 9 habitual snus users who refrained from use for 9 hours, and then were administered snus and measurements obtained at rest and following an exercise test. Both systolic and diastolic blood pressure were "markedly increased" after snuff intake, and heart rate increased by approximately 25% after 15-30 minutes. During exercise, heart rate, but not blood pressure increased when comparing snuff intake to no intake. The authors reported that the differences in blood pressure tended to disappear, and concluded that snuff intake was associated with significant short-term hemodynamic effects during rest but not during exercise.

In one additional clinical study, Sundstrom and colleagues (2012) investigated the acute effects of snuff use on ventricular heart function, heart rate and blood pressure among 27 men and four women who were habitual snus users (smoking habits were not described). Systolic and diastolic function was examined using echocardiography. The results of these measures were recorded at four different times: before snuff intake, 5 and 30 minutes after placing snuff in their mouth, and 30 minutes after snuff withdrawal from their mouth. Heart rate and blood pressure were not significantly increased at any of the three time points following snuff use compared to pre-snuff measurements. With respect to ventricular function, the authors reported a transient decrease in diastolic heart function attributed primarily to a statistically significant decrease in E/A ratio (the ratio between early (E) and late (atrial - A) ventricular filling velocity) at 5 and 30 minutes following snuff consumption and the delay in left- and right ventricular relaxation. The authors noted that these results, along with the finding that systolic function was unaffected following snuff use, are consistent with findings observed among cigarette smokers; the authors cited numerous references for this comparison to smokers, though this study did not examine and compare snuff users with either smokers or unexposed controls (nontobacco users). The authors also point out that even though the diastolic heart function parameters tended towards a pattern of impairment following the use of snuff compared to each participants own baseline, these parameters were still within the normal range of function (e.g. the E/A ratio following snuff use was not considered clinically abnormal). The authors state that potential effects of snuff use on diastolic heart function still remains to be explored, and that additional research is needed to determine whether long term snuff users have an increased risk of developing diastolic heart failure compared to non-snuffers. Because this study is not controlled and did not examine other tobacco use groups or an unexposed control in addition to the regular snus users (whose smoking habits were not described), the findings are limited, and additional study of this potential effect is needed. Altered E/A ratios have been identified, however, as a risk factor for sudden cardiac death.

Other data that support the conclusion that use of STP causes transient increases in blood pressure and increases in heart rate come from studies of smokeless tobacco in the US (Martin et al. 2010; Piano et al. 2010). Martin et al. (2010) studied the acute effects of STP on potential increases in central aortic pressure, which has recently been identified as a strong predictor of cardiovascular disease risk, and may not be strongly correlated with peripheral (brachial) blood pressure measurements. These authors found that heart rate, central aortic, blood pressure and peripheral blood pressure values were all significantly, but transiently, elevated after one time STP use. These authors, and Piano et al. (2010), point out that US smokeless tobacco is different from Swedish snus, and contains licorice, specifically glycyrrhetinic acid, which may increase sodium retention and blood pressure in a dose-dependent manner. They further note that differences in sodium content among the various forms of tobacco could also explain differences in effects which might also contribute to effects on heart rate and blood pressure.

***Effects on Blood Pressure and Heart Rate*:** Nonacute increases in blood pressure and heart rate were examined in one cohort study and a number of cross-sectional studies (see Table 5- 3 and Appendix I and Appendix J). In these studies, mean or median blood pressure and heart rates were compared among snus users and nontobacco users (and often among smokers as well), or relative risks for higher blood pressure or hypertension among snus users compared to nontobacco users were examined.

In another study of the fire fighters, reported by Bolinder and de Faire (1998), ambulatory blood pressure monitoring was recorded over a 24-hour period, blood pressure measurements obtained during the daytime (presumably while using "smokeless tobacco") were compared to those obtained at night (presumably while abstinent). The authors reported that diastolic blood pressure was significantly higher in snus users compared to nonusers of tobacco during the 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, and overall, the mean systolic blood pressure was higher than among 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 reported between blood pressure among the snus users and blood cotinine levels (the main nicotine metabolite), implying that the level of use was associated with these effects.

Several additional studies did not identify group differences in pulse rate (Bolinder et al. 1997a; Eliasson et al. 1991) or systolic or diastolic blood pressure between men who were either snuff users or nonusers of tobacco (Bolinder et al. 1997a; Eliasson et al. 1991; Wennmalm et al. 1991), and an additional study found no elevation in blood pressure of exclusive snus users compared to non-users of tobacco (Angman and Eliasson 2008, English-only abstract). In this study, male participants in the MONICA cohort who were exclusive snus users did not have higher mean systolic or diastolic blood pressure compared to never tobacco users.

Three additional studies examined blood pressure among snus users (Janzon and Hedblad 2009; Norberg et al. 2006; Sundstrom et al. 2012), however, none of the authors specified whether the participants are exclusive users of snus, and/or are not current smokers. Therefore, these studies were excluded from the summary table due to the potential confounding effect of current smoking.

In summary, though there appears to be acute increases in heart rate and blood pressure, it is not clear if blood pressure is elevated among regular snus users. A single cross sectional study reported higher mean blood pressure and heart rates among snus users, but several additional studies did not identify group differences when compared to nontobacco users.

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. Hypertension is generally defined as systolic blood pressure (BP) greater than or equal to 140 mm Hg, or diastolic BP greater than or equal to 90 mm Hg, observed on repeated measurements, or currently taking medication to lower high BP.

A single cohort study, reported by Hergens and colleagues (2008b) examined the risk of developing hypertension prospectively in the Swedish Construction Workers cohort with follow-up through 2003. In this prospective study, Hergens et al. (2008b) examined prevalent and incident hypertension among those participants 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 higher

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. For the overall cohort free of hypertension at baseline, the risk of developing hypertension during follow-up among the snus users was not elevated (RR=1.01, 95% CI: 0.91-1.33), and no dose-response was apparent. When the analysis was limited to the cohort participants with repeat visits, 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. Selection bias is possible if those who are more concerned with their blood pressure or health in general were more likely to participate in follow-up visits, which could overestimate the risk of hypertension among snus users. This study has also been criticized by Rodu and Heavner (2009) as containing errors and omissions that may have affected the study findings, including the exclusion of baseline participants who did not have a follow-up visit. In the response by Hergens and colleagues with some corrections, they presented relative risk estimates for those excluded from the analysis of those with repeat visits which was increased (RR=1.32, 95% CI: 0.98-1.79), but not statistically significant, and stood by their conclusion of increased risk of blood pressure effects among “snuff” users (Hergens et al. 2009).

Bolinder and colleagues (1992) also exampled the Swedish Construction Workers Cohort. The authors examined, cross-sectionally, the potential association between a disability pension for hypertension and snus use. Among 45-55 year old men, disability due to hypertension was significantly associated with the use of snus (OR=3.0, 95% CI: 1.9-4.9). The prevalence of Diastolic and systolic blood pressure above 90 and 160, respectively, was statistically significantly increased among snus users of two different age groups (45-55 and 56-65) compared to non-users of snus.

Hergens and colleagues (2005) used the controls from a case-control study of myocardial infarction that included 1,810 randomly selected men from two Swedish counties (Stockholm and Västernorrland) to conduct a cross-sectional analysis to describe the prevalence of certain risk factors for myocardial infarction among snuff users. Of the controls who used snuff, after controlling for smoking, the prevalence of hypertension was significantly higher compared to never-users of snuff (OR = 1.80; 95% CI: 1.30-2.50).

Two additional studies, conducted by Norberg and colleagues (2006) and Janzan and Hedblad (2009), were also considered, however, these were excluded from the table and analysis because neither appear to adequately adjust for current smoking. In both of these studies, no statistically significant increased risk of hypertension among snus users was observed, but the potential influence of smoking was either not controlled or not stated whether it was controlled for, and smokers are known to have a lower risk of hypertension than nontobacco users. Whether this is also the case for snus users is not clear. The single prospective study of hypertension, which did find an increased risk of hypertension among current snus users, was conducted in the Swedish Construction Workers cohort, which has tended to have positive findings in contrast to the findings of studies in other cohorts (Lee 2011).

5.4.1.2 CVD Biochemical Risk Factors

A number of different biomarkers are used to indicate CVD characteristics of individuals or to predict CVD risk. Several epidemiological studies were identified in which biochemical CVD markers were examined among snus users and compared to non-tobacco users. No prospective studies in which biochemical markers of CVD were measured repeatedly at different time points among snus users were identified. The one experimental study identified that measured a CVD marker was confounded by smoking (Rohani and Agewall 2004). There are several cross-sectional studies, and one case-control study in which the association between snus use and several CVD risk factors were examined among the controls (Hergens et al. 2005) (Table 5-3).

Lipid measurements (cholesterol, triglycerides): Several cross-sectional studies examined lipid measurements (high-density lipoprotein (HDL) or low-density lipoprotein (LDL)), triglycerides, or apolipoproteins (Bolinder 1997; Bolinder et al. 1997a; Eliasson et al. 1991; Eliasson et al. 1995). One case-control study examined whether controls who were snus users had increased risk of hyperlipidemia compared to controls who never used snus, controlling for smoking in multivariate analysis (Hergens et al. 2005). None of these studies reported increased prevalence of these lipid measurements among snus users compared to the nontobacco users.

Clotting measures and atherosclerosis: Several cross-sectional studies examined biochemical or physical measures of clotting or of atherosclerosis among snus users compared to nontobacco users; these include indicators such as carotid artery diameters and lumen thickness, to which may indicate increased risk of CVD events (Bolinder 1997; Bolinder et al. 1997a; Eliasson et al. 1991; Eliasson et al. 1995; Wennmalm et al. 1991). In these studies, none reported a significant difference between snus users and nontobacco users. 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 firefighters 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).

5.4.1.3 Studies of Insulin or Impaired Glucose Tolerance

The relationship between snus use and insulin resistance or impaired glucose tolerance has been examined in four cross-sectional studies of risk factors for CVD (Bolinder 1997; Eliasson et al. 1991; Eliasson et al. 1995; Persson et al. 2000) and one prospective cohort study (Eliasson et al. 2004) (that also had a cross-sectional component). All five studies that examined impaired glucose tolerance or glucose levels found no statistically significant differences between snus users and non-users of tobacco. Three out of the four studies that examined the relationship between snuff use and insulin resistance found no significant differences between snuff users and non-users of tobacco. Only Eliasson and colleagues (1991) suggested that serum insulin levels may be somewhat higher in snus users compared to nonusers of tobacco. Because of the cross-sectional nature of this study, it is not possible to determine whether the snus use preceded or followed the observed increase in insulin, but it

appears that snus is not associated with these measures of insulin resistance or glucose impairment.

5.4.1.4 Other Indicators of Cardiovascular Disease Risk

Numerous studies have examined the use of snus on other types of indicators of cardiovascular health, sometimes as part of studies of other related outcomes, such as diabetes (see Table 5-3 and Appendix I and Appendix J). A Swedish study of cardiovascular work capacity among healthy fire fighters 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. Using this same group of participants, Bolinder and de Faire (1998), reported that snuff users were not at an increased risk of having low physical capacity compared to non-users of tobacco. Wennmalm and colleagues (1991) also found that snuff users did not differ significantly with respect to maximum work capacity compared to non-users of tobacco.

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.

In a study of participants from the Northern Swedish Cohort, Gustafsson and colleagues (2011a) examined demographic and behavioral factors that affected allostatic load. In addition to biologic parameters such as systolic and diastolic blood pressure, fasting glucose, and blood lipid measurements in participants, salivary cortisol concentrations used as a measure of total cortisol secretion, were summed in an index used as a measure of allostatic load. Sociodemographic variables and behaviors, including snus use and smoking, were examined in a multivariate model as predictors of allostatic load. Smoking, but not snus use, was found to be a significant predictor of allostatic load (stress) in men. In women, neither tobacco type was significantly associated with allostatic load.

5.4.1.5 Risk Factors: Discussion and Conclusions

There were several studies excluded due to potential confounding due to smoking. Wallenfeldt et al. (2001) was excluded due to the fact that approximately 29% the population of snuff users examined in the study were current smokers, though 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) identified in this study. The only significant finding in this study was that never-snuff users had lower serum triglyceride levels than previous or current snuff-takers. Norberg et al. (2006) also examined triglyceride levels, fasting blood glucose and cholesterol levels among snus users, however, this study was also excluded because it is unclear that the authors adjusted for current smoking. Additionally, an experimental study of 20 healthy, middle-aged men and women suggests that acute use of Swedish snuff may be associated with endothelial dysfunction, but the study's authors do not

describe the smoking status of the participants, and therefore, the results of this study were excluded from the table (Rohani and Agewall 2004).

Marano and colleagues (2011) evaluated CVD biomarkers in adult healthy US smokeless tobacco consumers, utilizing data from two NHANES surveys as well as an internal cross-sectional study, compared to nontobacco users. In this analysis, CVD biomarkers in the smokeless tobacco users compared to the non-tobacco users were not found to be significantly different uniformly (consistently) from the nonusers in all three of the data sets for any of the various biomarkers considered, including lipid measurements, clotting measures or markers of inflammation like CRP and white blood cell counts. A few significant differences were observed among smokeless tobacco users and nontobacco users for any one of the individual data sets, for example, triglycerides were lower in smokeless tobacco users compare to nontobacco users for the NHANES 1999-2008 subset. Homocysteine was significantly higher among smokeless users compared to nontobacco users for the full NHANES dataset used in the study. The only exception, where a statistically significant difference was found for smokeless users compared to nonusers uniformly across the three datasets, was for folate. Smokeless tobacco users had lower blood folate compared to nontobacco users.

Generally, most of the available evidence does not indicate an association between use of snus and a wide range of risk factors for atherosclerosis or CVD. This included studies of risk factors for atherosclerosis (serum lipids, fibrinogen levels, fibrinolytic activity, glucose levels, insulin resistance, thromboxane A_2 production), which 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. 1991; Eliasson et al. 1995; Eliasson et al. 2004; Hergens et al. 2005; Persson et al. 2000; Wennmalm et al. 1991). One study reported a significantly higher level of serum insulin among snuff users compared to non-users of tobacco, and another study (which may have been confounded with smoking) serum triglyceride levels were lower in nontobacco users than previous or current snuff-takers.

Eriksson et al. (2011) reported on trends in major cardiovascular risk factors using data from repeated studies in the MONICA cohort. They noted that the incidence of cardiovascular disease in Northern Sweden had declined 69% in men and 45% in women since 1985. In this analysis, they examined trends in blood pressure and hypertension, tobacco use (smoking only), cholesterol, BMI/obesity, self-reported diabetes, and education. Among participants 25-64 years old, the prevalence of regular smoking declined significantly in both men and women from 1986-2009, and these observed significant reductions in systolic and diastolic blood pressure, the use of blood-pressure lowering medications among men, cholesterol levels and the use of cholesterol-lowering medications, and a significant increase in the overall education level of the study participants. Although the potential role of snus in CVD risk was not specifically examined, Eriksson and colleagues noted that the rate of smoking decline was "initially achieved by increasing use of snus, although recently all tobacco use has started to decrease." The authors further note, "As CVD risk with snus use is probably very low (36) [Wennberg et al. 2007], we believe that a reduction in smoking is an important explanation of the lower CVD mortality in northern Sweden" (Eriksson et al. 2011).

Data derived from cross-sectional 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. However, even with these cautions and limitations, it does not appear that the use of snus impacts indicators of long-term cardiovascular risk.

Table 5-3: Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users in which Current Cigarette Smoking is Adequately Addressed

Cardiovascular Outcome	Statistically Significant* Association with Snus Use Found	No Statistically Significant Association with Snus Use Found
Indicators of, or Risk Factors for Cardiovascular Disease		
Acute effects on heart rate	**4 Experimental studies** *Bolinder et al. (1997b)* *Hirsch et al. (1992)*[$] *Lunell and Curvall (2011)*[$] *Rohani and Agewall (2004)*[$]	**1 Experimental Study** *Sundstrom et al. (2012)*[$]
Acute effects on blood pressure	**3 Experimental studies** *Bolinder et al. (1997b)* *Hirsch et al. (1992)*[$] *Rohani and Agewall (2004)*[$]	**1 Experimental Study** *Sundstrom et al. (2012)*[$]
Heart rate[#]	**1 cross-sectional study** *Bolinder and de Faire (1998)*	**3 cross-sectional studies** *Eliasson et al. (1991)* Bolinder et al. (1997a) *Bolinder et al. (1997b)*
Blood pressure[#]	**1 cross-sectional study** *Bolinder and de Faire (1998)*	**4 cross-sectional studies** Ängman and Eliasson (2008) *Eliasson et al. (1991)* Wennmalm et al. (1991) Bolinder et al. (1997a) *Bolinder et al. (1997b)*
Hypertension	**1 cross-sectional study** Bolinder et al. (1992) **1 case-control study** *Hergens et al. (2005)*** **1 cohort study** Hergens et al. (2008b)	
Atherosclerosis or atherosclerotic index		**1 cross-sectional study** Bolinder et al. (1997a) Bolinder (1997)
Cholesterol/hyperlipidemia/high density lipoprotein (HDL) or low density lipoprotein (LDL)		**3 cross-sectional studies** Bolinder et al. (1997a) *Eliasson et al. (1991)* *Eliasson et al. (1995)* **1 case-control study** *Hergens et al. (2005)***

Table 5-3: Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users in which Current Cigarette Smoking is Adequately Addressed		
Cardiovascular Outcome	**Statistically Significant* Association with Snus Use Found**	**No Statistically Significant Association with Snus Use Found**
Triglycerides		**3 cross-sectional studies** Bolinder et al. (1997a) *Eliasson et al. (1991)* *Eliasson et al. (1995)*
Apolipoproteins		**1 cross-sectional study** Bolinder et al. (1997a)
Fibrinolytic activity		**3 cross-sectional studies** Bolinder et al. (1997a) *Eliasson et al. (1995)* *Eliasson et al. (1991)*
Thromboxane A_2 production (possibly reflecting platelet activation)		**1 cross-sectional study** Wennmalm et al. (1991)
Glucose levels		**5 cross-sectional studies** Bolinder (1997) *Eliasson et al. (1995)* *Eliasson et al. (1991)* Persson et al. (2000) Eliasson et al. (2004)
Insulin resistance or insulin response	**1 cross-sectional study** *Eliasson et al. (1991)*	**3 cross-sectional studies** Bolinder (1997) *Eliasson et al. (1995)* Eliasson et al. (2004)
High white blood cell count		**1 cross-sectional study** *Eliasson et al. (1991)*
Metabolic syndrome		**2 cross-sectional studies** Wandell et al. (2008) Gustafsson et al. (2011b)
Diabetes	**1 cross-sectional study** Persson et al. (2000) **1 cohort study** Ostenson et al. (2012)	**1 case-control study** *Hergens et al. (2005)* **2 cross-sectional studies** Wandell et al. (2008) Eliasson et al. (2004)
Oxygen uptake/work capacity		**3 cross-sectional studies** *Bolinder et al. (1997b)* *Bolinder and de Faire (1998)* Wennmalm et al. (1991)

Table 5-3: Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users in which Current Cigarette Smoking is Adequately Addressed

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)*** **1 cohort study** Hansson et al. (2011) **2 cross-sectional studies** Bolinder et al. (1992) *Vaezghasemi et al. (2012)*	**8 cross-sectional studies** Sundbeck et al. (2009) Bolinder et al. (1997a), *(1997b)* *Eliasson et al. (1995)* *Eliasson et al. (1991)* *Bolinder and de Faire (1998)* Engstrom et al. (2010) Aro et al. (2010) **1 cohort study** Rodu et al. (2004)
Waist-to-Hip Ratio		**5 cross-sectional studies** Bolinder et al. (1997a), *(1997b)* *Eliasson et al. (1995)* Sundbeck et al. (2009) *Bolinder and de Faire (1998)*
Cardiovascular Events		
Incidence of IHD, myocardial infarction (fatal or nonfatal), or heart failure		**4 case-control studies** Hergens et al. (2005) Huhtasaari et al. (1992) Huhtasaari et al. (1999) Wennberg et al. (2007) **5 cohort studies** Hergens et al. (2007) Janzon and Hedblad (2009) Johansson et al. (2005); updated by Haglund et al. (2007) Hansson et al. (2009) Arefalk et al. (2012)
Mortality from all cardiovascular disease	**1 cohort study** Bolinder et al. (1994)	**2 cohort studies** Hansson et al. (2009) Roosaar et al. (2008)
Fatal MI; Sudden cardiac death	**2 cohort studies** Bolinder et al. (1994) Hergens et al. (2007)	**2 case-control studies** Huhtasaari et al. (1999) Wennberg et al. (2007)

* Where available, effect estimates or p-values for current snus users were selected to determine significance, however if any particular subanalysis revealed a significant association for the specified outcome the corresponding study was placed in the statistically significant column.
**Cross-sectional analysis of this outcome in controls only
Some studies provided more than one measure of heart rate and/or blood pressure (e.g. at rest, 24 hours, after work, etc.).
$ Unadjusted for smoking but included for acute experimental studies
Italicized studies include those unadjusted for former smoking or those studies that do not specify that former smokers were excluded from the analyses

Table 5-3: Studies of Cardiovascular Disease Risk Factors and Events among Swedish Snus Users in which Current Cigarette Smoking is Adequately Addressed		
Cardiovascular Outcome	**Statistically Significant* Association with Snus Use Found**	**No Statistically Significant Association with Snus Use Found**
Studies excluded (except for acute effects) due to potential confounding from current smoking (or no information given about concurrent smoking): Attvall et al. (1993) (Insulin Resistance) Hirsch et al. (1992) (Oxygen Uptake/Work Capacity) Janzon and Hedblad (2009) (Blood Pressure, Hypertension) Nafziger et al. (2007) (Body Weight) Norberg et al. (2006) (Hypertension, Cholesterol, Triglycerides, Glucose Levels, Metabolic Syndrome, BMI) Persson et al. (2000) (Insulin Resistance or Insulin Response) Rohani and Agewall (2004) (Impaired Endothelial Function) Saarni et al. (2004) (Body Weight) Sundstrom et al. (2012) (Blood pressure, heart rate, ventricular heart function) Wallenfeldt et al. (2001) (Atherosclerosis, Cholesterol, Glucose Levels, Insulin Resistance or Insulin Response, C-Reactive Protein, Triglycerides, BMI, Waist-Hip Ratio)		

5.4.2 Chronic Cardiovascular Disease

Twelve 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 (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 relative risk for fatal MI observed among heavy "snuff" users using 50 or more g per day was 1.96 (95% CI: 1.08-3.58).

Arefalk and colleagues (2012) examined risk of hospitalization from heart failure in two independent Swedish prospective cohorts, including a sample of 118,425 never-smoking male construction workers from the Swedish Construction Worker Cohort (CWC) and a community based sample of 1,076 elderly men from the Uppsala Longitudinal Study of Adult Men (ULSAM). In the ULSAM study, all 50-year old men were sampled in 1970-73 and reinvestigated in 1991-95; mean age at follow-up was 71 years. Smokeless tobacco use was collected using a self-administered questionnaire. In this study, for the Construction Workers Cohort, participants included those who underwent regular 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. Participants included in this analysis were never-smoking men. The mean age was 31.5 years at baseline. Participants were followed until date of first hospitalization for heart failure, death, emigration, or December 31, 2003.

For both cohorts, the authors presented separate models with further adjustment for potential confounders. In models with full adjustment, current snus use was not significantly associated with risk of heart failure in either cohort, though some results were statistically significant in the simpler models. In particular, for the ULSAM cohort, though the fully adjusted model was not statistically significant, the risk for heart failure was doubled among snus users compared to those who did not use snus, after adjusting for past and current smoking. The risk was most apparent after about age 75 years old. The authors concluded that they observed an increased risk for subsequent heart failure among elderly male users of Swedish snus and a similar, but less pronounced association in a younger and larger cohort of never-smoking men (the Construction Worker Cohort). When snus use was analyzed by dose (in the Construction Workers Cohort only), no apparent trend was observed.

Hansson and colleagues (2009) followed participants in the Swedish Twin Registry, born between 1926-1958, for IHD 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 aged 45-73 years 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 male 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.

5.4.3 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 published; 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; Piano et al. 2010; 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. This was updated in 2011 to account for several additional studies (Haglund et al. 2007; Hansson et al. 2009; Janzon and Hedblad 2009). No evidence for an increased risk of CVD was observed. For ischemic heart disease/acute MI, the summary relative risk estimate was 1.01 (95% CI: 0.91-1.12), and for any CVD, the summary relative risk was 1.08 (0.92-1.27). Lee (2011) concluded that although a "weak effect of snus use on CID remains possible, the overall data are certainly consistent with no effect."

Hansson and colleagues (2012) investigated whether snus use is associated with risk of and survival after (28-day case-fatality) acute myocardial infarction (AMI). The authors conducted a pooled analysis in which the data from eight Swedish prospective cohort studies were pooled and reanalyzed. Relative risks were calculated for 130,361 men who never smoked and were adjusted for age and BMI. Current snus use was not significantly associated with risk of AMI (HR=1.04, 95% CI: 0.93-1.17) or the 28-day case fatality among snus users (OR=1.28, 95% CI: 0.99-1.68). Hazard ratios of, and trends in AMI risk were also not significantly elevated in analyses of intensity and duration of snus use. Snus users, however, had a higher probability of dying as compared to non-users ($p < 0.05$), which occurred during the first 24 hours. The authors stated that confounding by social or life style factors may have biased their findings. For example, they noted that early survival after AMI follow clear socioeconomic gradients, and that snus use, especially in older cohorts, is known to be more common in manual workers. The authors concluded that their findings, based on the largest sample to date, do not support any relationship between use of snus and development of AMI, and that the apparent increase in case fatality may be explained by confounding, though a small increased risk of sudden death from AMI among snus users cannot be ruled out.

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.

Additional information on the potential cardiovascular risk from smokeless tobacco comes from the US studies of STP users. Yatsuya and Folsom (2010) investigated whether current use of US STPs was associated with an increased incidence of CVD in the Atherosclerosis Risk in Communities Study. After adjusting for a limited number of potential confounders, such as demographic and lifestyle characteristics, the authors report a statistically significant increased risk of CVD among current smokeless tobacco users (HR=1.27, 95% CI: 1.06-1.52). After further adjustment for major CVD risk factors, the risk of CVD incidence was attenuated (HR=1.21, 95% CI: 1.00-1.45). The authors concluded that "current use of smokeless tobacco was associated with increased risk of CVD incidence among nonsmokers in the ARIC Study." The authors do not investigate the relationship between smokeless tobacco use and risk of specific CVD outcomes, which would be more useful in drawing stronger conclusions about whether this study finding is consistent with the evidence observed among snus users.

5.4.4 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 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 long-term risk of high blood pressure or hypertension. An increased risk of hypertension was observed in the only available prospective cohort study, but limited to participants with repeated visits, and not the entire cohort, and analyses of this cohort have often produced significant findings where other studies have not.
- Snus does not appear to be associated with atherosclerosis or risk factors for atherosclerosis (e.g. serum lipids, fibrinogen levels, fibrinolytic activity, insulin resistance).
- Most studies have not revealed an increased risk of MI or an overall increased risk of CVD. A single study found an increased risk only for fatal MI in an analysis of the Swedish Construction Worker cohort, and an analysis of heart failure among snus users controlled for smoking observed an increased risk especially in men ages 75 years and older.
- A large, pooled analysis, which pooled data from many of the major Scandinavian cohorts, confirmed previous findings that the use of snus is not associated with an increased risk of MI, and noted that slight increases in fatal MI may be explained by confounding.
- One recent US study observed an increased risk of cardiovascular risk among smokeless tobacco users. Meta-analyses that combine Scandinavian studies of snus users 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.

- Though there are known acute effects of nicotine on the cardiovascular system, no increased risk of cardiovascular disease has been detected epidemiologically, with the possible exception of a moderate increased risk of death due to a CV event. This increased risk of mortality due to a CV event among snus users has only been observed in the Construction Workers Cohort in Sweden, and in a US study of smokeless tobacco users (a category that is unlikely to include a significant proportion of snus users).

5.5 Stroke

5.5.1 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. 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.

5.5.2 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.

5.5.3 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 5-4. 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, and analyses of this cohort have often produced significant findings where other studies have not. In the study by Hansson et al. (2009), 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 5-4: 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. (2008a)* Asplund et al. (2003) Haglund et al. (2007) Hansson et al. (2009) Janzon and Hedblad (2009)
All Fatal		Hergens et al. (2008a) Haglund et al. (2007)
All Nonfatal		Hergens et al. (2008a)
Ischemic		Hergens et al. (2008a)
Ischemic Nonfatal		Hergens et al. (2008a)
Ischemic Fatal	Hergens et al. (2008a)	
Hemorrhagic		Hergens et al. (2008a)
Hemorrhagic Nonfatal		Hergens et al. (2008a)
Hemorrhagic Fatal		Hergens et al. (2008a)
Subarachnoid Hemorrhagic		Koskinen and Blomstedt. (2006)
Dose Response		
All: <12.5 g/day		Hergens et al. (2008a)
12.5-24.9 g/day		Hergens et al. (2008a)
25-49.9 g/day		Hergens et al. (2008a)
>50 g/day		Hergens et al. (2008a)
All: ≤ 4 cans/week		Hansson et al. (2009)
All: ≥ 4 cans/week		Hansson et al. (2009)
Ischemic: <12.5 g/day	Hergens et al. (2008a)	
12.5-24.9 g/day		Hergens et al. (2008a)
25-49.9 g/day		Hergens et al. (2008a)
>50 g/day		Hergens et al. (2008a)
Hemorrhagic: <12.5 g/day		Hergens et al. (2008a)
12.5-24.9 g/day		Hergens et al. (2008a)
25-49.9 g/day		Hergens et al. (2008a)
>50 g/day		Hergens et al. (2008a)
*Both studies report on the Swedish Construction Workers Cohort		

5.6 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 Risk Factors) 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).[109]

5.6.1 Studies of Insulin or Impaired Glucose Tolerance

The relationship between snus use and insulin resistance or impaired glucose tolerance has been examined in five 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), one experimental study (Attvall et al. 1993), and two cohort studies (Eliasson et al. 2004; Norberg et al. 2006), though the analysis in one of the studies is not clearly described (Norberg et al. 2006), included diagnosis of diabetes or impaired glucose levels, and additionally, may be confounded by smoking. Seven of the eight studies found no statistically significant associations between snus and impaired insulin or glucose tolerance, including two studies that examined the association by amount of snus used (Norberg et al. 2006; Persson et al. 2000). One of the cross-sectional studies (Eliasson et al. 1991) suggested that serum insulin levels may be somewhat higher in snus users compared to nonusers of tobacco. Because of the cross-sectional nature of many of these studies, it is not possible to determine whether the snus use preceded or followed the observed increase in insulin, but it appears that snus is not associated with these measures of insulin resistance or glucose impairment.

5.6.2 Studies on Diabetes

In addition to studies evaluating insulin or glucose impairment, five studies of varying designs have evaluated the relationship between Swedish snuff use and type 2 diabetes. These studies are summarized in Appendices M-1 (two descriptive studies), M-2 (two cohort studies), and M-4 (a case-control study).

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

The first cohort study (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. Of the 513 men with normal glucose tolerance at baseline, the risk of developing diabetes during follow-up was significantly increased 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.

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); stronger conclusions can be drawn from such data. The study also validated approximately half of the incident cases using an oral glucose tolerance test and tobacco use was validated biochemically in a subgroup of participants.

The second prospective study, conducted by Ostenson and colleagues (2012), examined the effect of snus use and smoking on type 2 diabetes among 2,383 middle-aged Swedish men without previously diagnosed diabetes. The authors considered cases of type 2 diabetes that were newly-identified via an oral glucose tolerance test (OGTT) at a final examination 10 years following baseline evaluation. Though newly-identified type 2 diabetes risk was not increased among all participants who reported consistent snus use (OR=1.1; 95% CI: 0.6-2.0), high consumption of snus (>5 boxes/week) was significantly associated with an increased risk of developing type 2 diabetes (OR=3.3; 95% CI: 1.4-8.1), but not among those using < 5 boxes per week. These risk estimates were adjusted for important potential confounders such as smoking, age, BMI, glucose tolerance at baseline, physical activity, alcohol consumption, socioeconomic position, and family history of diabetes, with the exception of any dietary variables. When the relationship between high consumption of snus and type 2 diabetes was restricted to never-smoking snus users (n=3), the risk was not significantly increased (OR=2.3; 95% CI: 0.5-9.8), but based on a very limited number of men in this category.

This study presents several limitations. Though the title implies that the study is prospective, in fact, participants who were free of type 2 diabetes at baseline but diagnosed prior to the follow-up examination were not considered in this study (n=84). Only the 99 participants who had newly-discovered type II diabetes following an OGTT at the final examination were included in the analysis of the study, which only tested for an association with self-reported tobacco use categories for outcomes determined at a single time point rather than taking into account risk of developing the disease over time. It is possible that these 84 individuals may have been different compared to the 99 included at follow-up, including tobacco use characteristics. The authors noted that a limitation of the study is the small number of cases developing diabetes, especially when attempting to evaluate the effects of snus in subjects who did not have a record of previous smoking (n=3). Never-smoking was significantly less prevalent among cases. It is

also possible that the results may have been influenced by other characteristics noted among snus users. Consistent snus users had a higher BMI, higher alcohol consumption and a higher frequency of individuals in the lowest socioeconomic position. Former smoking was more prevalent among cases than controls with high consumption of snus. This could have influenced BMI, waist circumference or waist-to-hip ratio that could have influenced risk of type 2 diabetes. Body weight (BMI) and central adiposity (waist circumference or waist-to-hip ratio) are both associated with smoking (where BMI tends to be lower in smokers but central adiposity tends to be higher in smokers). Central adiposity in snus users who were never smokers tends to be comparable to nontobacco users, as noted in section 5.8.

In contrast to the prospective study by 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. As discussed previously, Persson and colleagues did not observe impaired glucose tolerance among exclusive snus users, a condition which, as previously discussed, is a recognized precursor 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, though the study had a limited sample size and confidence intervals were imprecise.

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, consistent with the conclusion of Lee (2011), who states that an association between snus use and diabetes is not clearly established.

5.6.3 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 prospective study (a cohort study that generated both prevalence and incidence data) found that use of snus was not associated with increased risk of type 2 diabetes.
- Another prospective study with several limitations reported that the use of snus (adjusted for smoking) was associated with type 2 diabetes, while a significant association was not observed among never-smoking snus users.
- Of the other three epidemiological studies, one study observed a significant increase in the prevalence of type 2 diabetes among snus users, while the other two studies 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).

5.7 Metabolic Syndrome

Three epidemiology studies investigated the relationship between use of snus and 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 after 10 years of follow-up. Several factors were associated with increased risk of having developed MetSy (though it is not clear if those with MetSy at baseline were excluded, see below), 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 statistically significant finding in this study related to tobacco use was that ex-smokers had a significantly elevated prevalence of MetSy; the prevalence of MetSy 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). The number of snus users was small, thus limiting the power of this study.

Gustafsson and colleagues (2011b) investigated risk factors for metabolic syndrome, including use of snuff using a Swedish prospective cohort study that recruited participants at 16 years of age (N = 1071). While other risk factors were assessed periodically during follow-up, snuff use was only assessed at age 43. After controlling for risk factors such as SES, smoking, alcohol use, blood pressure, and BMI, snuff use was not a significant independent contributor to metabolic syndrome.

Lee (2011), in a critical review of the epidemiologic studies of snus, noted that an association of MetSy with high consumption in one study (Norberg et al. 2006) was not seen in another (Wandell et al. 2008). The remaining study, published after the Lee (2011) review, also did not observe an association between snus and MetSy.

5.7.1 Summary of Studies on Metabolic Syndrome

Three epidemiology studies investigated the relationship between use of snus and MetSy. One follow-up study suggests that MetSy may be associated with heavy use of snus while the two other studies did not find an association between MetSy and use of snus.

5.8 Effects on Body Weight

5.8.1 Overview

Multiple studies have examined weight (usually as mean weight or body mass index (BMI)), weight gain, and measures of central adiposity (waist-to-hip (WHR) ratios and waist circumference) in association with snus and smoking (Appendix O). It is recognized that smoking suppresses body weight, and that many people who quit smoking gain weight, and it has been suggested that although body weight is lower in smokers, body composition is different, with smokers having more abdominal fat than nonsmokers (Audrain-McGovern and Benowitz 2011; Chiolero et al. 2008). A dose-response among smokers has been observed, where heavier smokers have higher waist circumference compared to lighter smokers (Clair et al. 2011). Thus, the various measures of body weight (BMI, weight gain or loss) and waist to hip ratios should be examined separately, and the potential confounding effect of smoking, either former smoking or dual use, should be examined carefully as well. Therefore, the following table (Table 5-5) and discussion is limited to findings from studies that adequately account for past and current smoking use. Excluded studies are listed with the reason for exclusion provided.

Table 5-5: Available Studies to Address Questions Regarding Body Weight in Association with Tobacco Use and that Adequately Control for Past and Current Snus and Cigarette Use

Are snus users more likely to gain weight, or have a higher BMI compared to nonusers of tobacco?	Is the WHR or WC of snus users greater than nonusers of tobacco?	Do smokers who quit gain weight or do former smokers have a higher BMI than nonusers of tobacco?	Do smokers who quit cigarettes and switch to snus gain weight?
Yes: Bolinder et al. (1992) -CS: old age BMI >26 **Hansson et al. (2011)** -C Rodu et al. (2004) -CS: prevalence of overweight at entry	**Yes:** None	**Yes**: Aro et al. (2010) -CS (former smokers were heavier than current smokers, but were not significantly different from nonusers of tobacco) **Hansson et al. (2011)** -C **Rodu et al. (2004)** -C Sundbeck et al. (2009) -CS (BMI, WHR and WC)	**Yes:** None
No: Aro et al. (2010) -CS Bolinder et al. (1992) -CS: young age Bolinder et al. (1997a) -CS Engstrom et al. (2010) -CS **Rodu et al. (2004)** -C: development of overweight during follow up Sundbeck et al. (2009) -CS	**No:** Bolinder et al. (1997a) -CS Sundbeck et al. (2009) –CS (WHR and WC)	**No:** None	**No:** **Rodu et al. (2004)** -C Sundbeck et al. (2009) -CS

Abbreviations
CS: Cross-sectional study or cross-sectional analysis
C: Cohort study or prospective analysis (bolded in table)
WHR: Waist-to-hip ratio
WC: Waist circumference

Studies Included (sufficient control for past tobacco use):
Adjusted for former tobacco use: Engstrom et al. (2010)
Exclusive tobacco use (never smoking/snuff use): Aro et al. (2010), Bolinder et al. (1992), Bolinder et al. (1997a) (never-smoking snus users only), Hansson et al. (2011), Rodu et al. (2004), Sundbeck et al. (2009)

Studies Excluded (results for snus users with adequate control for current and/or past tobacco use not presented):
Bolinder et al. (1997a) (smoking only): 5 of 29 smokers were occasional smokeless tobacco users
Bolinder et al. (1997b): 21 of 50 smokeless users were ex-smokers, 7 of 33 smokers were ex- or current smokeless users
Bolinder and de Faire (1998): includes 20 ex-smokers out of 47 smokeless tobacco users, 5 of 29 smokers were ex- or current smokeless users

Table 5-5: Available Studies to Address Questions Regarding Body Weight in Association with Tobacco Use and that Adequately Control for Past and Current Snus and Cigarette Use
Eliasson et al. (1995): weight differences measured across tobacco groups only. Former smokers who quit > 1 year ago were included with snuff users Eliasson et al. (1991): 5 of 21 snuff users were ex-smokers, 1 of 19 smokers was a previous snuff user Hergens et al. (2005) : unclear if adjusted for past smoking; Hansson et al. (2011) stated that it includes current and/or former smokers) Saarni et al. (2004): intentional weight loss Janzon and Hedblad (2009): no control for smoking (included current and former smokers) Wallenfeldt et al. (2001): 29% and 67% of snuff users were current and ex-smokers respectively. Snuff-years among current smokers were 87±232 Norberg et al. (2006): unclear if adjusted for smoking (Hansson et al. 2011 stated that this study includes current and/or former smokers) Nafziger et al. (2007): does not appear to adjust for current or past smoking Vaezghasemi et al. (2012): unclear if adjusted for past smoking

There are two prospective studies of two individual cohorts that adequately controlled for past and current tobacco use. The results of these two studies were mixed. Rodu and colleagues (2004) studied 2,993 Swedish men who participated in the Northern Sweden MONICA study in 1986, 1990, or 1994; 1,650 of whom were followed up in 1999. The authors investigated the relationship between tobacco use (both smoking and use of snus), cessation of these habits, and subsequent weight gain and provided both cross-sectional and prospective data. Compared to participants who were nontobacco users at both baseline and at follow-up, and after adjusting for age and years of follow-up, the authors did not observe an increased risk of becoming overweight (BMI ≥ 27) during follow up among consistent, exclusive snus-using men who were not overweight at entry. Those who were formerly nonusers of tobacco and took up snus during follow-up also did not have an increased risk of gaining weight. Compared to participants who were nontobacco users at both baseline and at follow-up, smokers who quit all tobacco during follow-up (i.e., were smokers at baseline and nontobacco users at follow-up) were significantly more likely to become overweight, with an average annual gain of 0.96%. Baseline smokers who quit smoking and switched to snus were not at increased risk of becoming overweight, with an annual average gain of 0.51%. Though snus users at baseline who quit tobacco altogether during follow-up did not have a significant risk of becoming overweight (SIR=142, 95% CI: 78-264) compared to nontobacco users at both timepoints, an annual average weight gain of 0.70% was observed, which was statistically significant compared to baseline weight.

Hansson and colleagues (2011) studied 9,954 males living in Stockholm County, Sweden recruited in 2002 and reassessed in 2007 as part of the Stockholm Public Health Cohort. The authors examined weight gain and incident obesity in the participants over a 5-year period. Participants self-reported tobacco use and weight at both baseline and at follow-up. A statistically significant weight gain, defined as ≥ 5% increase in body weight, was observed among consistent, exclusive snus users compared to never tobacco users (OR=1.31, 95% CI: 1.04-1.65). In addition, current snus users had a statistically significant increased risk of developing obesity (defined as BMI ≥ 30 kg/m^2) during the follow-up period compared to never tobacco users (OR=1.93, 95% CI: 1.13-3.30). These risk estimates were controlled for several important confounders, including age, alcohol consumption, physical activity, education, and

dietary factors such as consumption of fruit and berries and frequency of having breakfast. Among former snus users and users who quit or began snus use during follow-up, no associations with incident weight gain or obesity were observed. Consistent with other studies, smokers who quit during follow-up had an increased risk of weight gain, and the incidence of becoming obese was of borderline statistical significance.

There are several potential explanations for the observed inconsistencies in results from the two cohort studies. First, it is possible that the results actually reflect a real effect (or lack of effect) of Swedish snus within two different populations during the different time periods of study. The Rodu et al. (2004) study was conducted over an 8-year period, from 1986 to 1994, whereas Hansson and colleagues studied weight gain later, over a 5-year period in the mid-2000s. Differences in the prevalence of overweight and obesity during the different time periods used in the two cohort studies described above have been described in the MONICA cohort. Eriksson and colleagues (2011) reported an increasing prevalence of overweight and obesity in this cohort from 1986 through 2004, which plateaued from 2004 to 2009. A second potentially important difference is the use of different cutpoints for the definition of obesity or overweight. Conventionally, the US Centers for Disease Control defines overweight as an adult who has a BMI between 25 and 29.9, and obesity as an adult who has a BMI ≥ 30 kg/m^2, which was the definition used by Hansson et al. (2011) for obesity; Rodu and colleagues (2004) used a cutpoint of BMI ≥ 27 kg/m^2) to define overweight. Rodu and colleagues (2004) noted that they used a cutpoint of 27 because a lower cutpoint of 25 resulted in a very high (60%) prevalence of overweight in the study population, which they state would have resulted in low specificity and obscured differences in the incidence and prevalence of overweight between tobacco-use groups. Additional potential sources of inconsistency between the two studies may include differences in sample size (9,954 vs. 1,650 for Hansson et al. 2011; Rodu et al. 2004 respectively), differences in control for sources of confounding or bias (Rodu et al. 2004) only adjusted for age and years of follow-up, while Hansson et al. (2011) adjusted for age, alcohol consumption, physical activity, education, and dietary factors such as consumption of fruit and berries and frequency of having breakfast), differences in mean age at baseline (which isn't provided by Rodu et al. 2004), and differences in retention rates (76% vs. 70% for Hansson et al. 2011; Rodu et al. 2004, respectively).

The remaining studies that investigated the relationship between the use of snus and body weight were cross-sectional. As with other health outcomes, cross-sectional studies, which collect outcome and exposure data simultaneously, are useful only to look at associations, and cannot establish a causal relationship between snus use and effects on body weight based on these studies alone. Temporality is difficult to establish as it is not possible to determine if the use of snus came before any observed difference in body weight between snus users and nonusers of tobacco. There are six studies with cross-sectional (prevalence) analyses that compare body-weight-related characteristics of snus users, smokers and non-users of snus at a single point (Aro et al. 2010; Bolinder et al. 1997a; Bolinder et al. 1992; Engstrom et al. 2010; Rodu et al. 2004; Sundbeck et al. 2009). Most of these studies did not have the specific intent of studying the association between weight characteristics and snus.

When comparison of BMI was used as the outcome, most studies reported that snus users did not differ significantly compared to non users, that is, snus users were not more likely to be

overweight (Aro et al. 2010; Bolinder et al. 1992-<36 years of age; Engstrom et al. 2010; Sundbeck et al. 2009). Three studies did report that snus users were either less likely to be underweight (Engstrom et al. 2010) or were more likely to be overweight (Bolinder et al. 1992; Rodu et al. 2004). Consistent with the scientific literature, Bolinder and colleagues (1992) observed that the risk of being overweight among snus users was associated with age, as a higher prevalence of being overweight was reported in those older than 35 years of age, while an increased prevalence was not observed in participants younger than 36 years of age. Rodu and colleagues (2004) observed a significantly increased prevalence of overweight among snus users at baseline, but snus users of normal weight at baseline, as described above, were not more likely than non-users to become overweight during follow-up.

Based on the only two studies that accounted for past and current smoking and assess the relationship between the use of snus and central adiposity (WHR and WC), snus users did not differ significantly from nonusers of tobacco (Bolinder et al. 1997a; Sundbeck et al. 2009).

One additional cross-sectional study (Saarni et al. 2004) found that Finnish men who had a history of using snuff were more likely to report that they had intentionally lost at least 5 kg at least twice. Snuff use was uncommon among women and there was no association with intentional weight loss among women.

Some studies (Aro et al. 2010; Bolinder et al. 1992; Engstrom et al. 2010) but not all (Hansson et al. 2011; Rodu et al. 2004; Sundbeck et al. 2009) observed the expected finding that smokers had lower BMI than nonsmokers.

The literature confirms the known influence of quitting smoking on weight gain (Aro et al. 2010; Hansson et al. 2011; Rodu et al. 2004; Sundbeck et al. 2009), and also suggests that substituting cigarettes with snus after quitting may prevent weight gain (Rodu et al. 2004; Sundbeck et al. 2009).

5.8.2 Summary of Studies on Effects on Body Weight

Numerous cross-sectional and prospective studies have examined the issue of body weight and obesity in association with snus and cigarette smoking. Among studies that controlled for past and current smoking, six of the seven found that BMI of snus users were no different than nontobacco users (Aro et al. 2010; Bolinder et al. 1997a (among younger snus users only); Bolinder et al. 1992; Engstrom et al. 2010; Rodu et al. 2004 (prospective analysis only); Sundbeck et al. 2009), while Hansson et al. (2011) observed that snus users were more likely to gain weight or become obese compared to non users of tobacco, but not among those who took up snus during the follow-up period. Additionally, Rodu et al. (2004) reported a significantly higher BMI of snus users compared to nonusers of tobacco in a cross-sectional analysis and Bolinder et al. (1992) reported a higher BMI among those older than 35 years of age. Two of the studies that looked only at exclusive snus users also reported that the WHR of snus users was not different from nonusers of tobacco, in contrast to the known relationship between smoking and central adiposity (Audrain-McGovern and Benowitz 2011; Chiolero et al. 2008). Another nearly consistent finding is that former smokers had a higher BMI compared to nonusers of tobacco (Aro et al. 2010 (not significantly higher compared to nonusers of tobacco but higher than current smokers); Sundbeck et al. 2009) or smokers who quit during follow-up

gained weight (Hansson et al. 2011; Rodu et al. 2004; Sundbeck et al. 2009). Weight gain among smokers who quit complicates the relationship between snus and weight gain as snus is often used as a smoking cessation aid so it is therefore difficult to examine the expected contribution of smoking cessation to weight gain independently from any potential contribution of snus use.

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

- There is some evidence that suggests snus may be associated with higher BMI or weight gain, among studies that control for past and current smoking. However, overall, the results are mixed.
- Though the results of the two prospective cohort studies that eliminated the effect that smoking (especially former smoking) has on body weight are contradictory, neither reported an increased risk of becoming overweight or obese among non-tobacco users who began using snus during the follow-up period.

A mechanism of how snus could influence body weight remains to be elucidated. None of the studies investigated the relationship between snuff use and total energy intake, a potential confounder. Though a possible association may exist, additional investigations that account for past smoking, energy intake, and other relevant lifestyle behaviors, and that examine the potential effect of snus on metabolism would help clarify the role of snus, if any, on body weight.

5.9 Gastrointestinal Effects

Because saliva produced during the use of snus is often swallowed instead of expectorated, studies of the relationship between snus use and potential gastrointestinal effects should be considered in an evaluation of the potential health effects of snus. Four 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) and Carlens et al. (2010) 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). Aro et al. (2010) examined the relationship between different forms of tobacco (including snus) and upper gastrointestinal (GI) symptoms, histology, and frequency of *H. pylori* infection. The findings of these studies are summarized in Appendices L-1, L-2, and L-3 and are discussed below.

5.9.1 Heartburn and Gastroesophageal Reflux Symptoms (GERS), 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 snuff (8.5) could be important when saliva is swallowed.

Aro and colleagues (2010) also investigated the relationship between the use of snus and GERS and peptic ulcer. The results from this population-based cross-sectional study of a 2,860 sample of adults from two northern Swedish municipalities indicate that current or former use of snus use is not significantly associated with GERS or overall peptic ulcer disease (along with gastric ulcer and duodenal ulcer) compared to never-users of tobacco among never-smokers.

5.9.2 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 and 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, and was no longer significant when the analysis was restricted to never-smokers. 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.

More recently, Carlens and colleagues (2010) conducted a cohort study, and examined the relationship between the use of snus and UC and CD among 277,777 male construction workers in Sweden. In this study, ever use of snus, adjusted for smoking, or among never-smokers was not associated with risk of UC (RR = 1.1, 95% CI: 0.9-1.2 and RR = 1.0, 95% CI: 0.8-1.2 respectively). With respect to CD, Carlens et al. found that ever use of snus, adjusted for smoking, or among never smokers, was not associated with risk of CD (RR = 0.9, 95% CI: 0.8-1.1 and RR = 1.0, 95% CI: 0.8-1.4 respectively). The authors also reported that a dose-response relationship of the amount of snus used was not observed.

5.9.3 Irritable Bowel Syndrome

Aro and colleagues (2010), described previously, also investigated the relationship between the use of snus and irritable bowel syndrome (IBS). The results indicate that current or former use

of snus among never-smokers is not significantly associated with IBS compared to never-users of tobacco.

5.9.4 Other Gastrointestinal Symptoms and Effects

In addition to the GI effects described above, Aro and colleagues (2010) investigated the relationship between the use of snus and other gastrointestinal symptoms including dyspepsia, epigastric pain, abdominal pain, and esophagitis. The results indicate that current or former exclusive use of snus is not significantly associated with any of these symptoms compared to never-users of tobacco. Statistically significant results were found only among study participants who also, or previously, smoked. Aro and colleagues (2010) also investigated the relationship between current snus use and *H. pylori* infection along with some histological markers in a subset of study participants. There was no significant association between current *H. pylori* infection and current snus use. However, current use of snus was significantly associated with hyperplasia of the basal cell layer (OR = 1.74, 95% CI: 1.02-3.00) and with elongation of papillae of the squamous epithelium at the esophago-gastric junction (OR = 1.79, 95% CI: 1.05-3.05) adjusted for GERS, *H. pylori* infection, categorized age, and sex (but does not appear to be controlled for other risk factors such as BMI and use of drugs, which were controlled in other analyses in this study. The authors noted that both of these outcomes are markers of cell turnover due to chronic chemical irritation such as occurs in gastroesophageal reflux disease, though GERS itself was not significantly elevated among current snus users.

5.9.5 Summary of Gastrointestinal Effects

Two descriptive studies of the relationship between snus and heartburn and peptic ulcer showed that users of snus did not have an excess risk of peptic ulcer or heartburn; furthermore, one of those studies showed that snus users had a significantly lower risk of heartburn. A case-control and a cohort study examined the relationship of IBD with oral moist snuff and cigarette smoking in Sweden. These studies found no increased risk of CD or UC associated with snuff use when the analysis was limited to never-smokers. One study found no association between snus use and gastrointestinal symptoms or peptic ulcer disease, but reported increased risk of altered histology of the esophago-gastric junction among exclusive snus users when examined macroscopically in a subset of study participants. This finding needs to be confirmed in additional studies.

5.10 Pregnancy Outcomes and Reproductive Effects

5.10.1 Overview

Six related cohort studies were identified that investigated the relationship between the use of snus and risk of adverse pregnancy outcomes. Five of these studies suggest that women who use snus on a daily basis while pregnant may have an increased risk of some adverse pregnancy outcomes. The earliest study, conducted by England and colleagues (2003), used self-reported data from the Swedish Medical Birth Register to compare the birth outcomes from 1999-2000 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.

More recently, five cohort studies have been published that included the original population investigated by England and colleagues (2003), and expanded this study population to include additional births through 2006 (Gunnerbeck et al. 2011; Wikstrom et al. 2010a; Wikstrom et al. 2010b; Wikstrom et al. 2010c), 2009 (Baba et al. 2012b), and 2010 (Baba et al. 2012a). These studies included approximately 610,000 women, of which approximately 7,600 (1%) were never-smoking snuff users and 58,000 (10%) were smokers (who never used snuff). Baba and colleagues (2012b) examined 776,836 women and Baba and colleagues (2012a) examined 846,411 women.

The findings of these studies are summarized in Appendix P-1 and described below.

- Birth weight (England et al. 2003): 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 (SGA) 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 SGA baby among snuff users was examined by England and colleagues (2003), and was found to be similar to that of nonusers of tobacco (OR=1.25; 95% CI: 0.72-2.17). By comparison, the risk was significantly increased among cigarette smokers (OR=2.99; 95% CI: 2.48-3.61). In the first expanded study, Wikström and colleagues (2010b) again observed that snuff use during pregnancy is not significantly associated with being SGA (OR = 1.17; 95% CI: 0.98-1.39). In the most recent expanded study, Baba and colleagues (2012b) concluded that both smoking, and to a lesser extent, use of snuff during pregnancy increased the risk of an SGA birth. The authors noted that both nicotine and tobacco combustion products are involved in the mechanisms by which maternal tobacco use during pregnancy increases the risk of SGA birth, and that products containing nicotine should be avoided during pregnancy. Women who used snuff (OR = 1.26; 95% CI: 1.09-1.46) or smoked (OR = 2.55; 95% CI: 2.43-2.67) during early pregnancy faced a significantly increased risk of SGA. Snuff use had a stronger association with preterm SGA (OR = 1.50; 95% CI: 1.13-1.98) than term SGA (OR = 1.21; 95% CI: 1.02-1.43), whereas the opposite was true for smoking (Preterm SGA OR = 1.85; 95% CI: 1.67-2.06, Term SGA OR = 2.76; 95% CI: 2.62-2.91). Women who stopped using snuff before their first visit to antenatal care had no increased risks of preterm or term SGA, and women who stopped using snuff later during pregnancy had no increased risk of term SGA. The authors also suggested that tobacco-related risk may have been influenced by unmeasured health-related factors. For example, they noted that compared with non-tobacco users, snuff users and especially smokers are more likely to have low education level and be overweight or obese during pregnancy.
- Stillbirth and antenatal bleeding: Wikström and colleagues (2010a) investigated the relationship between the use of snuff during pregnancy and stillbirth and antenatal bleeding. They found that snuff use was significantly associated with an increased risk of stillbirth (OR = 1.60; 95% CI: 1.13-2.29), but not antenatal bleeding (OR = 1.15; 95% CI: 0.92-1.44). Cigarette smoking was also significantly associated with both, stillbirth (1-9 cigarettes/day: OR = 1.40; 95% CI: 1.17-1.67, >9 cigarettes/day: OR = 2.42; 95% CI: 1.96-2.99) and

antenatal bleeding (1-9 cigarettes/day: OR = 1.51; 95% CI: 1.37-1.66, >9 cigarettes/day: OR = 1.88; 95% CI: 1.65-2.13).

- Preterm delivery: In the earlier study by England and colleagues (2003), 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. In the expanded study, Wikström and colleagues (Wikstrom et al. 2010a) confirmed that snuff use during pregnancy is significantly associated with increased risks of very (OR = 1.38; 95% CI: 1.04-1.83) and moderate (OR = 1.25; 95% CI: 1.12-1.40) preterm birth of both spontaneous (OR = 1.25; 95% CI: 1.10-1.41) and induced (OR = 1.33; 95% CI: 1.10-1.61) onsets, similar to risks observed among smokers. Baba and colleagues (2012b) further expanded this study population through 2009, and reported similar results. The authors also found that women who stop using snuff or stop smoking in early pregnancy reduce their risk of preterm birth. The authors noted that the similarities in risks between snuff users and smokers suggest that antenatal exposure to nicotine is involved in the mechanisms by which maternal use of tobacco increases the risks of preterm birth.
- Preeclampsia and gestational hypertension: In the earlier study, England and colleagues (2003) reported that 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). In the expanded study, Wikström and colleagues (2010a) found that snuff use was not significantly associated with preeclampsia (OR = 1.11; 95% CI: 0.97-1.28) or gestational hypertension (OR = 0.89; 95% CI: 0.68-1.15). In addition, snuff use was not associated with the severity of preeclampsia. Wikström and colleagues (2010b) note that the larger study sample made it possible to estimate preeclampsia risks with more precision and in more detail. Smoking during pregnancy was found to be inversely associated with the risk of preeclampsia in both the earlier and later studies; several hypotheses through which smoking could reduce the risk of preeclampsia have been proposed (USDHHS 2010).
- Neonatal apnea (Gunnerbeck et al. 2011): snuff use during pregnancy was significantly associated with an increased risk of neonatal apnea (OR = 1.96; 95% CI: 1.30-2.96), which appeared to be higher than that of heavy smokers (OR = 1.08; 95% CI: 0.76-1.52).

5.10.2 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.

5.10.3 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.

5.10.4 Summary of Pregnancy Outcomes and Reproductive Effects

The Swedish Medical Birth Register was used to examine birth outcomes in a large number of pregnancies. 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 (though less than smoking), small-for-gestational-age birth, and increased risk of preterm delivery, stillbirth, and neonatal apnea.
- Daily use of snus during pregnancy is not associated with risk of, preeclampsia, gestational hypertension, or antenatal bleeding.

These conclusions are consistent with the recent review by Rodu (2011), who also noted that while any form of nicotine should be avoided during pregnancy, the highest risks for the developing baby are associated with smoking.

In addition, breastfed infants of mothers who use snus were shown to be 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.

5.11 Other Health Effects

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), delayed bone healing (W-Dahl and Toksvig-Larsen 2007), rheumatoid arthritis, sarcoidosis and multiple sclerosis (MS) (Carlens et al. 2010), pain and post-operative nausea and vomiting (PONV) following surgery (Brattwall et al. 2010), respiratory death (Roosaar et al. 2008), musculoskeletal disorders (Bolinder et al. 1992; Heir and Eide 1997; Holmberg and Thelin 2006; Mattila et al. 2008), and all-cause mortality (Bolinder et al. 1994; Roosaar et al. 2008). These cohort studies are summarized in Appendix Q-1. One cross-sectional study explored the potential relationship between snus use and pain intensity among participants experiencing chronic pain (Jakobsson 2008). This cross-sectional study is summarized in Appendix Q-2. One case-control study examined the relationship between tobacco smoking and Swedish snuff use and the risk of developing MS (Hedstrom et al. 2009), while another examined the relationship between tobacco smoking and Swedish snuff use and risk of developing plaque psoriasis (Wolk et al. 2009). These case-control studies are summarized in Appendix Q-3. Information from a comprehensive review of the health effects of Swedish snus that provides data on general health and psychiatric disorders (Lee 2011) along with two relevant publications published subsequent to the review are also discussed briefly.

5.11.1 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.

5.11.2 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.

5.11.3 Delayed Bone Healing

A third analysis of the Swedish construction worker cohort was carried out 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.

5.11.4 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.

5.11.5 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, consistent with other research (Ferrea and Winterer 2009).

A cohort study carried out by Carlens and colleagues (2010) also examined the potential impact of tobacco smoking and snuff use on the risk of developing MS among 277,777 male construction workers from the Swedish Construction Workers Cohort. The authors found that ever use of snus, adjusted for smoking, was not associated with risk of MS (RR = 1.0; 95% CI: 0.8-1.4), however, the risk was marginally statistically significantly increased among never-smoking snus users (RR = 1.8; 95% CI: 1.1-2.9). A dose-response relationship was reportedly not observed (data not shown by authors). The authors note that this finding of an increased risk of MS among never smoking snus users could be due to a chance finding, and they point out that even if real, the combined use confers a lower risk than smoking alone. Additionally, the authors suggest that inhaled nonnicotinic components of cigarette smoke appear to be more important than nicotine itself in the etiology of chronic inflammatory diseases, including MS, which contradicts the finding of increased risk among never smoking snus users.

5.11.6 Rheumatoid Arthritis

The cohort study conducted by Carlens and colleagues (2010), described previously, investigated the relationship between tobacco smoking and snuff use and rheumatoid arthritis. Ever use of snus (adjusted for smoking) was not associated with risk of rheumatoid arthritis (RR = 1.0; 95% CI: 0.9-1.2), nor was the use of snus among never-smokers associated with risk of rheumatoid arthritis (RR = 1.2; 95% CI, 0.8-1.8). Smoking was significantly associated with an increased risk of developing rheumatoid arthritis.

5.11.7 Sarcoidosis

Carlens and colleagues (2010) also examined the relationship between tobacco smoking and snuff use and sarcoidosis. Ever use of snus, adjusted for smoking, or among never-smokers was not associated with risk of sarcoidosis (RR = 0.9; 95% CI: 0.8-1.5 for both). Smoking was protective against developing sarcoidosis, which the authors note is consistent with findings from other studies.

5.11.8 Plaque Psoriasis

Wolk and colleagues (2009) investigated the relationship between a variety of risk factors, including smoking and smokeless tobacco use and plaque psoriasis in a case-control study in

Stockholm, Sweden. No association was observed between current snus use and plaque psoriasis (OR = 1.0; 95% CI: 0.6-1.9).

5.11.9 Pain and Post-operative Nausea and Vomiting Following Surgery

Brattwall and colleagues (2010) examined the effects of snus use and smoking on pain and PONV following common day surgical procedures. The authors followed 355 patients during recovery and the first day at home, and found that PONV was significantly reduced during the early post-operative period among tobacco users (which included smokers and snuff users). With respect to post-operative pain, no significant impact on incidence was observed for regular tobacco use. The number of regular tobacco use was not sufficient for further sub-group analyses of snuff use or smoking individually.

5.11.10 Musculoskeletal Disorders

Heir and Eide (1997) investigated injury proneness in a prospective study of 480 male military conscripts. Snuff use was associated with a significantly increased risk of proneness to musculoskeletal injuries during training, adjusted for age and fitness (OR = 2.31; 95% CI: 1.34-3.99).

Bolinder and colleagues (1992) conducted a study among 37,722 Swedish construction workers and examined the prevalence of disability pension for musculoskeletal diagnoses among snus users. The risk of disability pension for musculoskeletal diagnoses was significantly increased in never-smoking snus users at both age 46-55 years (OR = 2.8, 95% CI: 1.6-4.8) and 56-65 years (OR = 1.5, 95% CI: 1.2-1.8). Bolinder and colleagues (1992) also examined the prevalence of low back pain within the past year among the 37,722 male Swedish construction workers. Among never-smoking snus users, the prevalence of low back pain within the past year was not significantly elevated (OR = 1.1; 95% CI: 1.0-1.2).

Holmberg and Thelin (2006)[110] 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.

Mattila and colleagues (2008) investigated low back pain in a cross-sectional study of 7,040 Finnish, male military conscripts. A significantly increased prevalence of low back pain was

[110] 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.

observed among smokeless tobacco users (not specified as Swedish snus), adjusted for age, perceived health, and disease during the past year (OR = 1.4; 95% CI: (1.2-1.7).

5.11.11 All-Cause Mortality

Two cohort studies have examined the relationship between the use of snus and all-cause mortality. Bolinder and colleagues (1994) investigated this relationship among 84,781 Swedish construction workers, and found a significant association between exclusive use of snus and all-cause mortality among all subjects (RR = 1.4; 95% CI: 1.3-1.8), and those aged 35-54 (RR = 1.9; 95% CI: 1.6-2.4) at study entry, but not among subjects aged 55-65 (RR = 1.2; 95% CI: 1.0-1.3) at study entry. This risk of all-cause mortality among exclusive snus users was lower than that observed among exclusive smokers (of who smoked less than 15 cigarettes per day: RR = 1.7; 95% CI: 1.6-1.9 and among those who smoked 15 or more cigarettes per day: RR = 2.2; 95% CI: 2.0-2.4).

Roosaar and colleagues (2008) also evaluated and compared the effects of the use of snus and smoking on all-cause mortality among 9,976 men from Uppsala County, Sweden. Ever daily use of snus (adjusted for smoking) was marginally significantly associated with an increased risk in all-cause mortality (HR = 1.10; 95% CI: 1.01-1.21). Ever daily use of snus among never-smokers was also marginally significantly associated with an increased risk of all-cause mortality (HR = 1.23; 95% CI: 1.09-1.40).

5.11.12 Respiratory Death

Roosaar and colleagues (2008), described above, also examined the relationship between ever daily snus use and death from respiratory diseases. Ever daily use of snus (adjusted for smoking and among never-smokers) was not significantly associated with an increased risk of death from respiratory disease among men under 80 years of age (HR = 0.8; 95% CI: 0.4-1.6 and HR = 0.8, 95% CI: 0.2-3.0 respectively). However, the authors found that ever daily use of snus (adjusted for smoking and among never-smokers) was significantly associated with an increased risk of death from respiratory disease among men aged 80 or older (HR = 1.8; 95% CI: 1.2-2.7 and HR = 2.0; 95% CI: 1.2-3.4 respectively). The authors note that the mechanisms of the observed excess risk of respiratory deaths in this age group could possibly be due to confounding from smoking and remain to be established.

5.11.13 Psychiatric Disorders and General Health

Lee (2011) published a review of the literature on snus, and considered studies of other "general health" and psychiatric outcomes. Those with psychiatric disorders are known to have increased rates of nicotine dependence (Grant et al. 2004), though Lee stated that there is no reliable indication that snus use affects the *onset* of psychiatric disorders based on the results from three publications on psychiatric outcomes. Subsequent to Lee's review, Edwards and colleagues (2011) reported that the prevalence of major depression was significantly associated with snus use for both male and female users, while Engstrom and colleagues (2010) reported that prevalence of snus use was not significantly associated with psychosocial distress. Though some studies suggest snus may be associated with psychiatric disorders, this has not been universally observed, and all the studies are cross-sectional in nature, and simply report an association; causality, including the issue of temporality cannot be determined based on these studies alone.

With respect to general health, Lee (2011) summarized two cross-sectional studies that investigated the relationship between the use of snus and general health outcomes that included frequent sick leave, long leave, and "best general health" (assessed by five indicators). Among these three outcomes, snuff use was significantly associated only with long leave. Another cross-sectional study reported that prevalence of snuff use was not significantly associated with poor or very poor self-rated health (Engstrom et al. 2010). Again, causality cannot be determined in these cross-sectional studies.

5.11.14 Summary of Studies of Other Health Effects

Fifteen studies were identified that evaluated the relationship between snus use and various potential health effects including ALS, complications after hernia surgery, pain and PONV following surgery, delayed bone healing, chronic pain intensity, musculoskeletal disorders, plaque psoriasis, rheumatoid arthritis, sarcoidosis, MS, all-cause mortality, and respiratory death. Among these studies, statistically significant positive associations with snus use and subsequent neck and low back pain, respiratory death, musculoskeletal injuries, and MS were observed in single studies, thus no strong conclusions about associations between snus use and these health outcomes can be drawn based only on single studies. Two studies observed small increases in risk of all-cause mortality among snus users, however, the potential for residual confounding from or misclassification of smoking in these studies remains a concern before strong conclusions from these studies can be drawn.

5.12 Summary and Conclusions

Studies have reported that the use of Swedish snus is associated with a characteristic type of oral mucosal lesion ("snus-induced 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. No other effects of snus use on periodontal disease, gingivitis, gingival recessions, and other dental conditions were consistently identified among studies that controlled for important confounders such as socioeconomic status and oral hygiene habits.

Evidence from clinical studies suggests that Swedish snus use acutely increases blood pressure and heart rate, almost certainly due to nicotine. An increased risk of developing hypertension was observed in the single available prospective cohort study, among Swedish Constructions Workers, but limited to participants with repeated visits, and not the entire cohort. No other consistent associations between biochemical measurements and other risk factors for cardiovascular disease were observed. Single epidemiological studies observed an increased risk of death from myocardial infarction and from one specific stroke type among Swedish snus users; however, multiple additional findings for risk of MI and stroke have consistently shown no association between use of snus and these cardiovascular outcomes.

Well controlled epidemiological evidence indicates that Swedish snus is not associated with oral cancer or with lung cancer. Though the studies are mostly consistent showing no association between Swedish snus use and esophageal cancer, a single recent study did observe an increased risk for this cancer site. 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: laryngeal, stomach, kidney, bladder, skin, colon,

anal, rectal, and 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 populations studied; there are inconsistencies between the two studies and the interpretation of the studies has been the topic of much scientific debate. A third analysis that pooled several studies of Western smokeless tobacco and pancreatic cancer did not observe an association with this cancer type. Though it is unlikely that Swedish snuff was a major product used in any of the populations included in the analysis, these results are potentially relevant with respect to Swedish snus in that smokeless tobacco used in North America and other western countries are expected to contain more TSNAs than Swedish snus. TSNAs are thought to be the components of tobacco products that are likely associated with an increased risk of pancreatic cancer.

Multiple studies have examined weight, weight gain, and measures of central adiposity in association with snus and smoking. Because smoking is known to suppress body weight, and many people who quit smoking gain weight, only studies that addressed the potential confounding effect of current or former smoking were examined. Some evidence suggests that snus use may be associated with higher BMI or weight gain among studies that account for past and current smoking. However, overall, the results are mixed; even those of the two studies of consistent snus users are contradictory.

Body weight and composition are important risk factors for type 2 diabetes and metabolic syndrome. One well-conducted prospective study found that use of Swedish snus was not associated with increased risk of diabetes, but two additional epidemiologic studies of the same population concluded that heavy users of moist snuff have an increased risk of type 2 diabetes; each study had significant limitations with respect to study design and sample size. 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. Other components of MetSy include body, weight, hypertension, and diabetes, which as discussed above, may be associated with snus use. Further research is needed to understand the potential mechanisms and causative factors to determine if snus use increases the risk of these metabolic-related health outcomes.

The literature indicates that use of Swedish snus is not associated with harmful gastrointestinal symptoms or diseases, including peptic ulcer, reflux, dyspepsia, or heartburn, Crohn's disease or ulcerative colitis. One study reported increased risk of altered histology of the esophago-gastric junction among exclusive snus users when examined macroscopically in a subset of study participants. This finding needs to be confirmed in additional studies.

Several epidemiological studies suggest that daily use of Swedish snus during pregnancy is associated with some adverse consequences (a modest reduction in average birth weight and small-for-gestational-age birth, and increased risk of preterm delivery, stillbirth, and neonatal apnea). Daily use of snus during pregnancy is not associated with risk of preeclampsia, gestational hypertension, or antenatal bleeding. 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 clearly not associated with lung cancer, oral cancer, or incident IHD or MI, and stroke. The most likely health risks associated with chronic use of Swedish snus appear to be acute, reversible increases in heart rate and blood pressure likely due to nicotine, and a characteristic, reversible lesion in the mouth of snus users. There is no evidence that snus is associated with other mouth and gum diseases. Several adverse pregnancy outcomes are also clearly associated with use of snus during pregnancy. 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 of more important public health significance where the available evidence has not yet reached the level of “definitive” for a lack of association, and thus firm conclusions cannot yet be drawn, include the relationship between Swedish snus use and possible weight gain issues, metabolic syndrome and diabetes, hypertension, and fatal myocardial infarction.

6 Conclusions

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® comprehensively through December 31, 2012 and selectively for important new studies through April 2013. 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 toxicological studies and epidemiological studies of Swedish snus.

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. Novel brands may deviate from these values. 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 a quality standard (GothiaTek®), for the entire manufacturing process including limits for certain "undesired" trace-level components in snus. The current list of "Harmful or Potentially Harmful Constituents (HPHC)" released by the FDA in April 2012 consists of 93 components, 43 of which are thought to originate mainly from combustion processes. In this section, published data available on the remaining 50 components and on additional components in STPs that have been quantified or were considered relevant were discussed. Where available, results from extraction studies were also presented.

Concentrations of TSNAs, traditionally the most frequently analyzed and reported trace-level components in STPs due to their carcinogenic potential in experimental animals have 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. The newest data indicates that TSNA concentrations have continued to decline and combined NNK and NNN concentrations currently appear to be approximately half the limit (2 µg/g dry weight) recommended by the WHO in 2009.

Published data for most other trace-level components other than TSNAs analyzed in STPs and snus have become available (e.g., PAHs, aldehydes, metals, and radioisotopes). PAH concentrations reported in recent studies demonstrate that B[*a*]P concentrations are generally lower than the limit recommended by the WHO in 2009 (5 ng/g dry weight). Limited data on the presence of other PAHs indicates that only phenanthrene, fluoranthene, pyrene, and possibly naphthalene were detected in higher quantities. Generally, the analytical data from recent published studies on the various components indicate that concentrations in traditional Swedish snus are below the GothiaTek® limits as well as existing WHO-recommended limits.

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, nicotine content and delivery between snus brands, as well as, extraction and absorption of the chemical substances from snus, which all need to be taken into account when conducting an exposure assessment.

A comparison of critical components in traditional Swedish snus with other STPs, such as new products marketed as snus and US-type moist snuff, other factors, including moisture content, pH and resulting free nicotine are provided in Appendix II.

For a risk assessment, patterns of use of any of the STPs 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.

Studies of exposure biomarkers in individuals who use various STPs have increasingly been reported in the scientific literature. Biomarkers of exposure may be used to assess the actual internal dose of a tobacco component to which a tobacco user might be exposed. While limitations to the available biomarkers of exposure exist, they can be used to supplement information from product analyses as they reflect total exposure, bypassing differences in routes of exposure and product use behavior. In addition, biomarker levels on a population basis may give an indication of general trends in internal exposure to certain components of a well characterized product. With respect to harm reduction, conclusions from these studies should be interpreted carefully and in the context of additional data from clinical and/or epidemiological studies

A panel of biomarkers of exposure to components in tobacco products has been recently proposed for the use in product regulations. Many biomarkers of exposure are less relevant for non-combusted tobacco products such as snus; however, the panel does include the following potentially relevant biomarkers of exposure for snus: nicotine, TSNAs, PAHs, aldehydes, cadmium, and acrylamide. To date, published studies are available that have investigated biomarkers of exposure to nicotine, TSNAs, cadmium, and selenium in regular users of traditional Swedish snus.

Commonly measured biomarkers of nicotine exposure are cotinine in plasma or serum. However, their levels may be impacted by the route of exposure, i.e., first pass metabolism of nicotine to cotinine via the oral route may result in higher blood concentrations of cotinine that do not necessarily reflect increased exposure to the parent compound, nicotine. Total nicotine equivalents in urine are considered to better represent the total nicotine dose absorbed. Information from nicotine pharmacokinetic parameters is relevant for nicotine delivery, total dose, and abuse liability assessments. The time to maximum plasma nicotine concentrations in snus users appears to be dependent on the usage time, but not on nicotine content or portion size. On the other hand, C_{max} and AUC appear mostly dependent on total nicotine content (per pouch or portion size) as well as pH of the product. Whether the snus was loose or pouched had no influence on these parameters.

A number of studies in regular snus users show that mean or median cotinine levels in plasma or serum range from 137 to 399 ng/mL depending on the amount of snus consumed (average 11-32 g/day). In the saliva, average levels ranged from 80 to 343 ng/mL. Urinary biomarkers of nicotine measured in regular users of snus were as follows: for nicotine itself, 29 µg/mmol creatinine; for cotinine, approximately 1000–1210 µg/L; for total cotinine, 5926 µg/L; and for nicotine equivalents ranged from 14-36 mg/24 hrs.

TSNAs and their metabolites have been determined in various human bodily fluids, including saliva, blood, and urine, as well as in toenails. Urinary NNAL is the most commonly-measured biomarker of TSNA exposure, and is considered to reflect 12-17% of the NNK dose.

Four studies of TSNA biomarkers in users of Swedish snus were identified. Of those, one publication from 1988 measured TSNA levels in saliva during snus use; snus in the 1980s contained considerably higher TSNA concentrations than more contemporary snus products. More recently, urinary total NNAL was measured in users of conventional US STPs that were switched to *General* snus use. Of the two clinical studies available, only one appears to have a sufficient duration to examine for and detect differences in levels before and after the switch. In this study, total NNAL levels decreased significantly (to half the concentration measured at baseline) by week 4. Importantly, urinary total cotinine levels in this study did not change significantly, indicating the decreased toxicant exposure could not be explained by a decrease in tobacco intake and mean product use was similar to that reported for regular snus users. No studies measuring biomarkers of NNN in snus users were identified. POB-DNA adducts were significantly increased in oral mucosa of Swedish snus based on information provided in a study abstract; however, the importance of these adducts in oral cancer development has been questioned.

With respect to the available studies of biomarkers of metals/metalloids, both levels of cadmium and selenium biomarkers in regular users of traditional Swedish snus were similar to those detected in non-tobacco users. Swedish snuff/snus has been investigated *in vitro* for genotoxic and cytotoxic endpoints in a variety of cell types, in animal models, including surgical lip canal in rats, cheek pouches in hamsters, and dietary studies in transgenic mice in comparison with wild-type strains. The available *in vitro* studies in cell types relevant to oral, cardiovascular, and immune systems indicate that snus extracts can cause concentration-dependent changes in cell morphology, viability, and other endpoints, including cell proliferation, gene expression, and expression and function of GPCR receptors. However, it is unknown to what extent the effects seen in vitro are relevant for the highly complex in vivo situation. In three sets of genotoxicity assays, most snus extracts, at best, showed weak and variable mutagenicity in bacteria, except for a snus extract in methylene chloride that was positive. No pattern of responses indicative of genotoxicity relevant for human snus users was observed in the available studies.

While of invasive nature, the seven experiments involving the surgical lip canal rat model appear to present a route of exposure sufficiently comparable to human use that they are considered informative for human risk assessment, despite several limitations. Although non-malignant oral lesions similar in histopathology to those seen in human snus users (“snus-induced lesions”) were observed in snus-treated rats, the incidence of oral cavity tumors in treated animals were not significantly different from controls.

Two studies in wild-type and transgenic mice strains may provide some mechanistic information related to gastrointestinal and pancreatic pathology potentially associated with ingestion of tobacco products. However, limitations in the data , i.e., the differences in exposure route, dose and study duration, make the data difficult to extrapolate to human risks. In the wild-type mouse strains, treatment with snus alone for 6 months did not cause any changes in the stomach wall except for an increased expression of an apoptosis marker and no changes in the pancreas were detected after 15 months.

Snus treatment for 6 months combined with hypergastrinemia in a transgenic mouse model of stomach cancer and/or *H. pylori* infection caused histopathological changes in the stomach wall, though the contribution of snus cannot be established due to the lack of a *H. pylori*-infected control group, and the small number of treated animals. Possible preneoplastic changes were observed; however no malignancies were observed,

The toxicology data base for effects of snus exposure to *in vitro* cell systems and various animal models is not large, compared to data for effects of other tobacco products. Nevertheless, the cellular pathology reported in the animal models, as well as the lack of snus-related tumor development, comports with the human data base for snus users. Thus, the nonclinical data are useful for informing on snus-related effect mechanisms in humans if care is taken to apply appropriate weight of evidence to the experimental models and the epidemiology data.

Epidemiological studies have reported that the use of Swedish snus is associated with a characteristic type of oral mucosal lesion (“snus-induced 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. No other effects of snus use on periodontal disease, gingivitis, gingival recessions, and other dental conditions were consistently identified among studies that controlled for important confounders such as socioeconomic status and oral hygiene habits.

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among specific subgroups of the populations studied; there are inconsistencies between the two studies and the interpretation of the studies has been the topic of much scientific debate. A third analysis that pooled several studies of Western smokeless tobacco and pancreatic cancer did not observe an association with this cancer type. Though it is unlikely that Swedish snuff was a major product used in any of the populations included in the analysis, these results are potentially relevant with respect to Swedish snus in that smokeless tobacco used in North America and other western countries are expected to contain more TSNAs than Swedish snus. TSNAs are thought to be the components of tobacco products that are likely associated with an increased risk of pancreatic cancer.

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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 clearly not associated with lung cancer, oral cancer, or incident IHD or MI, and stroke. The health risks

known to be associated with chronic use of Swedish snus appear to be acute, reversible increases in heart rate and blood pressure likely due to nicotine, and a characteristic, reversible lesion in the mouth of snus users. There is no evidence that snus is associated with other mouth and gum diseases. Several adverse pregnancy outcomes are also clearly associated with use of snus during pregnancy. 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 of more important public health significance where the available evidence has not yet reached the level of “definitive” for a lack of association, and thus firm conclusions cannot yet be drawn, include the relationship between Swedish snus use and possible weight gain issues, metabolic syndrome and diabetes, hypertension, and fatal myocardial infarction.

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Appendix I

Description of Literature Search

Appendix I: Description of Literature Search

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.

In its initial report, ENVIRON International Corporation (ENVIRON) conducted a comprehensive review of the relevant published chemistry, epidemiology, and toxicology studies available for Swedish snus, using the search strategies described above through December 31, 2009. Since that time, ENVIRON has systematically identified all literature as it is published through frequent searches and publication alerts using these same resources. The ENVIRON review summarizes studies of the potential health risks associated with the use of Swedish snus comprehensively through December 2012, and selectively for important new publications as available through April 2013.

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 2,153 citations and of those citations, maintains a library of 1,880 smokeless tobacco-related electronic copies of the publications.

Appendix II

Chemical Properties of Snus in Comparison to Other STPs

Appendix (II) to Chapter 2: Chemical Properties of Snus in Comparison to Other STPs

This Appendix supplements the information presented in Chapter 2 on the chemical properties of traditional Swedish snus with detailed analytical data as provided in the more recently published literature (2004-2012; presented in Tables A II-1 through 7) for traditional Swedish snus itself, including novel brands, new products marketed as snus, and US-type moist snuff. Data is also presented where these studies analyzed other new (such as lozenges) or traditional products (such as dry snuff and chewing tobacco) or nicotine replacement products; however this additional data is not discussed in detail. The studies on other STPs are not intended to be an exhaustive representation of all the available literature on these products, but were identified as part of the literature search for traditional Swedish snus, also called Swedish moist snuff, and new products marketed as snus. This appendix focuses on products available on the Swedish and US markets.

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. Much of the published literature that reports analyses of the chemical composition of Swedish snus also includes data on US-type oral moist snuff; the more recent studies (published 2004 to present) have also investigated newer products that are marketed as snus (hereafter referred to as 'new products marketed as snus') and novel brands of traditional Swedish 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 new products marketed as snus were not reported in the literature included in this review. On the other hand, novel brands of traditional Swedish snus are brands that might deviate from the traditional moisture content of approximately 50% or have somewhat lower pH values, but which are manufactured according to the GOTHIATEK® Standard.

The outline of this appendix closely follows that of Chapter 2, beginning at Section 2.3.1. Each section contains a brief description of how the different components analyzed in traditional Swedish snus compare with those measured in novels brands of traditional Swedish snus, new products marketed as snus, as well as US-type moist snuff.

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A II 2.3.1 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 novel brand of the traditional Swedish snus *Catch*, 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 ±6.61 mg per portion (0.3 g-portion, approximately 21 mg/g dry weight (wet weight multiplied by 1.33) for *Catch Dry Mini*). These concentrations are in the same range as those reported for the traditional Swedish snus products tested in the same study.

A II 2.3.2 Alkaloids Other Than Nicotine

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 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, per dry weight (Stepanov et al. 2008a).

When expressed as percentage of total nicotine content, nornicotine, anatabine, and anabasine levels in *General* snus were generally similar to those calculated for *Camel* snus and US-type moist snuff. 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 brands (Stepanov et al. 2008a).

A II 2.3.3 Nicotine, Free Nicotine, pH and Moisture

Table A II-1a summarizes concentrations of total nicotine, free nicotine or free-base nicotine (FBN) (where available), pH and moisture levels in traditional Swedish snus (including *General,* "general [sic] pouch"*, Granit, Nick and Johnny* and *Catch*), novel brands of traditional Swedish snus (*Catch Dry*) as well as several new products marketed as snus (such as*, 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; Stanfill et al. 2010; Lauterbach et al. 2010; Lunell & Curvall 2011; Borgerding et al. 2012; Caraway and Chen 2012; Digard et al. 2012).

Moisture

Compared to the moisture level in traditional Swedish snus, which is approximately 50%, novel brands and new products marketed as snus generally have lower moisture content. For example, *Catch Dry Mini* was reported to have a moisture content of 20-25% moisture; Moisture levels measured in *Marlboro Snus* and *Camel Snus* brands were approximately 10% and 32%, respectively (Stepanov et al. 2008a; Caraway and Chen 2012). Similarly, reported moisture levels in *Du Maurier* brands were 26 to 29% (Rickert et al. 2009; Lauterbach et al. 2010).

pH

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 5.54- 8.62, with most products having a pH below 8, whereas the pH in traditional Swedish snus brands were generally 7.5- 8.7 (Lunell and Lunell 2005; McNeill et al. 2006; Richter et al. 2008; Rickert et al. 2008, 2009; Stepanov et al. 2008a; Lauterbach et al. 2010; Lunnell & Curvall 2011; Borgerding et al. 2012). One study reported slightly lower pH values for traditional Swedish snus (pH 6.61-7.01) (Stanfill et al. 2010). The novel brands of traditional Swedish snus, *Catch Dry*) had pH values of 6.65 to 7.51 (Lunell and Lunell 2005; Borgerding et al. 2012). The pH of new products marketed as snus was also generally lower (below pH 8) than what has been measured for traditional Swedish snus. The lowest values were reported for six 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 (Stepanov et al. 2008a, 2012a; Borgerding et al. 2012).

Nicotine

Total nicotine concentrations on a per gram basis in new products marketed as snus were generally higher than those in traditional Swedish snus and *Catch Dry* brands, with *Camel Snus* being more in the range of US-type moist snuff products analyzed in the same studies (McNeill et al. 2006; Rickert et al. 2009; Stepanov et al. 2008a; Lauterbach et al. 2010; Faizi et al. 2010; Borgerding et al. 2012). The exception was *Marlboro Snus Mild* with a total nicotine concentration that was lower than concentrations detected in all other brands investigated (Stepanov et al. 2008a). Current data from Stepanov and colleagues supports generally higher total nicotine concentrations in *Marlboro Snus* compared to an older study by the same researchers; they also reported an increase in pouch size for all flavors of *Marlboro Snus* (Stepanov et al. 2008a, 2012a) (See Section A II 2.3.6.1.1 for related discussions).

Additionally, differences in portion sizes (less than 1 g as compared to many traditional Swedish snus brands) might provide in part an explanation for why total nicotine contents on a per gram basis were higher than those in traditional Swedish snus (Borgerding et al. 2012). According to Stepanov and colleagues (2008a) such differences in portion size could lead to “drastic differences in toxicant and carcinogen amounts per dose.”

Free Nicotine

In direct comparison studies, free nicotine concentrations (Free base nicotine, FBN) in traditional Swedish snus and *Catch Dry* brands were generally lower than those reported in *Camel Snus* but higher than those measured in *Marlboro Snus*[1] (McNeill et al. 2006;

[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.”

Stepanov et al. 2008a, 2012a; Borgerding et al. 2012). The pH values and the calculated FBN concentration of the aqueous extracts of the two *du Maurier* snus samples, new products marketed as snus, were generally in the same range as those measured for US-type moist snuffs (Lauterbach et al. 2010). 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 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]. Free nicotine concentrations were generally lower in US-type moist snuff brands compared to those measured in traditional Swedish snus, 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 (McNeill et al. 2006; Stepanov et al. 2008a; Borgerding et al. 2012).

A II 2.3.4 Nitrate and Nitrite

Table A II-1b summarizes concentrations of nitrate and nitrite in traditional Swedish snus (*General,* "general [sic] pouch"), novel brands of traditional Swedish snus (*Catch Dry*), new products marketed as snus in the US and Canada (*Camel Snus, Marlboro Snus,* and *du Maurier*) as well as US-type moist snuff as reported in five recent analyses of different STPs (McNeill et al. 2006; Rickert et al. 2009; Stepanov et al. 2008a; Faizi et al. 2010; Borgerding et al. 2012).

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. *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 lower concentrations in *du Maurier* snus compared to US-type moist snuff brands. 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 in *Camel Snus* and *Marlboro Snus* samples were similar to concentrations in *General* and *Catch Dry* or not detected and below or at (for *Camel Snus Spice*) the GOTHIATEK® Standard limit of 7 µg/g dry weight (Stepanov et al. 2008a; Borgerding et al. 2012). Similarly, McNeill and colleagues (2006) reported that the nitrite concentration in "general [sic] pouch" was below the detection limit of 0.2 µg/g. By contrast, concentrations in US-type moist snuff brands analyzed by the Stepanov and colleagues (2008a) and Borgerding and colleagues (2012) exceeded this limit considerably. Two *Wintergreen* brands had extremely high nitrite concentrations, several folds times higher than those observed in traditional Swedish snus.

[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.

A II 2.3.5 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 (including *General*), novel brands of traditional Swedish snus (*Catch Dry*), new products marketed as snus in the US and Canada (*Camel Snus, Marlboro Snus,* and *du Maurier*), and in US-type moist snuff as reported in three recent analyses of different STPs (Rickert et al. 2009; Stepanov et al. 2008a; Borgerding et al. 2012).

Concentrations of chloride in *Camel Snus,* and *Marlboro Snus* were lower than those detected in *General* and *Catch Dry* brands (Stepanov et al. 2008a; Borgerding et al. 2012). By contrast, chloride concentrations in traditional US-type moist snuff analyzed in the same studies were generally higher than concentrations measured in *General.* The one exception was *Cooper Wintergreen* with a chloride concentration that was within the range observed in *General*.

Concentrations of sulfate measured by Stepanov et al. (2008a) in *General* snus were similar to those measured in *Marlboro* and *Camel Snus*. In the same study, concentrations in US-type moist snuff were reported to be higher than those measured in *General* snus, *Camel* and *Marlboro Snus* brands. Phosphate concentrations measured by the same authors in *Marlboro Snus, Camel Snus* and US-type moist snuff were higher than those reported in *General* snus.

Ammonia and propylene glycol were not analyzed in traditional Swedish snus.

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 Original Portion 2006/07	Borgerding et al. 2012	50.9	NI	8.20	8.46 WWB	5.10 WWB (60.22%)	NI	NI	NI
General White Portion 2006/07		52.3	NI	8.21	7.92 WWB	4.81 WWB (60.77%)	NI	NI	NI
General Loose 2006/07		56.3	NI	7.57	7.15 WWB	1.87 WWB (26.19%)	NI	NI	NI
General Onyx 2006/07		51.9	NI	7.90	10.49 WWB	4.53 WWB (43.14%)	NI	NI	NI
Gustavus Original, Snuff 2006		47.3	NI	7.66	7.48 WWB	2.27 WWB (30.39%)	NI	NI	NI
Nick and Johnny, Snuff 2007		49.8	NI	7.98	10.55 WWB	5.03 WWB (47.7%)	NI	NI	NI
Rocker Black, Snuff 2007		48.4	NI	8.39	8.11 WWB	5.69 WWB (70%)	NI	NI	NI
Rocker Silver, Snuff 2007		47.1	NI	7.51	6.91 WWB	1.63 WWB (23.61%)	NI	NI	NI
Granit, Loose snus	Digard et al. 2012	50.5-53.5	NI	8-8.3	10.8 WWB	NI	NI	NI	NI
Lucky Strike Original, Brown, Pouched snus		48-52	NI	8-8.2	10.7^{Δ} WWB	NI	NI	NI	NI
Lucky Strike Bold, Pouched snus		47-51	NI	7.9-8.1	14.7^{Δ} WWB	NI	NI	NI	NI
General Onyx Pouch snus WL	Lunell & Curvall 2011	NI	NI	8.7	9.9 WWB	NI	NI	NI	NI
General Pouch snus WL		NI	NI	8.7	8.7 WWB	NI	NI	NI	NI

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)**
General Original	Stanfill et al. 2010	NI	NI	7.01	8.34 WWB	0.75 (8.98%) WWB	NI	NI	NI
General Loose		NI	NI	6.61	7.79 WWB	0.29 (3.77%) WWB	NI	NI	NI
General White Wintergreen		NI	NI	7.07	7.76 WWB	0.78 (10.0%) WWB	NI	NI	NI
General White Portion		NI	NI	6.86	8.09 WWB	0.52 (6.48%) WWB	NI	NI	NI
Catch Peppermint		NI	NI	7.21	15.2 WWB	2.03 (13.3%) WWB	NI	NI	NI
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		NI	NI	8.5	14[#]	NI	NI	NI	NI
Catch Mini Snus		NI	NI	8.4	18[#]	NI	NI	NI	NI

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)**
Snus (US/ Sweden)									
Pouched snus from US or Sweden 2008	Faizi et al. 2010	~21-50%; 5% (1 sample)	NI	NI	~0.6-1.2%, 1.7-2.4% (3 samples) WWB	NI	NI	NI	NI
Loose snus from US or Sweden 2008		~44-52%	NI	NI	~0.6-1% WWB	NI	NI	NI	NI
Novel Brands of Traditional Swedish Snus									
Catch Dry Eucalyptus, 2007	Borgerding et al. 2012	22.8	NI	7.21	15.63 WWB	2.10 WWB (13.41%)	NI	NI	NI
Catch Dry Eucalyptus, 2006		23.4	NI	7	15.93 WWB	1.39 WWB (8.72%)	NI	NI	NI
Catch Dry Licorice 2006/07		21.2	NI	6.65	16.70 WWB	0.68 WWB (4.09%)	NI	NI	NI
Catch Dry Cassis Menthol 2006/07		21.1	NI	7.51	15.28 WWB	3.61 WWB (23.61%)	NI	NI	NI
Catch Dry Vanilla Coffee 2006/07		20.1	NI	7.45	15.60 WWB	3.31 WWB (21.21%)	NI	NI	NI
Catch Dry Mini Snus	Lunell & Lunell 2005	NI	NI	7.3	21[#]	NI	NI	NI	NI
New Products Marketed as Snus									
Wise Citrus and Menthol, Dry 2007	Borgerding et al. 2012	7.4	NI	9.18	17.92 WWB	16.76 WWB (93.53%)	NI	NI	NI
Camel Original 2007		31.9	NI	7.95	13.49 WWB	6.2 WWB (45.98%)	NI	NI	NI

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 Original 2006		34.3	NI	7.73	13.87 WWB	6.65 WWB (33.9%)	NI	NI	NI
Camel Frost 2007		32.2	NI	7.72	14.10 WWB	4.71 WWB (33.39%)	NI	NI	NI
Camel Frost 2006		34.1	NI	7.76	13.25 WWB	4.7 WWB (35.46%)	NI	NI	NI
Camel Spice 2007		32.2	NI	7.81	13.35 WWB	5.09 WWB (38.14%)	NI	NI	NI
Camel Spice 2006		32.8	NI	8.03	13.16 WWB	6.65 WWB (50.58%)	NI	NI	NI
Camel Original	Caraway and Chen 2012	32.3%	NI	NI	11.5[∞] WWB	NI	NI	NI	NI
Camel Spice		32.3%	NI	NI	10.8[∞] WWB	NI	NI	NI	NI
Camel Frost		32.3%	NI	NI	12.2[∞] WWB	NI	NI	NI	NI
Marlboro Snus, Rich (2010)	Stepanov et al. 2012a	NI	NI	6.72	18.82	1.23	NI	NI	NI
Marlboro Snus, Mild (2010)		NI	NI	6.68	18.93	0.84	NI	NI	NI
Marlboro Snus, Spearmint (2010)		NI	NI	6.79	18.82	1.05	NI	NI	NI
Marlboro Snus, Peppermint (2010)		NI	NI	6.81	19.38	1.13	NI	NI	NI
Camel Snus, Frost (2010)		NI	NI	7.43	16.46	3.58	NI	NI	NI

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, Robust (2010)		NI	NI	7.78	13.93	5.09	NI	NI	NI
Camel Snus, Winterchill (2010)		NI	NI	7.68	14.65	4.59	NI	NI	NI
Camel Snus, Mellow (2010)		NI	NI	7.38	16.74	3.36	NI	NI	NI
Du Maurier Freshmint Pouched snus	Lauterbach et al. 2010	29.2	70.8	7.39	13.7 WWB	2.58 WWB	NI	NI	NI
Du Maurier Original Pouched snus		26.1	73.9	7.39	14.9 WWB	2.85 WWB	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		NI	73.9	7.39	18.1	NI	NI	NI	NI
Marlboro Snus Rich New STP (2006/07)	Stepanov et al. 2008a	10.1	NI	6.83	17.8	1.08	0.438	2.60	0.111
Marlboro Snus Mild New STP (2006/07)		NI	NI	6.47	12.8	0.350	0.484	1.82	0.072
Marlboro Snus Spice New STP (2006/07)		NI	NI	6.85	17.9	1.13	0.411	2.17	0.097
Marlboro Snus Mint New STP (2006/07)		NI	NI	6.58	20.0	0.701	0.454	1.97	0.063
Camel Snus Original New STP (2006/07)		31.2	NI	7.46	28.2	6.09	0.353	1.39	0.164
Camel Snus Spice New STP (2006/07)		NI	NI	7.75	25.4	9.16	0.314	1.09	0.183

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 Frost New STP (2006/07)		NI	NI	7.59	23.7	6.4	0.313	0.741	0.103
Other New Products									
Dissolvables (2007)	Borgerding et al. 2012	3.8 2.8-4.8	NI	7.3 7.2-7.4	6 WWB 3.9-8.2	0.9 WWB 0.7-1.2	NI	NI	NI
Twist (2007)		26.2 20.4-32	NI	5.3 5-5.6	23.3 WWB 21.1-25.4	0.05 WWB 0.02-0.07	NI	NI	NI
Taboka		9.8%	NI	6.36	16.73 WWB	0.36 WWB (2.14%)	NI	NI	NI
Taboka Green		9.8%	NI	6.60	13.01 WWB	0.48 WWB (3.66%)	NI	NI	NI
Ariva	Stepanov et al. 2012a	NI	NI	6.85-6.97	4.38-6.53	0.3-0.51	NI	NI	NI
Stonewall		NI	NI	7.10	7.06-7.17	0.75-0.76	NI	NI	NI
Hard pellets	Faizi et al. 2010	~2.1-2.2%	NI	NI	~0.7-1% WWB	NI	NI	NI	NI
Soft pellets		~13% (1 sample)	NI	NI	~2.7% (1 sample) WWB	NI	NI	NI	NI
Ariva Newer noncombusted oral tobacco product	Hatsukami et al. 2007	NI	NI	7.4	0.6 WWB	NI	NI	NI	NI
Revel Newer noncombusted oral tobacco product		NI	NI	7.2	1.1 WWB	NI	NI	NI	NI
Stonewall Newer noncombusted oral tobacco product		NI	NI	7.7	1.5 WWB	NI	NI	NI	NI

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)**
Ariva US STP	McNeill et al. 2006	2.40	NI	NI	NI	2.4	NI	NI	NI
US-Type Moist Snuff									
Moist snuff from US: Cooper, Copenhagen, Grizzly, Husky, Kayak, Kodiak, Longhorn, Red Seal, Renegades, Skoal, Timberwolf 2006/07	Borgerding et al. 2012	50.8-55.5	NI	7.2-8.53	7.97-14.13 WWB	1.09-8.18 WWB (13.6-76.4%)	NI	NI	NI
Moist snuff from US or Sweden 2008	Faizi et al. 2010	~46-52%	NI	NI	~1.1-1.55% WWB	NI	NI	NI	NI
Various fine-cut, long-cut, pouched brands: *Skoal, Rooster, Copenhagen*	Lauterbach et al. 2010	47.9-53.9	46.1-52.1	6.97-8.19	10.9-13.4 WWB	0.99-6.49 WWB	NI	NI	NI
Various fine-cut, long-cut, pouched brands: *Skoal, Rooster, Copenhagen*	Rickert et al. 2009	NI	46.1-52.1	6.97-8.18	22.6-31.2	NI	NI	NI	NI
Traditional Moist Snuff	Stepanov et al. 2008	NI	NI	7.45-8.23	19.6-26.7	4.88-12.1	0.157-0.248	0.438-1.43	0.037-0.150
Swedish Match moist snuff	Richter et al. 2008	50.2-54.4	NI	6.7-7.84	4.5-34.9%	0.56-5.04	NI	NI	NI
Moist snuff		27.4-54.5	NI	5.54-8.62	0.5-79.9%	0.12-7.81	NI	NI	NI

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)**
Copenhagen Smokeless tobacco	McNeill et al. 2006	48.10	NI	NI	NI	4.9	NI	NI	NI
US-Type Low-Moisture Snuff									
Dry snuff from US: Skoal, Bruton, Dental, Levi Garrett, Railroad Mills Plain, Red Seal. 2006/07	Borgerding et al. 2012	3.6-9.8	NI	5.65-7.42	11.14-23.13 WWB	0.13-2.39 WWB (1.19-20.1%)	NI	NI	NI
Dry snuff from US or Sweden 2008	Faizi et al. 2010	~8-10%	NI	NI	~1.7-2.5% WWB	NI	NI	NI	NI
Various *McChrystal's* brands	Lauterbach et al. 2010	11.8-19.6	80.4-88.2	9.09-9.68	5.45-8.63 WWB	5.25-7.96 WWB	NI	NI	NI
Various *McChrystal's* brands	Rickert et al. 2009	NI	80.9-88.2	9.40-9.68	5.47-9.02	NI	NI	NI	NI
US-Type Chewing Tobacco									
Chewing tobacco, Loose leaf 2007	Borgerding et al. 2012	23.9 21.9-29	NI	5.6 5.6-6.1	6.2 WWB 2.9-8.6	0.04 WWB 0.01-0.08	NI	NI	NI
Plug tobacco 2006		22.7	NI	6	6.4 WWB	0.1 WWB (0.90%)	NI	NI	NI
Chewing tobacco from US or Sweden 2008	Faizi et al. 2010	~19-28%	NI	NI	~0.4-1% WWB	NI	NI	NI	NI
Plug tobacco		~18% (1 sample)	NI	NI	~1.48% (1 sample) WWB	NI	NI	NI	NI
Red man, Apple plug	Lauterbach et al. 2010	17.1-21.6	78.4-82.9	4.90-5.85	7.65-12.6 WWB	0.01-0.05 WWB	NI	NI	NI

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)
Red man, Apple plug	Rickert et al. 2009	NI	78.4-82.9	4.9-5.85	8.86-13.9	NI	NI	NI	NI

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).
∞Values were calculated on a per gram basis from 0.6 g of pouched snus.
[Δ] Values were based on 1 g of pouched snus which included weight of the pouch
* as is (not per dry weight)
All amounts given as per dry weight unless otherwise noted. WWB: wet weight basis; 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 Original Portion 2006/07	Borgerding et al. 2012**	<LOD	NI	NI	NI	NI	69.5	NI	NI
General White Portion 2006/07		<LOD	NI	NI	NI	NI	87.4	NI	NI
General Loose 2006/07		<LOD	NI	NI	NI	NI	93.2	NI	NI
General Onyx 2006/07		<LOD	NI	NI	NI	NI	79.5	NI	NI
Gustavus Original, Snuff 2006		<LOD	NI	NI	NI	NI	75.9	NI	NI
Nick and Johnny, Snuff 2007		5.6	NI	NI	NI	NI	60.4	NI	NI
Rocker Black, Snuff 2007		<LOQ	NI	NI	NI	NI	64.3	NI	NI
Rocker Silver, Snuff 2007		<LOQ	NI	NI	NI	NI	58.5	NI	NI
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
Snus (US/Sweden)									
Pouched snus from US or Sweden 2008	Faizi et al. 2010	NI	~0.1-0.2% WWB	NI	NI	NI	NI	NI	NI
Loose snus from US or Sweden 2008		NI	~0.1-0.2% WWB	NI	NI	NI	NI	NI	NI

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)**
Novel Brands of Traditional Swedish Snus									
Catch Dry Eucalyptus, 2007	Borgerding et al. 2012**	<LOD	NI	NI	NI	NI	63	NI	NI
Catch Dry Eucalyptus, 2006		<LOD	NI	NI	NI	NI	65.5	NI	NI
Catch Dry Licorice 2006/07		<LOD	NI	NI	NI	NI	72.1	NI	NI
Catch Dry Cassis Menthol 2006/07		<LOD	NI	NI	NI	NI	61.3	NI	NI
Catch Dry Vanilla Coffee 2006/07		<LOD	NI	NI	NI	NI	63	NI	NI
New Products Marketed as Snus									
Wise Citrus and Menthol, Dry 2007	Borgerding et al. 2012**	<LOD	NI	NI	NI	NI	0.6	NI	NI
Camel Original 2007		NI	NI	NI	NI	NI	32.7	NI	NI
Camel Original 2006		NI	NI	NI	NI	NI	35.1	NI	NI
Camel Frost 2007		NI	NI	NI	NI	NI	30.8	NI	NI
Camel Frost 2006		NI	NI	NI	NI	NI	33	NI	NI
Camel Spice 2007		4	NI	NI	NI	NI	33.3	NI	NI
Camel Spice 2006		NI	NI	NI	NI	NI	33.1	NI	NI
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		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

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)**
Marlboro Snus Mild New STP		ND	1.54	NI	NI	1.56	7.28	6.86	1.28
Marlboro Snus Spice New STP		3	1.69	NI	NI	2.12	7.68	7.01	1.32
Marlboro Snus Mint New STP		3	1.58	NI	NI	1.51	7.41	6.63	1.31
Camel Snus Original New STP		ND	3.79	NI	NI	12.7	39.8	9.35	0.820
Camel Snus Spice New STP		7	3.79	NI	NI	14.7	39.7	8.42	0.725
Camel Snus Frost New STP		3	3.20	NI	NI	15.3	32.4	7.62	0.722
Other New Products									
Dissolvables 2007	Borgerding et al. 2012	3.7-6.1	NI	NI	NI	NI	2.2	NI	NI
Oliver Twist 2007		<LOQ-5.2	NI	NI	NI	NI	4.3-104	NI	NI
Taboka		<LOQ	NI	NI	NI	NI	2	NI	NI
Taboka Green		4.6	NI	NI	NI	NI	2.3	NI	NI
Hard pellets	Faizi et al. 2010	NI	~<0.1% WWB	NI	NI	NI	NI	NI	NI
Soft pellets	Faizi et al. 2010	NI	~0.25% (1 sample) WWB	NI	NI	NI	NI	NI	NI
Ariva Smokeless tobacco	McNeill et al. 2006	ND	NI	NI	NI	NI	NI	NI	NI

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)**
US-Type Moist Snuff									
Moist snuff from US: Cooper, Copenhagen, Grizzly, Husky, Kayak, Kodiak, Longhorn, Red Seal, Renegades, Skoal, Timberwolf 2006/07	Borgerding et al. 2012**	<LOD-1229	NI	NI	NI	NI	75-129	NI	NI
Moist snuff from US or Sweden 2008	Faizi et al. 2010	NI	~0.19-0.38% WWB	NI	NI	NI	NI	NI	NI
Various fine-cut, long-cut, pouched brands: *Skoal, Rooster, Copenhagen*	Rickert et al. 2009	NI	27.4-36.1	6.043-14.83	ND-23.4	NI	NI	NI	NI
Traditional Moist Snuff	Stepanov et al. 2008	11-55	6.60-7.96	NI	NI	1.11-13.5	107-150	9.03-12.3	0.455-0.975
Copenhagen Smokeless tobacco	McNeill et al. 2006	6.7	NI	NI	NI	NI	NI	NI	NI
US-Type Low-Moisture Snuff									
Dry snuff from US: Skoal, Bruton, Dental, Levi Garrett, Railroad Mills Plain, Red Seal. 2006/07	Borgerding et al. 2012**	<LOD-43.6	NI	NI	NI	NI	2-19.2	NI	NI
Dry snuff from US or Sweden 2008	Faizi et al. 2010	NI	~0.79-1.1% WWB	NI	NI	NI	NI	NI	NI
Various *McChrystal's* brands	Rickert et al. 2009	NI	4.72-6.79	0.114-0.302	ND-23.3	NI	NI	NI	NI

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)**
US-Type Chewing Tobacco									
Chewing tobacco, Loose leaf 2006/07	Borgerding et al. 2012**	<LOD-5	NI	NI	NI	NI	18.1-22	NI	NI
Plug 2007		<LOD	NI	NI	NI	NI	19	NI	NI
Chewing tobacco from US or Sweden 2008	Faizi et al. 2010	NI	~0.1-0.21% WWB	NI	NI	NI	NI	NI	NI
Plug tobacco	Faizi et al. 2010	NI	~0.1% (1 sample) WWB	NI	NI	NI	NI	NI	NI
Red man, Apple plug	Rickert et al. 2009	NI	7.18-8.76	1.285-2.663	10.6-15.9	NI	NI	NI	NI

Notes:
All amounts given as per dry weight unless otherwise noted. WWB: wet weight basis; ND: Not detected; NI: Not investigated;
** Limit of detection of Nitrite: 4.72 (2006) and 0.57 (2007) µg/g; *** Limit of quantification of Nitrite: 15.7 (2006) and 1.89 (2007) µg/g

A II 2.3.6 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 2.3.6.1 *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).

A II 2.3.6.1.1 Tobacco-Specific N-Nitrosamines

Table A II-2a summarizes concentrations of TSNAs in various brands of traditional Swedish snus, novel brands of traditional Swedish snus, and several new products marketed as snus presented in recent analyses of 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; Stanfill et al. 2010; Stepanov et al. 2006, 2008a, 2012a, 2012b; Caraway and Chen 2012; Borgerding et al. 2012).

Total TSNAs

Total TSNA concentrations as measured over the past decade by different investigators in new products marketed as snus were in the same range as those reported in traditional Swedish snus, ranging from approximately 1 to 4 μg/g dry weight. These levels were thus below the GOTHIATEK® Standard limit of 10 μg/g dry weight (McNeil et al. 2006; Stepanov et al. 2008a; Stanfill et al.2010; Borgerding et al. 2012).

In studies that presented analyses of *General* snus and *Catch Dry* compared to new products marketed as snus, TSNA concentrations in *Marlboro Snus, Camel Snus* brands were generally in the same range, with few exceptions for specific products (Stepanov et al. 2006, 2008a, 2012a; Hatsukami et al. 2007; Stanfill 2010; Caraway and Chen 2012; Borgerding et al. 2012). While total TSNA concentrations in *Camel Snus* seem to have not changed significantly, analytical data from a new study indicates that TSNA concentrations in *Marlboro Snus* have decreased considerably over the past years (Stepanov et al. 2008a, 2012a).

One exception is a report on the TSNA concentrations in *Exalt*, a product that has since been discontinued in the US. Rodu and Jansson (2004) reported that total TSNA concentrations were 5.8 μg/g dry weight. A separate study that analyzed *Exalt* samples as purchased in the US and also in Sweden reported slightly lower total TSNA concentrations (Hatsukami et al. 2007; Stepanov et al. 2006). Because moisture content was not reported by Stepanov and colleagues (2006), it is unclear if this indicates a true difference in TSNA content or may be due to interlaboratory variability.

Total TSNA concentrations in US-type moist snuff were generally higher than traditional Swedish snus and the range of concentrations was wider (Stepanov et al. 2006; 2008; Hatsukami et al. 2007; Richter et al. 2008; Rickert et al. 2009; Borgerding et al. 2012). The highest concentrations of TSNAs were reported in some brands of dry snuff, while the lowest concentrations were seen in other new products, such as dissolvable lozenges and other

pouched products (Rodu and Jansson 2004; McNeill et al. 2006; Stepanov et al. 2006; 2008; 2012a; Hatsukami et al. 2007; Borgerding et al. 2012).

In two studies, nicotine replacement therapy products were also analyzed (Stepanov et al. 2006; Hatsukami et al. 2007). NNK was detected in two strengths of nicotine patches (*NicoDerm CQ*) at 0.008 μg/g wet weight and in a 4 mg-nicotine gum (*Nicorette*), 0.002 μg/g wet weight NNN was detected. No other TSNAs were detected in these products.

Individual TSNAs

Borgerding and colleagues (2012) analyzed individual TNSAs in traditional Swedish snus, as well as in various STPs purchased in Sweden and in the US between 2006 and 2007 (total TSNA was not reported). They reported concentrations of NNN and NNK in traditional Swedish snus and *Catch Dry* brands that were similar or less than those measured in new products marketed as snus, *Camel Snus*. NNK concentrations were generally below the limit of quantitation (LOQ). In other studies (not head-to-head comparisons), concentrations of NNN and NNK in traditional Swedish snus reported by Stanfill and colleagues (2010) were lower than those reported in *Camel Snus* but comparable to those in *Marlboro Snus* brands by Stepanov and colleagues (2012a).

Stepanov and colleagues (2012a) analyzed TSNA concentrations in *Camel Snus, Marlboro Snus,* and various dissolvable *Camel* products purchased in different US regions during the 2010 summer months. Total TSNA levels among flavors of Marlboro Snus products did not differ significantly, but when *Marlboro Snus* products were combined, concentrations of NNK plus NNN showed variations across regions: products purchased in the Pacific Northwest had significantly lower levels than those from the Midwest or South. No significant differences between regions were reported for *Camel Snus* products.

In a subsequent study, Stepanov and colleagues (2012c) [3] re-examined data on NNN and NNK concentrations in *Camel Snus* and *Marlboro Snus* that had been analyzed in their laboratory from 2006 and 2010. Mean pouch sizes of the original products were 370 mg for *Camel Snus* introduced in 2006 and 240 mg for *Marlboro Snus* introduced in 2007. Subsequently, larger pouch sizes were introduced (in 2008 Medium *Camel Snus*: 531 mg; in 2009 Large *Marlboro Snus*: 410 mg; in 2010 Large *Camel Snus*: 970 mg). The authors reported that with the larger pouch size, there was a concurrent significant increase in TSNA content per *Camel Snus* pouch (mean NNK + NNN concentrations in Original, Medium, and Large: 0.36, 0.52, and 1.19 μg/pouch, respectively), though there was no significant difference in TSNA concentrations per product wet weight (0.98, 0.98, and 1.23 μg/g wet weight). Large pouches of *Marlboro Snus* contained slightly lower TSNAs than the original size (mean NNK + NNN concentrations in Original and Large: 0.31 and 0.21 μg/pouch, respectively) and the TSNA content was significantly lower per product wet weight (1.27 and 0.5 μg/g wet weight).

[3] Stepanov I, Jensen J, Biener L, Bliss RL, Hecht SS, and Hatsukami DK. 2012c. Increased Pouch Sizes and Resulting Changes in the Amounts of Nicotine and Tobacco-Specific N-Nitrosamines in Single Pouches of Camel Snus and Marlboro Snus. Nicotine Tob Res 14:1241-1245.

As seen for traditional Swedish snus, the combined NNN and NNK 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* (discontinued) and *Marlboro Snus Mint*, where NNN concentrations of more than 3 μg/g dry weight were detected (Rodu and Jansson 2004; Stepanov et al. 2008a).

While no published data on the enantiomeric composition of NNN is available for traditional Swedish snus, in their recent analysis of various tobacco products, Stepanov and colleagues (2012b) observed *S*-NNN to be on average 66.4% (range, 63.9-73.5%) of the NNN concentration detected in new products marketed as snus (*Camel Snus* and *Marlboro Snus* brands). The average NNN concentration in these products was given as 1.05 µg/g wet weight (range, 0.72-1.79 µg/g wet weight); with *S*-NNN accounting for an average of 0.70 µg/g wet weight (range, 0.47-1.19 µg/g wet weight). By comparison, the percentage of *S*-NNN of the NNN concentration detected in conventional moist snuff brands was lower with an average of 57% (range, 50.2-65.5%). However, the average NNN concentration in these products was higher than those in the new products (average, 2.18 (1.21-4.25) µg/g wet weight); therefore the average *S*-NNN concentration in the conventional moist snuff brands (average, 1.26 (0.71-2.5) µg/g wet weight) was higher than the average total NNN concentration in the new products marketed as snus.

As observed for total TSNA concentrations, NNN plus NNK concentrations in US-type moist snuff spanned a much larger range and were generally higher than concentrations observed in traditional Swedish snus brands, *Catch Dry* brands and new products marketed as snus. The highest concentrations of NNN and NNK were reported in some brands of dry snuff (on a per dry weight basis), while the lowest concentrations were seen in other new products, such as dissolvable lozenges and other pouched products (Rodu and Jansson 2004; McNeill et al. 2006; Stepanov et al. 2008, 2012a; Borgerding et al. 2012).

Comparison with the limited data available for nicotine replacement therapy (NRT) products indicates that combined NNN and NNK concentrations in traditional Swedish snus products are several-fold higher on a per wet weight basis (Stepanov et al. 2006; Hatsukami et al. 2007).

Table A II-2b summarizes concentrations of NNAL, *iso*-NNAL, and NAA in traditional Swedish snus, new products marketed as snus, US-type moist snuff and other different STPs (Stanfill et al. 2010; McAdam et al. 2011).

A study by BAT researchers presented as a poster at the 2011 SRNT Meeting analyzed NNAL, NNA and *iso*-NNAL in different STPs (McAdam et al. 2011). All concentrations were compared on a per wet weight basis. Similar to results for Swedish pouched and loose snus (unspecified brands), NNAL concentrations in “US snus” (likely new products marketed as snus) were below or close to the detection limit (LOD, 0.0084 μg/g wet weight) or quantitation limit (LOQ, 0.028 μg/g wet weight) with a few samples with approximately two times this concentration (McAdam et al. 2011). US-type moist snuff samples contained up to five times higher concentrations of NNAL compared to traditional snus. NNAL was detected in almost all STPs analyzed in this study with the highest concentrations detected in US-type dry snuff. Stanfill et al. (2010) analyzed only NNAL concentrations in traditional Swedish snus; their results were in range with those observed by McAdam et al. (2011).

Iso-NNAL concentrations in Swedish pouched and loose Swedish snus (except for one pouched product) were similar to "US snus" as well as other new products tested were at or below the LOD or well below the LOQ (0.029 μg/g wet weight). Several-fold higher concentrations were reported for some Swedish pouched snus, US-type moist and dry snuff (McAdam et al. 2011).

NNA was not detected in "US snus" (detection limit 345 μg/g wet weight). It was detectable, but below the LOQ (1.151 μg/g wet weight) in some pouched Swedish snus products and US-type moist snuff. The highest concentrations (more than 4 times the LOQ) were found in US-type dry snuff (McAdam et al. 2011).

A II 2.3.6.1.2 N-Nitrosamino Acids

Because of the lack of newer data, Table A II-2c summarizes concentrations of *N*-nitrosamino acids in traditional Swedish snus, US-type moist snuff, low moisture snuff, and chewing tobacco as reported in five older studies (Ohshima et al. 1985; Brunnemann et al. 1985; Tricker and Preussmann 1989; Brunnemann and Hoffmann 1991, 1992). One new study by BAT researchers investigated most of the *N*-nitrosamino compounds, listed in Table A II-2c, but did not provide the results (Essen et al. 2011).

NSAR

In head-to-head comparisons, concentrations of NSAR measured in traditional Swedish snus were generally similar or lower than those observed in US-type moist snuff (Tricker and Preussmann 1989; Hoffmann et al. 1991; Brunnemann and Hoffmann 1992). NSAR was either not detected or not investigated in dry snuff and chewing tobacco.

Other *N*-Nitrosamino Acids

Concentrations of MNBA, MNPA, *Iso*-NNAC,NHPRO, NPIPAC/NPIC, and NPRO in US-type moist snuff were generally higher than those reported in traditional Swedish snus in the same studies, with a few exceptions, and some highly variable results (e.g., Tricker and Preussmann 1989; Ohshima et al. 1985). MNBA, MNPA, *Iso*-NNAC and NPRO were measured in US dry snuff and chewing tobacco; reported concentrations were lower than those reported in US-type moist snuff but similar to those reported in traditional Swedish snus. There were no analytical data available on NAzCa, NMPhPA, and NMTCA in traditional Swedish snus.

A II 2.3.6.1.3 Volatile and Non-volatile N-Nitrosamines

Table A II-2d summarizes concentrations of volatile and non-volatile *N*-nitrosamino acids in traditional Swedish snus, novel brands of traditional Swedish snus, new products marked as snus, US-type moist snuff as well as low moisture snuff and chewing tobacco (Österdahl and Slorrach 1983; Hoffmann et al. 1984, 1991; Brunnemann et al. 1985; Tricker and Preussmann 1989; Brunnemann and Hoffmann 1992; McNeil et al. 2006; Borgerding et al. 2012).

Recent studies did not focus on individual volatile and non-volatile *N*-nitrosamino acids in STPs and only limited data on their presence in snus/STPs are available. Only NDMA has been mentioned recently (Borgerding et al. 2012; McNeil et al. 2006).

NDMA

Concentrations of NDMA measured in traditional Swedish snus and *Catch Dry* brands were below the LOD/LOQ, with few exceptions (Borgerding et al. 2012; McNeil et al. 2006). Concentrations in new products marketed as snus were also below the LOD/LOQ with a single exception. More variable NDMA concentrations were reported for US-type moist snuff and US low moisture snuff (Borgerding et al. 2012). McAdam et al (2010a) analyzed several *N*-nitroso compounds in pouched and loose Swedish snus, and in other STPs. Concentrations of NDMA in pouched or loose Swedish snus products were lower than those reported in the US-type moist snuff. NDMA concentrations reported in US-type dry snuff and chewing tobacco were generally similar to those reported in US-type moist snuff.

Other Volatile N-Nitrosamines

Several older studies analyzed NMOR and NPYR in traditional Swedish snus and other STPs. In head-to-head comparison and where analyzed, concentrations of NMOR and NPYR were generally lower in traditional Swedish snus compared to those reported in US-type moist snuff (Hoffmann et al. 1984, 1991; Brunnemann et al. 1985, 1992; Tricker and Preussmann 1989; Österdahl 1991), with a single exception (Österdahl and Slorrach 1983).

McAdam et al. (2010a) analyzed concentrations of several *N*-nitrosamino acids in pouched or loose Swedish snus and US-type moist snuff. NDPA was quantifiable in three samples of pouched Swedish snus, but non-quantifiable in loose Swedish snus and US-type moist snuff. Concentrations of NMOR and NPYR in loose Swedish snus was either non-detectable or below the LOD/LOQ. In pouched Swedish snus, concentrations of NPYR was below the LOD/LOQ; while similar concentrations of NMOR were observed in both pouched Swedish snus and US-type moist snuff. NPYR and NMOR concentrations reported in US-type dry snuff and chewing tobacco were generally similar to those reported in traditional Swedish snus. Other volatile *N*-nitrosamino acids analyzed were either non-detectable or non-quantifiable.

NDELA

Concentrations of NDELA in traditional Swedish snus were generally lower than those observed in US-type moist snuff in several older studies (Brunnemann et al.1982, 1985; Hoffmann et al. 1984; Tricker and Preussmann 1989). In a 2010 SRNT meeting, McAdam and colleagues reported concentrations of NDELA in pouched/loose Swedish snus and US-type moist snuff were non-quantifiable and non-detectable, respectively.

Table A II-2a: Trace-Level Components in Snus and Other STPs as Reported in the Literature: Tobacco-Specific Nitrosamines (µg/g)

Brand/ (Year of Purchase)	Citation	NNK	NNN	NAB	NAT	Total TSNAs
Traditional Swedish Snus						
General White Portion 2006/07	Borgerding et al. 2012***	<LOQ	0.728	<LOD	0.586	NR
General Original Portion 2006/07		<LOQ	0.875	<LOD	0.688	NR
General Loose 2006/07		<LOQ	0.659	<LOD	<LOQ	NR
General Onyx 2006/07		<LOQ	0.701	<LOQ	0.649	NR
Gustavus Original, Snuff 2006		<LOQ	0.808	<LOQ	0.656	NR
Nick and Johnny, Snuff 2007		<LOQ	0.885	<LOQ	0.754	NR
Rocker Black, Snuff 2007		<LOQ	0.684	<LOQ	0.448	NR
Rocker Silver, Snuff 2007		<LOQ	0.601	<LOQ	0.422	NR
General Original	Stanfill et al. 2010	0.0964 WWB	0.345 WWB	0.0208 WWB	0.248 WWB	0.723 WWB §
General Loose		0.105 WWB	0.293WWB	0.0177 WWB	0.224 WWB	0.652 WWB §
General White Wintergreen		0.0899 WWB	0.267 WWB	0.0171 WWB	0.214 WWB	0.601 WWB §
General White Portion		0.0968 WWB	0.296 WWB	0.0175 WWB	0.225 WWB	0.648 WWB §
Catch Peppermint		0.0845 WWB	0.295 WWB	0.0134 WWB	0.229 WWB	0.630 WWB §
General (2006/2007)	Stepanov et al.	0.464	1.66	0.008	0.969	3.1

Table A II-2a: Trace-Level Components in Snus and Other STPs as Reported in the Literature: Tobacco-Specific Nitrosamines (µg/g)

Brand/ (Year of Purchase)	Citation	NNK	NNN	NAB	NAT	Total TSNAs
	2008a					
"general [sic] pouch"	McNeill et al. 2006	NR	NR	NR	NR	0.478†
General (2003-2005)	Hatsukami et al. 2007/ Stepanov et al. 2006	0.18 WWB	0.98 WWB	0.06 WWB	0.79 WWB	2.0 WWB
General (2003)	Stepanov et al. 2006	0.075 WWB	0.78 WWB	0.049 WWB	0.65 WWB	1.6 WWB
General (2002)		0.28 WWB	1.2 WWB	0.076 WWB	0.93 WWB	2.5 WWB
General (2003)	Rodu and Jansson 2004	0.4	1.1	0.1	0.6	2.2
Ettan (2003)		0.3	1.1	0.1	0.6	2.1
Catch Licorice (2003)		0.4	1.0	0.0	0.6	2.0
Göteborgs Rapé (2003)		0.4	1.1	0.0	0.6	2.1
Grovsnus (2003)		0.5	1.1	0.1	0.6	2.3
Novel Brands of Traditional Swedish Snus						
Catch Dry Eucalyptus (2007)	Borgerding et al. 2012***	0.319	0.843	0.054	0.587	1.803 †
Catch Dry Eucalyptus (2006)		<LOQ	0.814	<LOQ	0.570	NR
Catch Dry Licorice 2006/07		<LOQ	0.855	<LOQ	0.579	NR
Catch Dry Cassis Menthol 2006/07		<LOQ	0.715	<LOQ	0.588	NR
Catch Dry Vanilla Coffee2006/07		<LOQ	0.704	<LOQ	0.521	NR
New Products Marketed as Snus						
Camel Original	Caraway and Chen 2012	0.23∞ WWB	0.708∞ WWB	0.048∞ WWB	0.363∞ WWB	1.35∞ WWB
Camel Spice		0.13∞ WWB	0.653∞ WWB	0.047∞ WWB	0.360∞ WWB	1.192∞ WWB

Table A II-2a: Trace-Level Components in Snus and Other STPs as Reported in the Literature: Tobacco-Specific Nitrosamines (µg/g)

Brand/ (Year of Purchase)	Citation	NNK	NNN	NAB	NAT	Total TSNAs
Camel Frost		0.24[∞] WWB	0.712[∞] WWB	0.048[∞] WWB	0.368[∞] WWB	1.367[∞] WWB
Wise Citrus and Menthol, Dry 2007	Borgerding et al. 2012***	<LOQ	<LOQ	<LOQ	<LOQ	NR
Camel Original 2007		0.322	1.082	0.1	0.964	2.468[†]
Camel Original 2006		<LOQ	1.123	<LOQ	0.807	NR
Camel Frost 2007		0.345	1.009	0.075	0.874	2.303[†]
Camel Frost 2006		<LOQ	1.068	<LOQ	0.745	NR
Camel Spice 2007		0.250	1.079	0.068	0.824	2.221[†]
Camel Spice 2006		<LOQ	0.984	<LOQ	0.735	NR
Marlboro Snus, Rich (2010)	Stepanov et al. 2012a	0.132	0.421	0.018	0.400	0.970
Marlboro Snus, Mild (2010)		0.160	0.420	0.003	0.358	0.941
Marlboro Snus, Spearmint (2010)		0.164	0.431	0.004	0.383	0.982
Marlboro Snus, Peppermint (2010)		0.162	0.470	0.005	0.389	1.03
Camel Snus, Mellow (2010)		0.404	0.859	0.022	0.327	1.61
Camel Snus, Frost (2010)		0.450	0.896	0.026	0.350	1.72
Camel Snus, Robust (2010)		0.595	1.28	0.027	0.482	2.39
Camel Snus, Winterchill (2010)		0.609	0.909	0.021	0.343	1.93
Du Maurier Freshmint	Rickert et al. 2009	NQ	1.214	NQ	0.905	2.119
Du Maurier Original		0.456	1.212	NQ	0.831	2.499
Marlboro Snus Rich (2006/07)	Stepanov et al. 2008a	0.259	1.27	ND	0.455	1.98
Marlboro Snus Mild (2006/07)		0.229	1.52	ND	0.234	1.98

Table A II-2a: Trace-Level Components in Snus and Other STPs as Reported in the Literature: Tobacco-Specific Nitrosamines (µg/g)

Brand/ (Year of Purchase)	Citation	NNK	NNN	NAB	NAT	Total TSNAs
Marlboro Snus Spice (2006/07)		0.257	1.56	ND	0.246	2.06
Marlboro Snus Mint (2006/07)		0.215	3.28	ND	0.221	3.72
Camel Snus Original (2006/07)		0.27	1.15	0.012	0.297	1.73
Camel Snus Spice (2006/07)		0.157	1.27	0.015	0.305	1.75
Camel Snus Frost (2006/07)		0.267	1.2	0.009	0.204	1.68
Camel Snus Original	Hatsukami et al. 2007 #	0.16 WWB	0.79 WWB	0.008 WWB	0.19 WWB	1.15 WWB
Camel Snus Spice		0.09 WWB	0.87 WWB	0.01 WWB	0.2 WWB	1.17 WWB
Camel Snus Frost		0.16 WWB	0.83 WWB	0.006 WWB	0.13 WWB	1.12 WWB
Exalt(purchased in Sweden)	Hatsukami et al. 2007/ Stepanov et al. 2006 #	0.27 WWB	2.3 WWB	0.13 WWB	0.98 WWB	3.7 WWB
Exalt (purchased in US)		0.24 WWB	2.1 WWB	0.05 WWB	0.68 WWB	3.1 WWB
Exalt	Rodu and Jansson 2004	1.1	3.1	0.2	1.5	5.8
Other New Products						
Dissolvables (2007)	Borgerding et al. 2012***	<LOD-<LOQ	<LOQ-0.139	<LOQ	0.113-0.236	NR
Twist (2007)		<LOQ-0.236	1.14-1.318	<LOQ-0.08	0.622-1.945	NR
Camel Orbs, Camel Sticks, Camel Strips	Stepanov et al. 2012a	0.269-0.353	0.185-0.304	0.006-0.023	0.192-0.300	0.65-0.98
Ariva (different flavors)		0.067-0.073	0.094-0.102	0.032-0.047	0.311-0.358	0.52-0.57
Stonewall (different flavors)		0.063-0.064	0.122-0.137	0.111-0.117	0.437-0.482	0.74-0.79
Ariva	Hatsukami et al. 2007	0.037 WWB	0.019 WWB	0.008 WWN	0.12 WWB	0.19 WWB
Revel Mint Flavor	Hatsukami et al.	0.033 WWB	0.62 WWB	0.018 WWB	0.32 WWB	0.99 WWB

Table A II-2a: Trace-Level Components in Snus and Other STPs as Reported in the Literature: Tobacco-Specific Nitrosamines (µg/g)

Brand/ (Year of Purchase)	Citation	NNK	NNN	NAB	NAT	Total TSNAs
	2007/ Stepanov et al. 2006					
Revel Wintergreen	Hatsukami et al. 2007/ Stepanov et al. 2006	0.032 WWB	0.64 WWB	0.017 WWB	0.31 WWB	1.0 WWB
Stonewall	Hatsukami et al 2007	0.043 WWB	0.056 WWB	0.007 WWB	0.17 WWB	0.28 WWB
Taboka		0.006 WWB	0.91 WWB	ND	0.30 WWB	1.27 WWB
Taboka Green		0.07 WWB	0.82 WWB	0.002 WWB	0.24 WWB	1.13 WWB
Ariva	McNeil et al. 2006	NI	NI	NI	NI	ND †
Ariva	Stepanov et al. 2006	0.037 WWB	0.019 WWB	0.008 WWB	0.12 WWB	0.19 WWB
Stonewall		0.043 WWB	0.056 WWB	0.007 WWB	0.17 WWB	0.28 WWB
Ariva	Rodu and Jansson 2004	<0.1	0.0	0.0	0.0	<0.1
Revel		0.2	1.3	0.1	0.7	2.3
US-Type Moist Snuff						
Moist snuff from US: Cooper, Copenhagen, Grizzly, Husky, Kayak, Kodiak, Longhorn, Red Seal, Renegades, Skoal, Timberwolf 2006/07	Borgerding et al. 2012	0.789-6.761	3.094-12.77	0.184-2.221	3.432-13.908	NR
Various fine-cut, long-cut, pouched brands: *Skoal, Rooster, Copenhagen*	Rickert et al. 2009	0.992-2.496	3.864-6.782	NQ-0.557	2.949-6.033	8.814-14.557
Traditional Moist Snuff	Stepanov et al. 2008	1.10-3.58	3.76-6.86	0.062-0.179	1.12-3.58	6.27-12.0
Moist Snuff, United States	Richter et al. 2008	ND-4.321 WWB	ND-42.554 WWB	ND-4.242 WWB	ND-31.866 WWB	ND-90.024 WWB
Moist Snuff, Swedish Match		0.653-2.287 WWB	2.432-9.556 WWB	0.169-1.196 WWB	2.543-12.056 WWB	6.096-25.218 WWB
Skoal, Copenhagen,	Hatsukami et al	0.17-1.6 WWB	0.9-4.5 WWB	0.24-4.1 WWB	0.014-0.22 WWB	1.3-9.2 WWB

Table A II-2a: Trace-Level Components in Snus and Other STPs as Reported in the Literature: Tobacco-Specific Nitrosamines (µg/g)

Brand/ (Year of Purchase)	Citation	NNK	NNN	NAB	NAT	Total TSNAs
Kodiak brands	2007/ Stepanov et al. 2006					
Copenhagen	McNeil et al. 2006	NI	NI	NI	NI	3.509 †
Moist Snuff, US	Rodu and Jansson 2004	0.4-1.6	2.4-6.4	0.1-0.4	1.1-5.0	4.5-12.3
Low Moisture Snuff						
Dry snuff from US: Skoal, Taboka, Bruton, Dental, Levi Garrett, Railroad Mills Plain, Red Seal. 2006/07	Borgerding et al. 2012***	<LOQ-7.387	0.870-14.424	<LOQ-3.023	0.682-16.124	NR
Various *McChrystal's* brands	Rickert et al. 2009	0.452-0.785	0.849-1.487	NQ-0.139	0.571-0.941	1.872-3.211
Dry snuff (*Bruton, Red Seal, Dental Sweet, Scotch*)	Rodu and Jansson 2004	6.5-922	19-287	1.2-32	14-210	41-1219
Chewing Tobacco						
Chewing tobacco, Loose leaf 2006/07	Borgerding et al. 2012***	<LOQ-0.840	0.662-2.853	<LOQ-0.179	0.503-1.316	NR
Plug 2007		1.230	5.053	0.353	1.702	8.338
Red man, Apple plug	Rickert et al. 2009	NQ-0.378	1.021-2.179	ND-NQ	0.619-0.829	1.640-3.385
Beech Nut, Oliver Twist, Red Man	Rodu and Jansson 2004	0.1-0.8	0.9-3.0	0-0.1	0.5-1.3	1.5-4.7
Nicotine Replacement Therapy Products						
Nicoderm CQ (patch, 24-mg nicotine) Nicotine replacement therapy products	Hatsukami et al 2007	0.008 WWB	ND	ND	ND	0.008 WWB
NicoDerm CQ (patch, 4-mg nicotine) Nicotine replacement	Stepanov et al. 2006	0.008 WWB	ND	ND	ND	0.008 WWB

Table A II-2a: Trace-Level Components in Snus and Other STPs as Reported in the Literature: Tobacco-Specific Nitrosamines (µg/g)

Brand/ (Year of Purchase)	Citation	NNK	NNN	NAB	NAT	Total TSNAs
therapy products						
Nicorette (gum, 4-mg nicotine) Nicotine replacement therapy products	Hatsukami et al 2007/ Stepanov et al. 2006	ND	0.002WWB	ND	ND	0.002 WWB
Commit (lozenge, 2-mg nicotine) Nicotine replacement therapy products	Hatsukami et al 2007/ Stepanov et al. 2006	ND	ND	ND	ND	ND

Notes:
All amounts given as per dry weight, unless otherwise noted. WWB: wet weight basis; ND: Not detected; NQ: Not quantifiable; NR: Not reported; NI: Not Investigated; LOD: limit of detection; LOQ: limit of quantification.
∞Values were calculated based on 0.6g of pouched camel snus.
† Total TSNA = NNK + NNN + NAB + NAT; § Total TSNA = NNK + NNN + NAB + NAT + NNAL
*** Limit of Detection of NNK: 0.0815 (2006) and 0.033 (2007) µg/g; Limit of quantification of NNK: 0.272 (2006) and 0.109 (2007) µg/g;
*** Limit of Detection of NNN: 0.054 (2006) and 0.022 (2007) µg/g; Limit of quantification of NNN: 0.18 (2006) and 0.072 (2007) µg/g;
*** Limit of Detection of NAB: 0.031 (2006) and 0.012 (2007) µg/g; Limit of quantification of NAB: 0.103 (2006) and 0.041 (2007) µg/g;
*** Limit of Detection of NAT: 0.064 (2006) and 0.026 (2007) µg/g; Limit of quantification of NAT: 0.213 (2006) and 0.085 (2007) µg/g

Table A II-2b: Trace-Level Components in Snus and Other STPs as Reported in the Literature: Tobacco-Specific Nitrosamines (µg/g)

Brand/ STP Type Specified by Study Authors	Citation	NNAL	Iso-NNAL	NAA
Traditional Swedish Snus				
Swedish pouched snus	McAdam et al. 2011	<LOD (0.0084)-~0.080 WWB	<LOQ (0.029)-~0.320 WWB	<LOD (0.345)-<LOQ (1.151) WWB
Swedish loose snus		<LOQ (0.028)-~0.06 WWB	< LOQ (0.029)	< LOD (0.345)
General Original	Stanfill et al. 2010	0.0125 WWB	NI	NI
General Loose		0.0128 WWB	NI	NI
General White Wintergreen		0.0128 WWB	NI	NI
General White Portion		0.0131 WWB	NI	NI
Catch Peppermint		0.00857 WWB	NI	NI
New Products Marketed as Snus				
US snus	McAdam et al. 2011	<LOD (0.0084)-~0.070 WWB	< LOD (0.0087) WWB	< LOD (0.345) WWB
Other New Products				
Pellet moist	McAdam et al. 2011	<LOD (0.0084) WWB	<LOD (0.0087) WWB	<LOD (0.345) WWB
Pellet hard		<LOD (0.0084) WWB	<LOD (0.0087) WWB	<LOQ (1.151) WWB
US-Type Moist Snuff				
Moist snuff	McAdam et al. 2011	<LOQ (0.028)-~0.35 WWB	<LOQ (0.029)-~0.54 WWB	<LOD (0.345)-<LOQ (1.151) WWB
US-Type Dry Snuff				
Dry snuff	McAdam et al. 2011	~0.4-2.4 WWB	<LOQ (0.029)-~0.39 WWB	<LOD (0.345)-~4.3 WWB

Table A II-2b: Trace-Level Components in Snus and Other STPs as Reported in the Literature: Tobacco-Specific Nitrosamines (µg/g)

Brand/ STP Type Specified by Study Authors	Citation	NNAL	Iso-NNAL	NAA
US-Type Chewing Tobacco				
Chewing tobacco	McAdam et al. 2011	<LOD (0.0084)-~0.150 WWB	<LOD (0.0087)-LOQ (0.029) WWB	<LOD (0.345) WWB
Plug		<LOQ (0.028)	<LOD (0.0087)	<LOD (0.345) WWB

Notes:
All amounts given as per dry weight, except if otherwise indicated.
WWB: wet weight basis; LOQ: Limit of quantitation; LOD: Limit of detection; ND: Not detected; NQ: Not quantifiable; NR: Not reported; NI: Not Investigated;
NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; *iso*-NNAL: 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol; NNA: 4-(methylnitrosamino)-4-(3-pyridyl)-butanal

Table A II-2c: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: N-Nitrosamino Acids (µg/g)

Brand/ STP Type Specified by Study Authors	Citation	MNBA	MNPA	Iso-NNAC	NAzCa	NHPRO	NMPhPA	NMTCA	NPIPAC/ NPIC	NPRO	NSAR	NTCA
Traditional Swedish Snus												
Moist Snuff, Sweden (1989-1991)	Brunnemann and Hoffmann 1992	0.05-0.23	1.0-3.3	0.04-0.11	NI	NI	NI	NI	NI	0.63-8.3	0.01-0.68	NI
Moist Snuff, Sweden 3 brands,1989-91	Hoffmann et al. 1991	0.19-0.23	3.10-3.28	0.04-0.11	NI	NI	NI	NI	NI	4.91-8.33	0.03-0.68	NI
Moist Snuff, Sweden 3 brands	Brunnemann and Hoffmann 1991	NI	NI	0.1	NI	NI	NI	NI	NI	NI	NI	NI
Swedish moist snuff	Tricker and Preussmann 1989	0.07 (0.053-0.094)	1.34 (1.04-1.82)	NI	ND	0.140 ND-0.23	NI	NI	0.036 ND-0.13	1.1 0.63-1.82	0.019 0.008-0.031	0.021 ND-0.069
Smokeless Tobacco Products, Sweden Moist Snuff	Brunnemann et al. 1985	NI	NI	NI	NI	NI	NI	NI	NI	3.12-8.21	NI	NI
Swedish snuff (49-55% moisture)	Ohshima et al. 1985	ND-0.24	2.92-4.40	NI	NI	NI	NI	NI	0.22-5.56	6.21-29.5	NI	NI
US- Moist Snuff												
Moist Snuff, United States (1989-1991)	Brunnemann and Hoffmann 1992	0.09-9.10	2.2-66.0	0.05-21.00	NI	NI	NI	NI	NI	1.3-60	ND-2.5	NI
Moist Snuff, United States (1990-1991)		0.09-9.1	2.20-65.7	NI	NI	NI	NI	NI	NI	NI	ND-2.5	NI
Moist Snuff, United		0.06-8.00	1.4-19.9	ND	NI	NI	NI	NI	NI	0.33-5.0	0.03-1.10	NI

Table A II-2c: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: N-Nitrosamino Acids (µg/g)

Brand/ STP Type Specified by Study Authors	Citation	MNBA	MNPA	Iso-NNAC	NAzCa	NHPRO	NMPhPA	NMTCA	NPIPAC/ NPIC	NPRO	NSAR	NTCA
Kingdom (1989-1991)												
Moist Snuff, United States	Brunnemann and Hoffmann 1991	NI	NI	0.1-10.5	NI	NI	NI	NI	NI	NI	NI	NI
Moist Snuff, Sweden 3 brands,1989-91	Hoffmann et al. 1991	0.09-3.09	2.72-25.3	0.07-10.5	NI	NI	NI	NI	NI	0.74-21.0	<0.01-0.60	
English Moist Snuff	Tricker and Preussmann 1989	2.12 0.062-8.03	6.89 1.36-18.6	NI	ND	0.410 0.092-0.73	NI	NI	0.9 0.083-2.36	2.260 0.33-4.95	0.31 0.029-1.05	0.019 ND-0.069
Smokeless Tobacco Products, Canada Moist Snuff (>45% moisture)	Brunnemann et al. 1985	NI	NI	NI	NI	NI	NI	NI	NI	8.8-16.6	NI	NI
Smokeless Tobacco Products, US Moist Snuff (>45% moisture)		NI	NI	NI	NI	NI	NI	NI	NI	7.8-14.0	NI	NI
US- Low Moisture Snuff												
Dry Snuff, United States (1989-1991)	Brunnemann and Hoffmann 1992	0.14-0.46	1.2-4.5	0.05-0.21	NI	NI	NI	NI	NI	NI	ND	NI
Dry Snuff, United States	Brunnemann and Hoffmann 1991	NI	NI	0.1	NI	NI	NI	NI	NI	NI	NI	NI

Table A II-2c: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: N-Nitrosamino Acids (µg/g)

Brand/ STP Type Specified by Study Authors	Citation	MNBA	MNPA	Iso-NNAC	NAzCa	NHPRO	NMPhPA	NMTCA	NPIPAC/ NPIC	NPRO	NSAR	NTCA
US- Style Chewing Tobacco												
Chewing tobacco, United States (1989-1991)	Brunnemann and Hoffmann 1992	0.03	0.6	0.01	NI	NI	NI	NI	NI	NI	ND	NI
Chewing tobacco, United States	Brunnemann and Hoffmann 1991	NI	NI	0.01	NI	NI	NI	NI	NI	NI	NI	NI

Notes: Due to the lack of new data, data from older published studies is presented.
All amounts given as per dry weight, except otherwise noted.
ND: Not detected; NQ: Not quantifiable; NR: Not reported
MNBA: 4-(methylnitrosamino)butyric acid; MNPA: 3-(methylnitrosamino)propionic acid; *iso*-NNAC: 4-(N-methylnitrosamino)-4-(3-pyridinyl)-1-butyric acid; NAzCa: *N*-Nitrosoazetidine 4-carboxylic acid; NHPRO: *N*-Nitrosohydroxyproline; NMPhPA: *N*-Nitroso-N-methyl-phenylalanine; NMTCA: *N*-Nitroso-2-methylthiazolidine-4carboxylic acid; NPIPAC/NPIC: *N*-Nitrosopipecolic acid; NPRO: *N*-Nitrosoproline; NSAR: *N*-Nitrososarcosine; NTCA: *N*-Nitrosothiazolidine 4-carboxylic acid

Table A II-2d: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Volatile and Non-Volatile N-Nitrosamines (ng/g)												
Brand/ STP Type Specified by Study Authors	**Citation**	**NDBA**	**NDEA**	**NDIPLA**	**NDIPA**	**NDMA**	**NDPA**	**NEMA**	**NMOR**	**NPYR**	**NPIP**	**NDELA**
Traditional Swedish Snus												
General Original Portion 2006/07	Borgerding et al. 2012***	NI	NI	NI	NI	<LOD	NI	NI	NI	NI	NI	NI
General White Portion 2006/07		NI	NI	NI	NI	<LOD	NI	NI	NI	NI	NI	NI
General Loose 2006/07		NI	NI	NI	NI	<LOD	NI	NI	NI	NI	NI	NI
General Onyx 2006/07		NI	NI	NI	NI	<LOQ	NI	NI	NI	NI	NI	NI
Nick and Johnny, Snuff 2007		NI	NI	NI	NI	<LOQ	NI	NI	NI	NI	NI	NI
Gustavus Original, Snuff 2006		NI	NI	NI	NI	<LOD	NI	NI	NI	NI	NI	NI
Rocker Black, Snuff 2007		NI	NI	NI	NI	24.5	NI	NI	NI	NI	NI	NI
Rocker Silver, Snuff 2007		NI	NI	NI	NI	19.8	NI	NI	NI	NI	NI	NI
"general [sic] pouch"/ Snus (Sweden)	McNeill et al. 2006	NI	NI	NI	NI	ND	NI	NI	NI	NI	NI	NI
Moist Snuff, Sweden (1981-1990)	Brunnemann and Hoffmann 1992	NI	NI	NI	NI	0.1-50.0	NI	NI	ND-44.0	ND-95.0	NI	NI
Three unspecified brands/ Snuff from	Hoffmannn et al. 1991	NI	NI	NI	NI	51 - 63	NI	NI	NI	ND - 155	NI	NI

Table A II-2d: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Volatile and Non-Volatile N-Nitrosamines (ng/g)

Brand/ STP Type Specified by Study Authors	Citation	NDBA	NDEA	NDIPLA	NDIPA	NDMA	NDPA	NEMA	NMOR	NPYR	NPIP	NDELA
Sweden 1989-1990												
Unspecified brands/ Swedish snuff available on Swedish market 1983-1986 (average of 32 samples analyzed)	Österdahl 1991	NI	NI	NI	NI	0.7	NI	NI	Trace	5.1 (WWB)	Trace	NI
Swedish moist snuff	Tricker & Preussmann, 1989**	NI	NI	NI	NI	1.5 (1.0-2.5)	NI	ND	1.0 (ND-1.0)	5.0 (4.5-6.0)	ND	19 (8-31)
Unspecified brands/ Smokeless Tobacco Products, Sweden Moist Snuff	Brunnemann et al. 1985	NI	ND	NI	NI	ND	NI	NI	ND-9.1	12.2-22.1	NI	230-300
Five unspecified brands/ Snuff Sweden	Hoffmann et al. 1984	NI	NI	NI	NI	ND – 60	NI	NI	ND – 44	ND – 210	NI	225-390
Unspecified brands/ Snuff available on Swedish market 1982	Österdahl & Slorrach1983	NI	NI	NI	NI	0.7 ND-1.6 WWB	NI	NI	0.6 ND-4.0 WWB	6.9 4.4-9.6 WWB	ND	NI
Unspecified brands/ Snuff available on Swedish market 1981		NI	NI	NI	NI	7.8 0.1-50.0 WWB	NI	NI	ND-1.2 WWB	17.1 2.6-95.1 WWB	0.2 ND-0.9 WWB	NI

Table A II-2d: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Volatile and Non-Volatile N-Nitrosamines (ng/g)												
Brand/ STP Type Specified by Study Authors	**Citation**	**NDBA**	**NDEA**	**NDIPLA**	**NDIPA**	**NDMA**	**NDPA**	**NEMA**	**NMOR**	**NPYR**	**NPIP**	**NDELA**
Five unspecified brands/ Swedish snuff	Brunnemann et al. 1982	NI	NI	NI	NI	<2-60	NI	NI	<2-44	<2-210	NI	225-390
Snus (Sweden)												
Pouched snus from 2008	McAdam et al. 2010a	<LOQ	<LOQ	<LOD	<LOQ	<LOQ/LOD-9 WWB	<LOQ-24-57 WWB	<LOQ	<LOQ-13-20 WWB	<LOD/LOQ	<LOQ	<LOD
Loose snus from 2008	McAdam et al. 2010a	<LOQ	<LOQ	<LOD	<LOQ	<LOQ/LOD	<LOQ	<LOQ	<LOQ	<LOD/LOQ	<LOQ	<LOD
Novel Brands of Traditional Swedish Snus												
Catch Dry Eucalyptus, 2007	Borgerding et al. 2012	NI	NI	NI	NI	<LOQ	NI	NI	NI	NI	NI	NI
Catch Dry Eucalyptus, 2006		NI	NI	NI	NI	<LOD	NI	NI	NI	NI	NI	NI
Catch Dry Licorice 2006/07		NI	NI	NI	NI	<LOD	NI	NI	NI	NI	NI	NI
Catch Dry Cassis Menthol 2006/07		NI	NI	NI	NI	<LOD	NI	NI	NI	NI	NI	NI
Catch Dry Vanilla Coffee 2006/07		NI	NI	NI	NI	<LOD	NI	NI	NI	NI	NI	NI
New Products Marketed as Snus												
Wise Citrus and Menthol, Dry 2007	Borgerding et al. 2012	NI	NI	NI	NI	15	NI	NI	NI	NI	NI	NI
Camel Original 2007		NI	NI	NI	NI	<LOQ	NI	NI	NI	NI	NI	NI

Table A II-2d: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Volatile and Non-Volatile N-Nitrosamines (ng/g)												
Brand/ STP Type Specified by Study Authors	**Citation**	**NDBA**	**NDEA**	**NDIPLA**	**NDIPA**	**NDMA**	**NDPA**	**NEMA**	**NMOR**	**NPYR**	**NPIP**	**NDELA**
Camel Original 2006		NI	NI	NI	NI	<LOD	NI	NI	NI	NI	NI	NI
Camel Frost 2007		NI	NI	NI	NI	<LOQ	NI	NI	NI	NI	NI	NI
Camel Frost 2006		NI	NI	NI	NI	<LOD	NI	NI	NI	NI	NI	NI
Camel Spice 2007		NI	NI	NI	NI	<LOQ	NI	NI	NI	NI	NI	NI
Camel Spice 2006		NI	NI	NI	NI	<LOD	NI	NI	NI	NI	NI	NI
Other New Products												
Dissolvables 2007	Borgerding et al. 2012***	NI	NI	NI	NI	<LOQ	NI	NI	NI	NI	NI	NI
Oliver Twist 2007		NI	NI	NI	NI	<LOQ	NI	NI	NI	NI	NI	NI
Hard pellets	McAdam et al. 2010a	<LOD	<LOD	<LOD	<LOQ	<LOQ/LOD	<LOD	<LOD	<LOQ	<LOQ/LOD	<LOD	<LOD
Soft pellets	McAdam et al. 2010a	<LOD	<LOD	<LOD	NR	<LOQ/LOD	<LOD	<LOD	<LOD	<LOQ/LOD	<LOD	<LOD
Ariva	McNeill et al. 2006	NI	NI	NI	NI	ND	NI	NI	NI	NI	NI	NI
US-Type Moist Snuff												

Table A II-2d: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Volatile and Non-Volatile N-Nitrosamines (ng/g)

Brand/ STP Type Specified by Study Authors	Citation	NDBA	NDEA	NDIPLA	NDIPA	NDMA	NDPA	NEMA	NMOR	NPYR	NPIP	NDELA
Moist snuff from US: Cooper, Copenhagen, Grizzly, Husky, Kayak, Kodiak, Longhorn, Red Seal, Renegades, Skoal, Timberwolf 2006/07	Borgerding et al. 2012***	NI	NI	NI	NI	<LOQ-39.8	NI	NI	NI	NI	NI	NI
Moist snuff from US 2008	McAdam et al. 2010a	NR	NR	<LOD	NR	<LOQ-110 WWB	NR	NR	<LOQ-13-20 WWB	<LOD-~60 WWB	NR	<LOD
Copenhagen	McNeill et al. 2006	NI	NI	NI	NI	ND	NI	NI	NI	NI	NI	NI
US moist snuff	Brunnemann and Hoffmann 1992	NI	NI	NI	NI	3.8-215.0	NI	NI	ND-690.0	7.4-360.0	NI	NI
Five unspecified brands/ Snuff from USA 1989-1990	Hoffmann et al. 1991	NI	NI	NI	NI	<0.01-265	NI	NI	NI	44-575	NI	NI
Unspecified brands/ US snuff available on Swedish market 1983-1986 (average of 5 samples analyzed)	Österdahl 1991	NI	NI	NI	NI	27	NI	NI	ND	31	ND	NI
English Moist Snuff	Tricker and Preussmann 1989	NI	NI	NI	NI	40 6.0-82	NI	1.5 ND-3	0.5 ND-1.5	270 64-860	20 ND-40	230 ND-740
Unspecified brands/	Brunnemann et al.	NI	ND	NI	NI	23.0-72.8	NI	NI	21.9-32.8	321-337	NI	1180-

Table A II-2d: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Volatile and Non-Volatile N-Nitrosamines (ng/g)												
Brand/ STP Type Specified by Study Authors	**Citation**	**NDBA**	**NDEA**	**NDIPLA**	**NDIPA**	**NDMA**	**NDPA**	**NEMA**	**NMOR**	**NPYR**	**NPIP**	**NDELA**
Smokeless Tobacco Products, Canada Moist Snuff (>45% moisture)	1985											2720
Unspecified brands/ Smokeless Tobacco Products, USA Moist Snuff (>45% moisture)		NI	ND	NI	NI	46.5-46.9	NI	NI	ND-19.5	41.7-93.8	NI	880-890
Five unspecified brands/ Snuff USA	Hoffmann et al. 1984	NI	NI	NI	NI	ND-215	NI	NI	24-690	ND-360	ND	290-3300
Unspecified brands/ US snuff available on Swedish market 1981-1982	Österdahl & Slorrach1983	NI	NI	NI	NI	0.4-0.8 (WWB)	NI	NI	ND	ND-1.4 (WWB)	ND	NI
Five unspecified brands/ US snuff products	Brunnemann et al. 1982	NI	NI	NI	NI	<2-215	NI	NI	24-690	<2-360	NI	290-3300
US Low-Moisture Snuff												

Table A II-2d: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Volatile and Non-Volatile N-Nitrosamines (ng/g)

Brand/ STP Type Specified by Study Authors	Citation	NDBA	NDEA	NDIPLA	NDIPA	NDMA	NDPA	NEMA	NMOR	NPYR	NPIP	NDELA
Dry snuff from US: Skoal, Taboka, Bruton, Dental, Levi Garrett, Railroad Mills Plain, Red Seal. 2006/07	Borgerding et al. 2012***	NI	NI	NI	NI	<LOQ-222	NI	NI	NI	NI	NI	NI
Dry snuff from US 2008	McAdam et al. 2010a	NR	NR	<LOD	NR	<LOQ-30 WWB	NR	NR	NR	30-200 WWB	NR	<LOD
US dry snuff	Brunnemann and Hoffmann 1992	NI	NI	NI	NI	ND – 19	NI	NI	ND – 39	72 - 148	NI	NI
US-Style Chewing Tobacco												
Chewing tobacco, Loose leaf 2006/07	Borgerding et al. 2012***	NI	NI	NI	NI	<LOQ/LOD	NI	NI	NI	NI	NI	NI
Plug 2007		NI	NI	NI	NI	<LOQ	NI	NI	NI	NI	NI	NI
Chewing tobacco from US 2008	McAdam et al. 2010a	<LOQ	<LOD	<LOD	<LOQ	<LOQ/LOD	<LOD	<LOQ	<LOQ	LOQ/LOD	<LOQ	<LOD
Plug tobacco	McAdam et al. 2010a	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Chewing tobacco, United States (1981-1990, average of 6 samples)	Brunnemann and Hoffmann 1992	NI	NI	NI	NI	64.0	NI	NI	0.6	0.8	NI	NI
Chewing tobacco, Sweden (1981-1990,	Brunnemann and Hoffmann 1992	NI	NI	NI	NI	0.2	NI	NI	0.4	0.8	NI	NI

Table A II-2d: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Volatile and Non-Volatile N-Nitrosamines (ng/g)

Brand/ STP Type Specified by Study Authors	Citation	NDBA	NDEA	NDIPLA	NDIPA	NDMA	NDPA	NEMA	NMOR	NPYR	NPIP	NDELA
average of 4 samples)												
Chewing tobacco, Denmark (1981-1990, average of 8 samples)	Brunnemann and Hoffmann 1992	NI	NI	NI	NI	5.5	NI	NI	ND	16.0	NI	NI
Unspecified brands/ Danish and Swedish chewing tobacco available on Swedish market 1981-1982	Osterdahl & Slorrach1983	NI	NI	NI	NI	ND – 3.3 WWB	NI	NI	ND – 0.8 WWB	0.9 – 25.5 WWB	ND – 0.5 WWB	NI

Notes:
All amounts given as per dry weight, except otherwise noted. ** Dry or wet weight was not specified. ND: Not detected; NQ: Not quantifiable; NR: Not reported; LOD: limit of detection; LOQ: limit of quantification; WWB: wet weight basis.
N-Nitrosodi-n-butylamine (NDBA), *N*-Nitrosodiethylamine (NDEA), *N*-Nitrosodiisopropanolamine (NDIPLA), *N*-Nitrosodiisopropylamine (NDIPA), *N*-Nitrosodimethylamine (NDMA)*, *N*-Nitrosodi-n-propylamine (NDPA), *N*-Nitrosoethylmethylamine (NEMA), *N*-Nitrosomorpholine (NMOR), *N*-Nitrosopyrrolidine (NPYR), *N*-Nitrosospiperidine (NPIP), *N*-Nitrosodiethanolamine (NDELA)
*** Limit of Detection of NDMA: 3.54 (2006) and 1.17 (2007) µg/g; Limit of quantification of NDMA: 11.7 (2006) and 3.9 (2007) µg/g
McAdam et al. 2010a (LOD; LOQ): NDBA (2.11; 7.04), NDEA (1; 4.67), NDIPLA (0.634; 2.14), NDIPA (0.579; 1.93), NDMA (1.18; 3.9), NDPA (1.51; 5.05), NEMA (1.35; 4.51), NMOR (0.53; 1.77), NPYR (1.66; 5.53), NPIP (2.29; 7.63), NDELA (0.78; 2.61)

A II 2.3.6.2 Polycyclic Aromatic Hydrocarbons

Table A II-3a, Table A II-3b, and Table A II-3c list PAHs that are either on the current HPHC list of the FDA or because they were quantified in STPs. Table A II-3a provides a summary of total PAHs and B[*a*]P concentrations in traditional Swedish snus, novel brands of traditional snus, and new products marketed as snus in the US and Canada as reported in six recent analyses of different STPs (McNeill et al. 2006; Rickert et al. 2009; Stepanov et al. 2008a; 2010; McAdam et al. 2010b, Borgerding et al. 2012). Table A II-3b and Table A II-3c details concentrations of individual specific PAHs in STPs. Results for PAH concentrations for other new (such as lozenges) or traditional products (such as moist and dry snuff and chewing tobacco) as reported in these studies are also presented.

B[*a*]P

B[*a*]P concentrations in traditional Swedish snus, including novel brands, most samples of new products marketed as snus and other new products ranged from below the LOD/LOQ to 5 ng/g dry weight, the WHO recommended limit (McNeill et al. 2006; Rickert et al. 2009; Stepanov et al. 2008a, 2010, WHO 2009, Borgerding et al. 2012). As noted in Table A II-3a, exceptions were observed for some samples of Swedish pouched snus, *Camel Snus, Grand Prix, and Triumph* products (Stepanov et al. 2008a, 2010; McAdam et al. 2010b). B[*a*]P concentrations in US moist snuff were 5-50 times higher than those detected in traditional Swedish snus, including novel brands, as well as most samples of new products marketed as snus, and other new products.

All Other PAHs

Stepanov and colleagues (2008a) reported concentrations of seven additional PAHs in various STPs, including two IARC-classified Group 2B carcinogens PAHs. B[*b*]F and B[*k*]F were generally not detected (in *General* snus or in new products marketed as snus), with the exception of some *Marlboro Snus* brands, where the sum of B[*b*]F and B[*k*]F was approximately 3 ng/g dry weight. In comparison, concentrations of B[*b*]F and B[*k*]F in US-type moist snuff samples were up to 19 times higher. No other classified carcinogenic PAHs were analyzed in traditional Swedish snus.

Stepanov and colleagues (2010) analyzed 23 PAHs (including eight IARC-classified Group 2A/2B carcinogens and B[*a*]P) in 17 brands of spit-free tobacco pouches (new products marketed as snus and produced by US companies: *Marlboro Snus, Camel Snus, Tourney, Grand Prix, Triumph, and Nordic Ice Snus*) and in brands of US-type moist snuff; no traditional Swedish snus products were included in this study.

They concluded that, in agreement with their previous results (as reported above), “the levels of PAHs in spit-free tobacco pouches were very low”. Specifically, they 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 mean sum of PAHs in US-type moist snuff. 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. Naphthalene was 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)."

Also, McAdam and colleagues (2010b), at the ACS Fall Meeting in Boston, presented the analysis of 21 PAHs (8 IARC-classified Group 2A/2B carcinogens in addition to B[*a*]P) in Swedish pouched and loose snus and other STPs. Except for B[*a*]P, the results of the individuals PAHs were not provided. The authors noted that "[h]ighest total PAH contents were found with moist and dry snuff, and with soft pellet products. Other smokeless products had considerably lower contents."

Table A II-3c summarizes the concentrations of PAHs *not classifiable as to their carcinogenicity* (IARC Group 3) or not evaluated as reported in STPs. Stepanov et al. (2008a) reported that similar to *General* samples, anthracene concentrations were below the detection limit in all *Marlboro Snus* and *Camel Snus* samples, whereas higher concentrations were observed in US-type moist snuff. Also, 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 below the LOD to approximately twice of what was detected in the *General* samples (Stepanov et al. 2008a). In addition to the above discussed Group 2A/B carcinogens, Stepanov et al. (2010) also reported nine other PAHs in STPs; however, traditional Swedish snus products were not analyzed. The range of concentrations of these other PAHs measured in US moist snuff was generally higher than those observed in new products marketed as snus.

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Table A II-3a:Trace-Level Components in Snus and STPs as Reported in the Literature: Polycyclic Aromatic Hydrocarbons				
Brand/ STP Type Specified by Study Authors	**Citation**	**B[*a*]P (ng/g)**	**PAHs Analyzed**	**Total PAH (ng/g)**
Traditional Swedish Snus				
General Original Portion	Borgerding et al. 2012	1	B[*a*]P only	NI
General White Portion		0.6		
General Original Loose		1.1		
General Onyx		0.3		
Gustavus Original		4.1		
Nick and Johnny		2.1		
Rocker Black		1.3		
Rocker Silver		1.6		
Swedish pouched snus	McAdam et al. 2010b	~0-7	21 PAHs	~50-700*
Swedish loose snus		~0		~50-500*
General/ Swedish snus	Stepanov et al. 2008a	<LOD	8 PAHs	NR
"general [sic] pouch"/ Snus (Sweden)	McNeill et al. 2006	1.99	B[*a*]P only	NI
Novel Brands of Traditional Swedish Snus				
*Catch Dry***	Borgerding et al. 2012	1-1.6	B[*a*]P only	NI
New Products Marketed as Snus				
*Camel Snus***	Caraway and Chen 2012	0.883-1.416∞ WWB	B[*a*]P only	NI
Wise Citrus and Menthol, Dry	Borgerding et al. 2012	0.8	B[*a*]P only	NI
Camel Snus **		1.2-1.9		
Marlboro Snus **	Stepanov et al. 2010	<LOQ	23 PAHs	749-1260
*Camel Snus***		<LOQ-15.2		1170-1430

Table A II-3a:Trace-Level Components in Snus and STPs as Reported in the Literature: Polycyclic Aromatic Hydrocarbons				
Brand/ STP Type Specified by Study Authors	**Citation**	**B[*a*]P (ng/g)**	**PAHs Analyzed**	**Total PAH (ng/g)**
*Tourney***		<LOQ		1150-1300
Grand Prix		13.3-15.6		1120–1340
*Triumph***		<LOQ-5.9		1720-1940
Nordic Ice		LOQ		1410
Average for spit-free tobacco**		12.3 (<LOQ-15.6)		1280 (749-1940)
Du Maurier Freshmint/ Swedish snus	Rickert et al. 2009	1.59	B[*a*]P only	NI
Du Maurier Original/ Swedish snus		2.08		NI
Marlboro Snus/ New STP	Stepanov et al. 2008a	<LOD-2.06	8 PAHs	NR
Camel Snus/ New STP		<LOD-10.5		NR
Other New Products				
Dissolvables**	Borgerding et al. 2012	0.3-0.4	B[*a*]P only	NI
*Twist***		1.8-88.5		
Skoal Dry		1.1		
*Taboka***		0.7-0.8		
Hard tobacco pellets	McAdam et al. 2010b	~0-5	21 PAHs	~0*
Soft tobacco pellets		~100		~15,000*
*Skoal Dry***	Stepanov et al. 2008a	<LOD-2.1	8 PAHs	NI
*Taboka***		<LOD		NI
Ariva	McNeill et al. 2006	0.40	B[*a*]P only	NI
US-Type Moist Snuff				
US Moist snuff: *Cooper, Copenhagen, Grizzly, Husky,*	Borgerding et al. 2012	49.8-198	B[*a*]P only	NI

Table A II-3a:Trace-Level Components in Snus and STPs as Reported in the Literature: Polycyclic Aromatic Hydrocarbons				
Brand/ STP Type Specified by Study Authors	**Citation**	**B[*a*]P (ng/g)**	**PAHs Analyzed**	**Total PAH (ng/g)**
Kayak, Kodiak, Longhorn, Red Seal, Renegades, Skoal, Timberwolf				
US moist snuff	McAdam et al. 2010b	~30-180	21 PAHs	~4,000-20,000*
Average for various brands and types, including: *Skoal, Copenhagen, Grizzly, Kayak, Timber Wolf, Red Seal, Longhorn*, and *Hawken*	Stepanov et al. 2010	56 (13-102)	23 PAHs	11600 (1250-20000)
Various fine-cut, long-cut, pouched brands: *Skoal, Rooster, Copenhagen*	Rickert et al. 2009	21.1-83.2	B[*a*]P only	NI
Various brands and types, including: *Skoal, Copenhagen, and Kodiak*	Stepanov et al. 2008a	30.1-57.3	8 PAHs	NI
Copenhagen	McNeill et al. 2006	19.33	B[*a*]P only	NI
US-Type Dry Snuff				
US Dry snuff: *Bruton, Dental, Levi Garrett, Railroad Mills Plain, Red Seal.*	Borgerding et al. 2012	0.7-118	B[*a*]P only	NI
US dry snuff**	McAdam et al. 2010b	~5-130	21 PAHs	~200-13,000*
Various *McChrystal's* brands	Rickert et al. 2009	11.8-18.6	B[*a*]P only	NI
US-Type Chewing Tobacco				
Chewing tobacco, Loose leaf	Borgerding et al. 2012	1.2-5.1	B[*a*]P only	NI
Plug		5.4		
US chewing tobacco	McAdam et al. 2010b	~0-5	21 PAHs	~100-1000*
US plug tobacco		~5		~20*

Table A II-3a:Trace-Level Components in Snus and STPs as Reported in the Literature: Polycyclic Aromatic Hydrocarbons

Brand/ STP Type Specified by Study Authors	Citation	B[*a*]P (ng/g)	PAHs Analyzed	Total PAH (ng/g)
Red man, Apple plug	Rickert et al. 2009	<LOD-<LOQ	B[*a*]P only	NI

Notes:
All amounts given as per dry weight, unless otherwise noted. ∞Values were based on 0.6g of pouched snus. LOD: limit of detections; LOQ: limit of quantification; NI: Not investigated; NR: Not reported
* Not specified if per dry or wet weight, potentially wet weight basis. ***Multiple brands*

Table A II-3b:Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: IARC-Classified Group 2A/2B Carcinogens Polycyclic Aromatic Hydrocarbons**											
Brand/ STP Type Specified by Study Authors	**Citation**	**B[*a*]A (ng/g)**	**B[*a*]P (ng/g)**	**B[*b*]F + B[*k*]F (ng/g)**	**B[*b*]F + B[*j*]F (ng/g)**	**B[*k*]F (ng/g)**	**Chrysene (ng/g)**	**DB[*ah*]A (ng/g)**	**I[*cd*]P (ng/g)**	**Methyl-chrysene Isomers/ 5-MC (ng/g)**	**Naphthalene (ng/g)**
Traditional Swedish Snus											
Swedish pouched snus, 2008	McAdam et al. 2010b	NR	~0-7	NR	NR	NR	NR	NR	NR	NI	NI
Swedish loose snus, 2008	McAdam et al. 2010b	NR	~0	NR	NR	NR	NR	NR	NR	NI	NI
General/ Swedish snus	Stepanov et al. 2008a	NI	ND	ND	NI	NI	NI	NI	NI	NI	NI
New Products Marketed as Snus											
Marlboro snus, Rich	Stepanov et al. 2010	1.7	<LOQ	NI	<LOQ	<LOQ	1.9	<LOQ	<LOQ	<LOQ	866
Marlboro snus, Mild		1.1	<LOQ	NI	<LOQ	<LOD	1.3	<LOQ	<LOQ	<LOQ	722
Marlboro snus, spearmint		<LOQ	<LOQ	NI	<LOQ	<LOD	1.4	<LOD	<LOQ	<LOQ	1070
Marlboro snus, peppermint		1.1	<LOQ	NI	<LOQ	<LOD	1.5	<LOQ	<LOQ	<LOQ	1230
Camel Snus, Original		5.9	15.2	NI	38.8	3.1	13.3	19.8	<LOQ	<LOD	1110
Camel Snus Spice		5.5	15	NI	30.2	3.1	9.1	15.2	<LOQ	<LOD	1080
Camel Snus		5.4	14.9	NI	31.5	3.1	12.4	8.8	<LOQ	<LOD	1070

Table A II-3b:Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: IARC-Classified Group 2A/2B Carcinogens Polycyclic Aromatic Hydrocarbons**											
Brand/ STP Type Specified by Study Authors	**Citation**	**B[*a*]A (ng/g)**	**B[*a*]P (ng/g)**	**B[*b*]F + B[*k*]F (ng/g)**	**B[*b*]F + B[*j*]F (ng/g)**	**B[*k*]F (ng/g)**	**Chrysene (ng/g)**	**DB[*ah*]A (ng/g)**	**I[*cd*]P (ng/g)**	**Methyl-chrysene Isomers/ 5-MC (ng/g)**	**Naphthalene (ng/g)**
Frost											
Camel Snus Mellow		2.5	<LOQ	NI	<LOQ	<LOD	3.2	<LOD	<LOQ	<LOQ	1060
Tourney Original		2.6	<LOQ	NI	6.6	<LOQ	5.6	<LOQ	<LOQ	<LOD	1060
Tourney Spearmint		5.5	<LOQ	NI	9.8	3.4	9.9	<LOQ	4.2	<LOD	1130
Tourney Wintergreen		5.1	<LOQ	NI	9.9	3.2	8.7	3.6	4	<LOD	993
Grand Prix Original		2.9	13.3	NI	7.6	<LOQ	5.1	4.2	<LOQ	<LOD	1100
Grand Prix Spearmint		7.1	15	NI	11.9	3.6	11.1	3.7	4.4	<LOD	1100
Grand Prix Wintergreen		7.6	15.6	NI	12.6	4.4	11.5	<LOQ	<LOD	<LOD	932
Triumph Original		5.3	<LOQ	NI	45.1	<LOQ	11.7	68.7	<LOQ	<LOD	1560
Triumph Mint		4.1	5.9	NI	21.2	<LOQ	8.6	8.6	<LOQ	<LOD	1510
Nordic Ice		3.2	<LOQ	NI	2.9	<LOQ	3.7	<LOD	<LOQ	3.4	1310
Average for spit-free tobacco		4.0 (<LOQ-7.6)	12.3 (<LOQ-15.6)	NI	19 (<LOQ-38.8)	2.8 (<LOD-4.4)	7.1 (1.3-13.3)	16.6 (<LOQ-68.7)	4.4 (<LOD-4.4)	NA (<LOD-3.4)	1110 (722-1560)
Marlboro Snus/ New	Stepanov et al. 2008a	NI	ND-2.06	ND-2.93	NI	NI	NI	NI	NI	NI	NI

Table A II-3b:Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: IARC-Classified Group 2A/2B Carcinogens Polycyclic Aromatic Hydrocarbons**											
Brand/ STP Type Specified by Study Authors	**Citation**	**B[*a*]A (ng/g)**	**B[*a*]P (ng/g)**	**B[*b*]F + B[*k*]F (ng/g)**	**B[*b*]F + B[*j*]F (ng/g)**	**B[*k*]F (ng/g)**	**Chrysene (ng/g)**	**DB[*ah*]A (ng/g)**	**I[*cd*]P (ng/g)**	**Methyl-chrysene Isomers/ 5-MC (ng/g)**	**Naphthalene (ng/g)**
STP											
Marlboro Snus Rich		NI	1.55	2.59	NI	NI	NI	NI	NI	NI	NI
Marlboro Snus Mild		NI	2.06	2.93	NI	NI	NI	NI	NI	NI	NI
Marlboro Snus Spice		NI	ND	ND	NI	NI	NI	NI	NI	NI	NI
Marlboro Snus Mint		NI	1.02	ND	NI	NI	NI	NI	NI	NI	NI
Camel Snus Original		NI	10.5	ND	NI	NI	NI	NI	NI	NI	NI
Camel Snus Spice		NI	ND	ND	NI	NI	NI	NI	NI	NI	NI
Camel Snus Frost		NI	ND	ND	NI	NI	NI	NI	NI	NI	NI
Other New Products											
Hard tobacco pellets, US 2008	McAdam et al. 2010b	NR	~0-5	NR	NR	NR	NR	NR	NR	NI	NI
Soft tobacco pellets, US 2008		NR	~100	NR	NR	NR	NR	NR	NR	NI	NI
Skoal Dry Regular	Stepanov et al. 2008a	NI	1.48	ND	NI	NI	NI	NI	NI	NI	NI

Table A II-3b:Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: IARC-Classified Group 2A/2B Carcinogens Polycyclic Aromatic Hydrocarbons**											
Brand/ STP Type Specified by Study Authors	**Citation**	**B[*a*]A (ng/g)**	**B[*a*]P (ng/g)**	**B[*b*]F + B[*k*]F (ng/g)**	**B[*b*]F + B[*j*]F (ng/g)**	**B[*k*]F (ng/g)**	**Chrysene (ng/g)**	**DB[*ah*]A (ng/g)**	**I[*cd*]P (ng/g)**	**Methyl-chrysene Isomers/ 5-MC (ng/g)**	**Naphthalene (ng/g)**
Skoal Dry Cinnamon		NI	ND	ND	NI	NI	NI	NI	NI	NI	NI
Skoal Dry Menthol		NI	2.10	ND	NI	NI	NI	NI	NI	NI	NI
Taboka Original		NI	ND	ND	NI	NI	NI	NI	NI	NI	NI
Taboka Green		NI	ND	ND	NI	NI	NI	NI	NI	NI	NI
US Moist Snuff											
Average for various brands and types, including: *Skoal, Copenhagen, Grizzly, Kayak, Timber Wolf, Red Seal, Longhorn*, and *Hawken*	Stepanov et al. 2010	194 (5.3-346)	56 (13-102)	NI	107 (7.4-281)	20 (<1.6-37)	232 (7.8-477)	7.5 (<3.9-11)	21 (<2.3 – 49)	93 (57-217)	1730 (886-2270)
Moist snuff brands, US 2008	McAdam et al. 2010b	NR	~30-180	NR	NR	NR	NR	NR	NR	NI	NI
Various brands and	Stepanov et al. 2008a	NI	30.1-57.3	28.6-57.1	NI	NI	NI	NI	NI	NI	NI

Table A II-3b:Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: IARC-Classified Group 2A/2B Carcinogens Polycyclic Aromatic Hydrocarbons**

Brand/ STP Type Specified by Study Authors	Citation	B[*a*]A (ng/g)	B[*a*]P (ng/g)	B[*b*]F + B[*k*]F (ng/g)	B[*b*]F + B[*j*]F (ng/g)	B[*k*]F (ng/g)	Chrysene (ng/g)	DB[*ah*]A (ng/g)	I[*cd*]P (ng/g)	Methyl-chrysene Isomers/ 5-MC (ng/g)	Naphthalene (ng/g)
types, including: *Skoal, Copenhagen and Kodiak*											
US-Type Low-Moisture Snuff											
US dry snuff	McAdam et al. 2010b	NR	~5-130	NR	NR	NR	NR	NR	NR	NI	NI
US-Type Chewing Tobacco											
US chewing tobacco	McAdam et al. 2010b	NR	~0-5	NR	NR	NR	NR	NR	NR	NI	NI
US plug tobacco		NR	~5	NR	NR	NR	NR	NR	NR	NI	NI

Notes:
All amounts given as per dry weight, unless otherwise noted.
B[*a*]A - Benz[*a*]anthracene; B[*a*]P - Benzo[*a*]pyrene; B[*b*]F - Benzo[*b*]fluoranthene; B[*k*]F - Benzo[*k*]fluoranthene; B[*j*]F - Benzo[*j*]fluoranthene; DB[*ah*]A - Dibenz[*a,h*]anthracene; I[*cd*]P - Indeno[*1,2,3-cd*]pyrene. **Per IARC Classifications
∞Values were based on 0.6g of pouched snus.
ND: Not detected; NI: Not investigated; NR: Not reported, but included in total PAHs, see Table A II-3a

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

Brand/STP Type Specified by Study Authors	Citation	Acenaphthene (ng/g)	Acenaphthylene (ng/g)	Anthracene (ng/g)	B[e]P (ng/g)	B[ghi]Py (ng/g)	Fluoranthene (ng/g)	Fluorene (ng/g)	Phenanthrene (ng/g)	Pyrene (ng/g)
Traditional Swedish Snus										
Swedish pouched and loose snus in 2008	McAdam et al. 2010b	NR	NR	NR	NR	NR	NR	NR	NR	NR
General/ Swedish snus	Stepanov et al. 2008a	NI	1.7	ND	NI	NI	31.1	NI	55.3	29.7
"general [sic] pouch"/ Snus (Sweden)	McNeill et al. 2006	NI	NI	NI	NI	NI	NI	NI	NI	NI
New Products Marketed as Snus										
Marlboro Snus Rich	Stepanov et al. 2010	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	9	<LOQ	13.5	9
Marlboro Snus Mild		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.9	<LOQ	9.7	7.6
Marlboro Snus Spearmint		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.7	<LOQ	10	7.0
Marlboro Snus Peppermint		<LOQ	8.1	<LOQ	<LOQ	<LOQ	5.6	<LOQ	9.4	6.0
Camel Snus Original		<LOQ	<LOQ	6.9	15.2	<LOQ	60.1	9	68	46.5
Camel Snus Spice		<LOQ	9	8.1	15	<LOQ	59.7	11.8	79.4	45.4
Camel Snus Frost		<LOQ	<LOQ	6.9	14.9	<LOQ	60.5	9.7	68.7	46.3

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

Brand/STP Type Specified by Study Authors	Citation	Acenaphthene (ng/g)	Acenaphthylene (ng/g)	Anthracene (ng/g)	B[e]P (ng/g)	B[ghi]Py (ng/g)	Fluoranthene (ng/g)	Fluorene (ng/g)	Phenanthrene (ng/g)	Pyrene (ng/g)
Camel Snus Mellow		<LOQ	<LOQ	6.1	<LOQ	<LOQ	33	<LOQ	44.8	25.3
Tourney Original		17.7	<LOQ	<LOQ	<LOQ	<LOQ	8.8	5.2	36.2	17.3
Tourney Spearmint		19.5	<LOQ	<LOQ	<LOQ	<LOQ	23.7	5.6	41.9	28.2
Tourney Wintergreen		20.7	<LOQ	<LOQ	<LOQ	<LOQ	19.4	5.9	34.9	25.4
Grand Prix Original		129	<LOQ	<LOQ	<LOQ	<LOQ	10.9	6.1	42.4	18.5
Grand Prix Spearmint		16	<LOQ	<LOQ	3.2	<LOQ	37	6.4	43.2	35.4
Grand Prix Wintergreen		<LOQ	<LOQ	<LOQ	2.9	<LOQ	36.4	5.7	51.7	37.5
Triumph Original		<LOQ	<LOQ	<LOQ	75.9	<LOQ	53.1	9.2	65.5	48.5
Triumph Mint		<LOQ	<LOQ	<LOQ	32.4	<LOQ	33.6	7.4	44.4	36.1
Nordic Ice		<LOQ	<LOQ	5.7	<LOQ	<LOQ	15.6	2.6	36.7	17.5
Average for spit-free tobacco		35.4 (<LOQ -129)	8.5 (<LOQ-9.0)	6.7 (<LOQ-8.1)	23.0 (<LOQ-75.9)	<LOQ	28.2 (5.6-60.5)	6.3 (<LOQ-11.8)	41.2 (9.4-68.7)	26.9 (6.0-48.5)
Marlboro Snus Rich	Stepanov et al. 2008a	NI	ND	ND	NI	NI	5.54	NI	14.8	7.24
Marlboro Snus Mild		NI	ND	ND	NI	NI	4.42	NI	9.44	4.43
Marlboro		NI	ND	ND	NI	NI	5.38	NI	15.9	6.24

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

Brand/STP Type Specified by Study Authors	Citation	Acenaphthene (ng/g)	Acenaphthylene (ng/g)	Anthracene (ng/g)	B[e]P (ng/g)	B[ghi]Py (ng/g)	Fluoranthene (ng/g)	Fluorene (ng/g)	Phenanthrene (ng/g)	Pyrene (ng/g)
Snus Spice										
Marlboro Snus Mint		NI	3.15	ND	NI	NI	5.86	NI	14.6	5.68
Camel Snus Original		NI	3.95	ND	NI	NI	20.5	NI	41.7	20.1
Camel Snus Spice		NI	4.14	ND	NI	NI	19.2	NI	33.7	16.4
Camel Snus Frost		NI	4.99	ND	NI	NI	22.5	NI	40.7	20.3
Other New Products										
Taboka Original	Stepanov et al. 2008a	NI	2.28	ND	NI	NI	9.56	NI	15.6	9.23
Taboka Green		NI	2.04	ND	NI	NI	11.0	NI	19.8	7.52
Skoal Dry Regular		NI	1.27	ND	NI	NI	3.78	NI	10.7	5.08
Skoal Dry Cinnamon		NI	0.849	ND	NI	NI	8.38	NI	24.3	7.37
Skoal Dry Menthol		NI	0.986	ND	NI	NI	4.25	NI	12.8	4.54
US-Type Moist Snuff										
Moist snuff brands from the US in 2008	McAdam et al. 2010b	NR	NR	NR	NR	NR	NR	NR	NR	NR
Various	Stepanov et	105	111	844	52	18	1400	827	4700	1290

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

Brand/STP Type Specified by Study Authors	Citation	Acenaphthene (ng/g)	Acenaphthylene (ng/g)	Anthracene (ng/g)	B[e]P (ng/g)	B[ghi]Py (ng/g)	Fluoranthene (ng/g)	Fluorene (ng/g)	Phenanthrene (ng/g)	Pyrene (ng/g)
brands and types, including: *Skoal, Copenhagen, Grizzly, Kayak, Timber Wolf, Red Seal, Longhorn,* and *Hawken*	al. 2010	(<10.9-200)	(37-174)	(8.6-440)	(<2.1-111)	(<2.4-41)	(45-2540)	(4.8-1440)	(58-8660)	(46-2250)
Various brands and types, including: *Skoal, Copenhagen, and Kodiak*	Stepanov et al. 2008a	NI	16.7-67.5	148-639	NI	NI	277-872	NI	528-3920	323-1060

Notes:
* PAHs that were not classifiable as to their carcinogenicity to humans (IARC Group 3) or not evaluated by IARC
All amounts given as per dry weight, unless otherwise noted.
B[e]P: Benzo[e]pyrene; B[ghi]Py: Benzo [g,h,i] perylene
ND: Not detected; NI: Not investigated; NR: Not reported, but included in total PAHs, see Table A II-3a

A II 2.3.6.3 Aldehydes

Table A II-4 summarizes concentrations of aldehydes in traditional Swedish snus and in new products marketed as snus in the US as reported by two recent analyses of various STPs (Stepanov et al. 2008a; Faizi et al. 2009).

Concentrations of formaldehyde and acetaldehyde in *General* snus were in the range of those detected in US-type moist snuff investigated in the same study and slightly lower in *Marlboro Snus* and *Camel Snus* (Stepanov et al. 2008a). Acrolein concentrations in *General* were slightly lower than those reported in US-type moist snuff, and in the same range or lower in *Marlboro Snus* and *Camel Snus.* Crotonaldehyde concentrations were similar in *General* compared to new products marketed as snus and US-type moist snuff, 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".

BAT researchers at the CORESTA Joint Study Group meeting, presented information on aldehydes in various Swedish and US products (data not shown in the table below). Although this data has not been peer reviewed, it provides additional information to the existing limited data on aldehydes in STPs (Faizi et al. 2009). These researchers did not specify whether any of the snus analyzed was traditional Swedish. Concentrations of formaldehyde in Swedish pouched and loose snus were lower than those reported in US-type moist snuff, per dry and wet weight. Acetaldehyde concentrations in Swedish pouched or loose snus were slightly higher or comparable to concentrations reported in US moist snuff, per wet and dry weight. Concentrations of crotonaldehyde in all Swedish and US samples analyzed were below the LOQ/LOD (LOQ: 0.024 µg/g; LOD: 0.007 µg/g). The highest acrolein concentrations were detected in US moist snuff, while those in almost all other STPs were below the LOQ/LOD (LOQ: 0.033 µg/g; LOD: 0.01 µg/g). Overall, aldehyde concentrations reported by Faizi et al (2009) were lower than those reported by Stepanov and colleagues (2008a).

Table A II-4:Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Aldehydes					
Brand	**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	Stepanov et al. 2008a	4.66	5.88	0.483	17.1
Marlboro Snus Mild		4.09	3.33	0.591	18.4
Marlboro Snus Spice		7.04	8.08	0.383	10.4
Marlboro Snus Mint		5.35	10.5	0.726	4.83
Camel Snus Original		1.51	6.64	0.31	0.552
Camel Snus Spice		4.11	13.3	4.42	3.37
Camel Snus Frost		3.02	16.4	3.31	3.56
Other New Products					
Taboka Original	Stepanov et al. 2008a	3.14	1.83	0.4	19.4
Taboka Green		2.3	1.96	0.52	16.5
Skoal Dry Regular		1.76	2.51	0.269	3.49
Skoal Dry Cinnamon		0.207	0.970	0.619	8.95
Skoal Dry Menthol		1.58	2.53	ND	2.74
US-Type Moist Snuff					
Various brands and types, including: *Skoal, Copenhagen, and Kodiak*	Stepanov et al. 2008a	6.58-10.6	17.1-72.3	2.58-7.85	0.984-6.35
Notes: All amounts given as per dry weight unless otherwise noted. ND: not detected					

A II 2.3.6.4 Metals/Metalloids

Table A II-5 summarizes concentrations of selected heavy metals in traditional Swedish snus, including novel brands, and new products marketed as snus reported in recent analyses of various STPs (McNeill et al. 2006; Rickert et al. 2009; McAdam et al. 2010c; Caraway and Chen 2012; Borgerding et al. 2012).

Concentration of arsenic, cadmium, chromium, lead, mercury, and nickel measured in traditional Swedish snus were below, or for cadmium, near the GOTHIATEK® Standard limits (McNeil et al. 2006; Fisher 2007; Borgerding et al. 2012). Concentrations of these metals in traditional Swedish snus, including novel brands were similar to those in new products marketed as snus (*Camel Snus*), and were generally lower than those in US-type moist snuff. The exceptions were nickel and arsenic, where concentrations in traditional Swedish snus were in the range of those detected in US-type moist snuff brands analyzed in the same studies; lead concentrations in traditional Swedish snus were on the lower end of the range of those detected in US-type moist snuff brands (McNeil et al. 2006; Fisher 2007; Borgerding et al. 2012).

Also, Rickert and colleagues (2009) measured selenium, an essential trace element, in STPs on the Canadian market, including new products marketed as snus (*du Maurier brands);* traditional Swedish snus was not included in this study. In most of the investigated US-type long-cut moist snuff brands, selenium concentrations were not detected; Selenium concentrations in fine-cut and pouched US-type moist snuff brands were approximately half of those detected in the two brands of *du Maurier*.

Lastly, at the 2010 SRNT Meeting, McAdam and colleagues (2010c) presented the results of a study that investigated concentrations of eight metals in several Swedish and US snus products; however, it was unclear which STPs products were from Sweden or the US. Concentrations of arsenic, cadmium, lead, nickel and selenium in pouched and loose snus were all lower than concentrations observed in moist snuff, per wet weight, except beryllium, chromium and mercury, where concentrations were generally comparable. Exceptions were three snus samples that had considerably higher beryllium concentrations than moist snuff. Mercury concentrations were determined in only one to three samples per product category.

No studies were identified that evaluated cobalt and barium in traditional Swedish snus, including novel brands, or new products marketed as snus; the only data available was for US-type moist snuff (Pappas et al. 2008).

Table A II-5: Trace-Level Components in Snus and other STPs as Reported in the Literature: Metals/ Metalloids (µg/g dry weight unless indicated otherwise)

Brand/ STP Type Specified by Study Authors	Citation	Arsenic (As)	Beryllium (Be)	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Lead (Pb)	Mercury (Hg)	Nickel (Ni)	Barium (Ba)	Selenium (Se)
Traditional Swedish Snus											
General Original Portion 2006/07	Borgerding et al. 2012	0.152	NI	0.365	1.058	NI	0.208	NI	1.471	NI	NI
General White Portion 2006/07		0.118	NI	0.355	1.178	NI	0.180	NI	1.384	NI	NI
General Loose 2006/07		0.153	NI	0.437	0.877	NI	0.209	NI	1.322	NI	NI
General Onyx 2006/07		0.084	NI	0.615	1.822	NI	0.193	NI	2.781	NI	NI
Gustavus Original, Snuff 2006		0.160	NI	0.363	1.334	NI	0.244	NI	1.182	NI	NI
Nick and Johnny, Snuff 2007		0.120	NI	0.564	1.044	NI	0.157	NI	2.121	NI	NI

Table A II-5: Trace-Level Components in Snus and other STPs as Reported in the Literature: Metals/ Metalloids (µg/g dry weight unless indicated otherwise)

Brand/ STP Type Specified by Study Authors	Citation	Arsenic (As)	Beryllium (Be)	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Lead (Pb)	Mercury (Hg)	Nickel (Ni)	Barium (Ba)	Selenium (Se)
Rocker Black, Snuff 2007		0.111	NI	0.430	1.229	NI	0.228	NI	1.651	NI	NI
Rocker Silver, Snuff 2007		0.078	NI	0.362	0.870	NI	0.162	NI	1.923	NI	NI
Swedish snus products	Fisher 2007	~0.15-0.20	NI	~0.30-0.75	~0.45-1.70	NI	~0.35-0.50	NI	~0.20-1.90	NI	NI
"general [sic] pouch"/ Snus (Sweden)	McNeill et al. 2006	0.30	NI	NI	1.54	NI	0.50	NI	2.59	NI	NI
Snus (US/Sweden)											
Pouched snus from US or Sweden 2008	McAdam et al. 2010c	~0.04-0.11, ~0.275 (1 sample) WWB	~0.002-0.020, ~0.067 & ~0.087 (1 sample each) WWB	~0.150-0.730 WWB	~0.40-1.10, ~4.400 (1 sample) WWB	NI	~0.12-0.36, ~0.900 & ~1.300 WWB (1 sample each)	~0.0105-0.0145 WWB (3 samples)	~0.60-1.70, ~2.30 (1 sample) WWB	NI	~0.055-0.10,~0.125 (2 samples) WWB
Loose snus from US or Sweden 2008	McAdam et al. 2010c	~0.03-0.90 WWB	~0.006-0.014, ~0.062 (1 sample) WWB	~0.15-0.41 WWB	~0.4-1.6 WWB	NI	~0.18-0.45, ~1.5 WWB	0.0105 (1 sample) WWB	~0.60-1.95 WWB	NI	~0.05-0.09 WWB

Table A II-5: Trace-Level Components in Snus and other STPs as Reported in the Literature: Metals/ Metalloids (µg/g dry weight unless indicated otherwise)

Brand/ STP Type Specified by Study Authors	Citation	Arsenic (As)	Beryllium (Be)	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Lead (Pb)	Mercury (Hg)	Nickel (Ni)	Barium (Ba)	Selenium (Se)
Novel Brands of Traditional Swedish Snus											
Catch Dry Eucalyptus 2007	Borgerding et al. 2012	0.105	NI	0.672	0.955	NI	0.198	NI	1.584	NI	NI
Catch Dry Eucalyptus 2006		0.149	NI	0.527	1.270	NI	0.218	NI	1.920	NI	NI
Catch Dry Licorice 2006/07		0.157	NI	0.494	1.198	NI	0.221	NI	1.946	NI	NI
Catch Dry Cassis Menthol 2006/07		0.096	NI	0.420	0.986	NI	0.240	NI	1.545	NI	NI
Catch Dry Vanilla Coffee 2006/07		0.113	NI	0.426	1.035	NI	0.254	NI	1.668	NI	NI
New Products Marketed as Snus											
Wise Citrus and Menthol, Dry 2007	Borgerding et al. 2012	0.325	NI	0.278	4.452	NI	0.737	NI	2.318	NI	NI
Camel Original 2007		<LOQ (0.025)	NI	0.512	1.209	NI	0.221	NI	1.379	NI	NI

Table A II-5: Trace-Level Components in Snus and other STPs as Reported in the Literature: Metals/ Metalloids (µg/g dry weight unless indicated otherwise)

Brand/ STP Type Specified by Study Authors	Citation	Arsenic (As)	Beryllium (Be)	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Lead (Pb)	Mercury (Hg)	Nickel (Ni)	Barium (Ba)	Selenium (Se)
Camel Original 2006		0.157	NI	0.566	1.823	NI	0.276	NI	2.084	NI	NI
Camel Frost 2007		0.136	NI	0.641	1.451	NI	0.225	NI	1.498	NI	NI
Camel Frost 2006		0.188	NI	0.540	1.900	NI	0.220	NI	2.187	NI	NI
Camel Spice 2007		0.108	NI	0.740	1.540	NI	0.216	NI	1.708	NI	NI
Camel Spice 2006		0.151	NI	0.526	1.466	NI	0.220	NI	1.734	NI	NI
Camel Original	Caraway and Chen 2012	0.083[∞] WWB	NI	0.328[∞] WWB	0.43[∞] WWB	NI	0.118[∞] WWB	NI	0.612[∞] WWB	NI	NI
Camel Spice		0.0773[∞] WWB	NI	0.325[∞] WWB	0.42[∞] WWB	NI	0.112[∞] WWB	NI	0.0.572[∞] WWB	NI	NI
Camel Frost		0.083[∞] WWB	NI	0.332[∞] WWB	0.437[∞] WWB	NI	0.12[∞] WWB	NI	0.643[∞] WWB	NI	NI
Du Maurier Freshmint/ Swedish snus mint-flavored	Rickert et al. 2009	0.175	NI	0.994	1.575	NI	0.242	NI	1.446	NI	0.159
Du Maurier Original/ Swedish snus	Rickert et al. 2009	0.143	NI	0.967	1.985	NI	0.233	NI	1.536	NI	0.153
Philip Morris	Fisher 2007	~0.10-0.15	NI	~0.50-0.75	~1.60-1.80	NI	~0.20-0.25	NI	~0.70-1.6	NI	NI

Table A II-5: Trace-Level Components in Snus and other STPs as Reported in the Literature: Metals/ Metalloids (µg/g dry weight unless indicated otherwise)

Brand/ STP Type Specified by Study Authors	Citation	Arsenic (As)	Beryllium (Be)	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Lead (Pb)	Mercury (Hg)	Nickel (Ni)	Barium (Ba)	Selenium (Se)
snus products											
Other New Products											
Dissolvables (2007)	Borgerding et al. 2012	0.072-0.181	NI	0.251-0.471	1.418-2.040	NI	0.181-2.040	NI	0.807-1.928	NI	NI
Twist (2007)	Borgerding et al. 2012	0.115-0.307	NI	0.436-1.496	0.726-0.956	NI	0.386-0.501	NI	0.770-2.141	NI	NI
Moist pellets	McAdam et al. 2010c	~0.18 WWB (1 sample)	~0.011 WWB	~0.65 WWB (1 sample)	~0.4 WWB (1 sample)	NI	~0.4 WWB (1 sample)	0.01 WWB (1 sample)	~1.1 (1 sample) WWB	NI	0.09 WWB (1 sample)
Hard pellets	McAdam et al. 2010c	~0.14-0.16 WWB (2 samples)	~0.01-0.011 WWB (2 samples)	~0.39-0.5 WWB (2 samples)	~2.0-2.2 WWB (2 samples)	NI	~0.37 WWB (1 sample)	0.01 WWB (1 sample)	~1.5 WWB (1 sample)	NI	~.025 WWB (2 samples)
Ariva	McNeill et al. 2006	0.12	NI	NI	1.40	NI	0.28	NI	2.19	NI	NI

Table A II-5: Trace-Level Components in Snus and other STPs as Reported in the Literature: Metals/ Metalloids (µg/g dry weight unless indicated otherwise)

Brand/ STP Type Specified by Study Authors	Citation	Arsenic (As)	Beryllium (Be)	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Lead (Pb)	Mercury (Hg)	Nickel (Ni)	Barium (Ba)	Selenium (Se)
US Moist Snuff											
US Moist snuff: *Cooper, Copenhagen, Grizzly, Husky, Kayak, Kodiak, Longhorn, Red Seal, Renegades, Skoal, Timberwolf* 2006/07	Borgerding et al. 2012	0.132-0.325	NI	0.881-1.537	1.430-2.285	NI	0.222-0.471	NI	1.887-2.648	NI	NI
Moist snuff from US or Sweden 2008	McAdam et al. 2010c	~0.075-0.135 WWB	~0.008-0.018 WWB	~0.58-0.90 WWB	~0.50-1.50 WWB	NI	~0.17-0.38 WWB	~0.010 (1 sample) WWB	~0.90-1.40 WWB	NI	~0.03-0.05 WWB
Various fine-cut, long-cut, pouched brands: *Skoal, Rooster,*	Rickert et al. 2009	0.218-0.366	NI	0.806-1.086	0.797-1.416	NI	0.302-0.419	NI	1.145-1.627	NI	NQ-0.082

Table A II-5: Trace-Level Components in Snus and other STPs as Reported in the Literature: Metals/ Metalloids (µg/g dry weight unless indicated otherwise)

Brand/ STP Type Specified by Study Authors	Citation	Arsenic (As)	Beryllium (Be)	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Lead (Pb)	Mercury (Hg)	Nickel (Ni)	Barium (Ba)	Selenium (Se)
Copenhagen											
Various brands and types, including: *Hawken, Kodiak, Cougar, Copenhagen, Skoal, Red Seal, Rooster, Timberwolf, Silver Creek,* and *Redwood*	Pappas et al. 2008	0.13-0.36	0.010-0.038	0.66-1.88	0.86-3.20	0.26-1.22	0.28-0.85	NI	1.39-2.73	37.9-158.1	NI
Copenhagen	McNeill et al. 2006	0.23	NI	NI	1.69	NI	0.45	NI	2.64	NI	NI

Table A II-5: Trace-Level Components in Snus and other STPs as Reported in the Literature: Metals/ Metalloids (µg/g dry weight unless indicated otherwise)

Brand/ STP Type Specified by Study Authors	Citation	Arsenic (As)	Beryllium (Be)	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Lead (Pb)	Mercury (Hg)	Nickel (Ni)	Barium (Ba)	Selenium (Se)
US Low-Moisture Snuff											
US Dry snuff: *Skoal, Taboka, Bruton, Dental, Levi Garrett, Railroad Mills Plain, Red Seal.* 2006/07	Borgerding et al. 2012	0.0695-0.294	NI	0.356-1.794	1.184-5.740	NI	0.179-0.791	NI	1.223-7.540	NI	NI
Dry snuff from US or Sweden 2008	McAdam et al. 2010c	~0.15-0.195, 0.27, 0.34 (2 samples) WWB	~0.0198-0.40 WWB	~0.98-1.58 WWB	~0.7-1.1 WWB	NI	~0.4-1.1 WWB	~0.01 WWB	~2.0-4.5 WWB	NI	~0.06-0.1 WWB
Various *McChrystal's* brands	Rickert et al. 2009	0.356-0.437	NI	0.300-0.365	1.307-2.186	NI	0.627-1.202	NI	1.509-2.045	NI	NQ-<LOD

Table A II-5: Trace-Level Components in Snus and other STPs as Reported in the Literature: Metals/ Metalloids (µg/g dry weight unless indicated otherwise)

Brand/ STP Type Specified by Study Authors	Citation	Arsenic (As)	Beryllium (Be)	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Lead (Pb)	Mercury (Hg)	Nickel (Ni)	Barium (Ba)	Selenium (Se)
US-Style Chewing Tobacco											
Chewing tobacco, Loose leaf 2006/07	Borgerding et al. 2012	0.074-0.157	NI	0.469-0.811	0.585-1.432	NI	0.227-0.424	NI	0.648-1.399	NI	NI
Plug 2007	Borgerding et al. 2012	0.149	NI	0.681	1.009	NI	0.364	NI	1.331	NI	NI
Chewing tobacco from US or Sweden 2008	McAdam et al. 2010c	~0.07-0.16 WWB	~0.002-0.011, 0.058 WWB	~0.38-0.7 WWB	~0.2-0.6 WWB	NI	~0.18-0.39 WWB	~0.01 WWB	~0.4-1.1 WWB	NI	~0.04-0.15, 0.22 (1 sample) WWB
Red man, Apple plug	Rickert et al. 2009	0.168-0.238	NI	0.478-0.528	0.714-1.210	NI	0.301-0.365	NI	0.844-1.712	NI	0.082-0.085
Plug tobacco	McAdam et al. 2010c	~0.1 WWB (1 sample)	~0.019 WWB (1 sample)	~0.65 WWB (1 sample)	~0.4 WWB (1 sample)	NI	~0.15 WWB (1 sample)	NI	~1.95 (1 sample) WWB	NI	~0.05 (1 sample) WWB

Notes:
All amounts given as per dry weight unless otherwise noted
WWB: wet weight basis; DWB: dry weight basis; NI: Not investigated; NQ: Not quantified
∞Values were based on 0.6g of pouched snus.

A II 2.3.6.5 Radioisotopes

Table A II-6 summarizes levels of nine radioisotopes in pouched or loose Swedish snus samples, US moist snuff, and other STPs (Mola et al. 2009; McAdam et al. 2010c). Data on radioisotopes in new products marketed as snus has not been identified in the more recent studies.

Two studies by BAT researchers presented as posters at the 2009 and 2010 SRNT Meetings analyzed radioisotopes in Swedish snus samples and other STPs (Mola et al. 2009; McAdam et al. 2010c). Polonium-210, and radium-226 activity (mBq/g) in pouched or loose Swedish snus samples were comparable to levels observed in US-type moist snuff, with snus having the lowest polonium-210 activity of all STP tested (Mola et al. 2009). Radium-226 activity spanned a wider range in snus samples compared to moist snuff. Levels of thorium-232,-230 and uranium-238 in pouch or loose Swedish snus and in US-type moist snuff were below the LOD. An older analysis by Hoffmann et al. (1987) of polonium-210 in the five most popular moist snuff brands on the market in the US in 1985/1986 showed that activity ranged from 0.006 to 0.045 Bq/g per dry weight, while in the newer study the activity was below 10 mBq/g wet weight (0.01 Bq/g) (Mola 2009).

Radioisotope concentrations in pouched and loose snus were generally in the same range as those measured in moist snuff samples; in the analysis by McAdam and colleagues (2010c) it was unclear whether the products were from either the US or Sweden.

Table A II-6: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Radioactive Isotopes

Brand/ STP Type Specified by Study Authors	Citation	Polonium-210	Lead-210	Radium-226	Thorium-232	Thorium-230	Thorium-228	Uranium-238	Uranium-234	Uranium-235
Snus (Sweden)										
Pouched snus	Mola et al. 2009	~5 mBq/g WWB, median α-activity	NI	~4 (3.5-5) mBq/g WWB, median α-activity	<LOD	<LOD	~1.5 (0-4) mBq/g WWB, median α-activity	<LOD	<LOD	<LOD
Loose snus	Mola et al. 2009	~4.8 mBq/g WWB, median α-activity	NI	~1.9 (1.8-5.5) mBq/g WWB, median α-activity	<LOD	<LOD	~2 (0-4.5) mBq/g WWB, median α-activity	<LOD	<LOD	<LOD
Snus (US/Sweden)										
Pouched snus from US or Sweden 2008	McAdam et al. 2010c	~10-45 ag/g WWB	~5-42 fg/g WWB β-activity	~75-155 fg/g WWB	ND	ND	~98-170 ag/g WWB	ND	~9.5 pg/g (1 sample) WWB	NI
Loose snus from US or Sweden 2008	McAdam et al. 2010c	~10-30 ag/g WWB	~2-10 fg/g WWB β-activity	~45-245 fg/g WWB	ND	ND	~55-150, ~260 ag/g (1 sample) WWB	ND	ND	NI
Other New Products										

Table A II-6: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Radioactive Isotopes

Brand/ STP Type Specified by Study Authors	Citation	Polonium-210	Lead-210	Radium-226	Thorium-232	Thorium-230	Thorium-228	Uranium-238	Uranium-234	Uranium-235
Moist pellets	McAdam et al. 2010c	~150 ag/g (1 sample) WWB	~28 fg/g (1 sample) WWB β-activity	~145 fg/g (1 sample) WWB	ND	ND	ND	ND	ND	NI
Hard pellets	McAdam et al. 2010c	~60-65 ag/g (2 samples)	~60 fg/g (1 sample) WWB β-activity	~160-180 fg/g WWB	ND	~5.5 pg/g (1 sample) WWB	~70-110 ag/g (2 samples) WWB	~230, 800 ng/g (2 samples) WWB	~10 -38 pg/g (1 sample) WWB	NI
Pellets	Mola et al. 2009	~11 (10-17) mBq/g WWB, median α-activity	NI	~5.7 (5 -7) mBq/g WWB, median α-activity	<LOD	~3 (4-9.2) mBq/g WWB, median α-activity	~2 (1.5-3.5) mBq/g WWB, median α-activity	~3 (2.9-10) mBq/g WWB, median α-activity	~2.5 (2.6-9) mBq/g WWB, median α-activity	<LOD
Plug tobacco	Mola et al. 2009	~10 mBq/g WWB, median α-activity	NI	~3.5 mBq/g WWB, median α-activity	<LOD	<LOD	~5 (WWB, median α-activity	<LOD	<LOD	<LOD
US-Type Moist Snuff										
Moist snuff from US or Sweden 2008	McAdam et al. 2010c	~35-60 ag/g (2 samples)	~8-21 fg/g WWB β-activity	~50-95 fg/g WWB α-activity	ND	~1-1.5 pg/g (2 samples)	~48-245 ag/g WWB	~60 ng/g (1 sample) WWB	~3-6 pg/g (2 samples) WWB	NI

Table A II-6: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Radioactive Isotopes

Brand/ STP Type Specified by Study Authors	Citation	Polonium-210	Lead-210	Radium-226	Thorium-232	Thorium-230	Thorium-228	Uranium-238	Uranium-234	Uranium-235
Moist snuff	Mola et al. 2009	~7 (6.1-9.4) mBq/g WWB	NI	~2.5 (2.5-3) mBq/g WWB, median α-activity	<LOD	<LOD	~2 (1.5-3.5) mBq/g WWB, median α-activity	<LOD	<LOD	<LOD
US Low-Moisture Snuff										
Dry snuff from US or Sweden 2008	McAdam et al. 2010c	~65-100 ag/g (2 samples)	~10-29 fg/g (3 samples) WWB β-activity	~40-240 fg/g WWB	ND	ND	~150-280 ag/g WWB	ND	ND	NI
Dry snuff	Mola et al. 2009	~14.5 (11-16) mBq/g WWB, median α-activity	NI	~2.7 (3.1-8) mBq/g WWB, median α-activity	<LOD	<LOD	~5 (2.2-7.2) mBq/g WWB, median α-activity	<LOD	<LOD	<LOD
US-Style Chewing Tobacco										
Chewing tobacco from US or Sweden 2008	McAdam et al. 2010c	~21-42, 60 ag/g (1 sample)	~8-31 fg/g WWB β-activity	~10-80, ~160 fg/g (1 sample) WWB α-activity	~270 fg/g (1 sample) WWB	~2 pg/g (1 sample) WWB	~50-170 ag/g (2 samples) WWB	ND	ND	NI

Table A II-6: Trace-Level Components in Snus and New Products Marketed as Snus as Reported in the Literature: Radioactive Isotopes

Brand/ STP Type Specified by Study Authors	Citation	Polonium-210	Lead-210	Radium-226	Thorium-232	Thorium-230	Thorium-228	Uranium-238	Uranium-234	Uranium-235
Chewing tobacco from	Mola et al. 2009	~6 (4-6.5) mBq/g WWB, median α-activity	NI	~2.1 (0.1-2.9) mBq/g WWB, median α-activity	<LOD	<LOD	~3.2 (1-4.5) mBq/g WWB, median α-activity	<LOD	<LOD	<LOD
Plug tobacco	McAdam et al. 2010c	~60 ag/g (1 sample)	NI	~95 fg/g (1 sample) WWB	ND	ND	~170 ag/g (1 sample) WWB	ND	ND	NI

Notes:
All amounts given as per dry weight unless otherwise noted. LOD: limit of detection, NI: Not investigated, ND: Not detected.

A II 2.3.6.6 Other Trace-Level Components

Table A II-7 summarizes concentrations of ethyl carbamate (urethane) in traditional Swedish snus and other STPs (Faizi et al. 2010).

This study by BAT researchers presented as a poster at the 2010 CORESTA conference analyzed concentrations of ethyl carbamate in pouched or loose Swedish snus and other STPs. The range of concentrations of ethyl carbamate in pouched or loose snus were lower than those reported in US moist snuff, per wet and dry weight.

No other recent published analyses of other trace-level components, such as mycotoxins, acrylamide, and hydrazine, in traditional Swedish snus and other STPs were identified.

Table A II-7: Other Compounds (ng/g dry weight unless indicated otherwise)

Brand/ STP Type Specified by Study Authors	Citation	Ethyl Carbamate (Urethane)
Snus (Sweden)		
Pouched snus 2008	Faizi et al. 2010	<20 (RL)-~90 WWB; (>RL: 29-~155 DWB)
Loose snus 2008	Faizi et al. 2010	<20 (RL)-~40 WWB; (>RL: 35-~74 DWB)
Other New Products		
Hard pellets	Faizi et al. 2010	<20 (RL) WWB
Soft pellets	Faizi et al. 2010	<20 (RL) WWB (1 sample)
US Moist Snuff		
Moist snuff 2008	Faizi et al. 2010	<20 (RL)-~200 WWB; (>RL: ~56 – 400, 1398 DWB, 1 sample)
US Low-Moisture Snuff		
Dry snuff 2008	Faizi et al. 2010	<20 (RL) WWB
US-Style Chewing Tobacco		
Chewing tobacco from US or Sweden 2008	Faizi et al. 2010	<20 (RL) WWB
Plug tobacco	Faizi et al. 2010	<20 (RL) WWB 1 sample)

Notes:
All amounts given as per dry weight unless otherwise noted
DWB: dry weight basis, RL: Reporting limit, WWB: wet weight basis.

A II 2.3 Summary and Discussion of Chemical Properties

It is well established that the production process for traditional Swedish snus is distinctively different from that of traditional US-type oral moist snuff. The manufacturing process of new products marketed as snus is not well documented in the published scientific literature. Based on analytical results from the chemical composition of these STPs, moist snuff and products marketed as snus, as published in the scientific literature; there are considerable differences between these products and traditional Swedish snus for many components.

Swedish snus and other STPs differ on free nicotine content, pH and moisture levels; some of the new products may deliver considerably less nicotine. Additionally, nicotine content in traditional Swedish snus somewhat differed from those found in US-type moist snuff products.

Swedish snus and other STPs also differ in TSNA concentrations, those detected in US-type moist snuff products were highly variable but generally higher than those measured in traditional Swedish snus, including novel brands, and new products marketed as snus.

Similar to TSNA concentrations, most analyzed PAHs (including B[*a*]P) concentrations in traditional Swedish snus, novel brands 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

Biomarkers of Exposure to Snus, Other Tobacco Products, and NRTs

Appendix (III) to Chapter 3: Biomarkers of Exposure to Snus, Other Tobacco Products, and NRTs

In this Appendix, the available data on biomarkers of exposure for traditional Swedish snus users, supplemented with available data for users of new products marketed as snus, is discussed in comparison with data for smokers and Nicotine Replacement Therapy (NRT) users. Where no data was identified for users of snus or new products marketed as snus, select studies of traditional US STPs users are discussed.

The above noted supplementation of data for traditional Swedish snus with that from studies of non-snus STPs has several limitations. Differences that need to be considered in the evaluation and extrapolation of the data to the users of traditional Swedish snus are as follows:

- Differences in product manufacture and formulation and resulting differences in chemical composition of the products (e.g., nicotine content, pH, trace level components)
- Differences in portion size
- Differences in resulting use characteristics, including use time (also referred to as usage time)
- Difference in user characteristics

In addition, comparability among studies is limited due to possible differences in study design and type of biomarker measured (e.g. nicotine equivalents vs. serum cotinine).

This appendix follows the same outline and format as used in Chapter 3 for Swedish snus. Summaries of the respective studies are presented in **Table A III-7** in chronological order, starting with studies that investigated traditional Swedish snus use, followed by studies of use of new products marketed as snus and select studies that focused on US STP use. Comparisons of biomarker levels are made as follows:

- Snus use as compared to smoking
- The changes for smokers who switch to using snus
- Smokers switching to snus as compared to quitting the use of tobacco products
- Using snus in conjunction with smoking (dual use)
- Switching to snus as compared to using NRTs
- Snus use as compared to not using tobacco products

Reduced exposure to certain components as reflected in the reduced respective biomarkers levels seen with snus use as compared to smoking likely contributes to the reduced health risk seen in epidemiological studies. As described in more detail in Section 3 of the Main Report, however, conclusions from the biomarker studies with respect to harm reduction should be interpreted carefully and in the context of additional data from clinical or epidemiological studies. As the IOM (2012) stated “If the panel of biomarkers presented were decreased to the levels found in nonsmokers, it is likely that there would be a beneficial effect on health, but this has not been proven.”

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A III 3.1 Biomarkers of Exposure to Tobacco Alkaloids: Nicotine

Details on the significance of nicotine biomarkers are described in Section 3.1 of the main report.

A III 3.1.1 Nicotine Pharmacokinetics

Details on the significance of nicotine pharmacokinetics are described in Section 3.1.1 of the main report.

A III 3.1.1.1 Nicotine Pharmacokinetics in Users of Snus, New Products Marketed as Snus, Select STPs, NRT and Smokers

Pharmacokinetic (PK) parameters of nicotine in blood absorbed from traditional Swedish snus, including novel brands in comparison with NRT, and cigarette smoking have been investigated in several studies by US and Swedish university researchers, researchers from British American Tobacco, and Swedish Match (Digard et al. 2012; Holm et al. 1992; Lunell and Curvall 2011; Lunell and Lunell 2005). This data is supplemented with available studies of new products marketed as snus and select traditional US STPs that provided comparisons between different products (Cobb et al. 2010; Fant et al. 1999; Kotlyar et al. 2007). In general, in these studies test products were administered once or a specified number of times after an overnight period of abstinence from any tobacco products, followed by an observation period. Nicotine PK parameters from the above studies are provided in **Table A III-1**.

Nicotine delivery kinetics, i.e., speed and peak height of internal nicotine exposure, are thought to be involved in a product's abuse liability/addictive potential, therefore, if together with nicotine PK parameters related subjective effects were reported, a brief summary of these outcome is included at the end of the following section.

Rise of Nicotine Blood Concentration and Time to Maximum Concentration (t_{max})

PK parameters (rise of nicotine blood concentration and t_{max}) – snus use versus smoking

A recent study by Digard et al. (2012) suggests that the time to maximum (t_{max}) plasma nicotine concentration in users of loose snus as well as pouched snus products is dependent on the amount of usage time following placement in the mouth, but not nicotine content or portion size (see also Section 3.1.1). In agreement with this observation, when experimental usage time has been 30 minutes, as common in previous studies, t_{max} values observed were approximately 30 minutes with use of snus as well as moist snuff products. A study that tested new tobacco products in regular smokers, including the new products marketed as snus, *Camel Snus* and *Marlboro Snus,* with two cycles (15-minute usage time plus 30-minute observation periods) separated by a 60-minute break, reported the highest plasma nicotine concentration for *Camel Snus* 15 minutes after the second administration (Cobb et al. 2010). In the study by Digard and colleagues (2012) the usage time was 60 minutes and the median t_{max} was 60 minutes. The authors concluded that "The data showed that nicotine was continually absorbed from the snus portions over the entire 60-min period" (Digard et al. 2012).

During smoking, rapid nicotine absorption, reflected in the steep rise in nicotine blood level, is followed by a steady decrease in nicotine blood level - until the next cigarette is smoked. The t_{max} measured for smokers, who smoked a cigarette for 5 minutes or who were asked to take 10

puffs in 20-second intervals was 7 minutes (median) or 5 minutes, respectively (Cobb et al. 2010; Digard et al. 2012).

The rise of nicotine plasma concentrations was less steep for the snus products tested than after smoking a cigarette (Digard et al. 2012). However, the rise of nicotine plasma concentrations after snus use during the first few minutes of snus was somewhat comparable. While Lunell and Curvall (2011) did not measure nicotine plasma concentrations during smoking, they noted that "At 8 min after start of administration, the mean nicotine plasma concentration exceeded 7 ng/ml for both snus preparations […]" and added that "[t]he corresponding increase in plasma nicotine concentration after smoking a single cigarette is 5–14 ng/ml". The rise in nicotine plasma concentration following those first minutes of snus use as well as the decrease after the end of the use period were less steep than those seen with smoking.

- The nicotine t_{max} for snus users was dependent on the amount of usage time following placement in the mouth, but not nicotine content or portion size. The t_{max} for smokers ranged between 5 and 7 minutes.
- Snus use resulted in a less steep overall rise and – after the end of use period – decrease of the nicotine plasma concentration compared with smoking, although the increase in concentration within the first minutes of use was somewhat comparable to smoking.

PK parameters (rise of nicotine blood concentration and t_{max}) - snus use versus NRT use

Nicotine gum chewed for 30 minutes resulted in t_{max} values of approximately 45 minutes for 4-mg gums and 30 minutes for 2-mg gum (Digard et al. 2012; Lunell and Curvall 2011; Lunell and Lunell 2005). Based on these studies, it is unclear if the difference for the 2-mg nicotine gum is due to the different study conditions or indication of an influence of nicotine content. The t_{max} was approximately 30 minutes for use of lozenges with either 2 or 4 mg nicotine (Cobb et al. 2010; Kotlyar et al. 2007). These results indicate that unlike what was observed for snus use, the t_{max} for NRT use was less dependent on usage time.

Lunell and Curvall (2011) noted that the rise of the nicotine plasma concentration was faster for portion snus than for nicotine chewing gum users, but this was not significant (the 8-min nicotine concentration for the gum was ~70% of that measured for snus). Based on their nicotine plasma concentration versus time curve, results from the snus products tested by Digard and colleagues (2012) appear to follow a similar trend.

- Unlike what was observed for Swedish snus use, the nicotine t_{max} for nicotine gum and lozenges appear less dependent on usage time and ranged from 30 to 45 minutes.
- Snus use tended to result in faster rise of the nicotine plasma concentration than use of nicotine gum, although this difference was not significant.

Maximum and Total Nicotine Blood Concentration (C_{max} and AUC)

The maximum plasma concentrations (C_{max}) following the use of 1-g portions of pouched and loose snus brands tested were only slightly higher compared to those following use of the new product marketed as snus *Camel Snus* (1-g portion) (Cobb et al. 2010; Digard et al. 2012; Lunell and Curvall 2011), but considerably higher than following use of *Marlboro Snus*, consistent with its lower nicotine content per portion during the time of product sampling (Cobb et al. 2010). Loose snus portions of 2 g and more (instead of 1-g portions) resulted in respective higher C_{max} values (Digard et al. 2012; Holm et al. 1992). Moist snuff use (2 g portions) resulted in C_{max} values in the same range (Fant et al. 1999; Kotlyar et al. 2007).

The C_{max} was correlated to the total nicotine content of a portion when products had similar pH (Digard et al. 2012; Lunell and Curvall 2011; Lunell and Lunell 2005) and to product pH (decreasing with lower pH) (Fant et al. 1999; Lunell and Lunell 2005). Similarly, the AUC was correlated with these parameters (Lunell and Lunell 2005). The increase of internal exposure (as $AUC_{(0\text{-}30\ min)}$) with increased product pH was also shown in a study by Fant and colleagues (1999), who tested several moist snuff products with similar nicotine content.

PK parameters (nicotine C_{max} and AUC) - snus use versus smoking

Two studies measured nicotine PK parameters following both smoking and use of snus/new products marketed as snus: One in Swedish snus users that occasionally smoked (Digard et al. 2012) and one in regular smokers (Cobb et al. 2010). In these studies, the average C_{max} values following smoking was in the range of those observed for snus users and as high as those seen with 2 g portions of moist snuff, respectively (Cobb et al. 2010; Digard et al. 2012; Fant et al. 1999; Kotlyar et al. 2007; Lunell and Curvall 2011). However, as noted above, in the study by Cobb and colleagues (2010) smoking resulted in significantly higher C_{max} values than use of the two new products marketed as snus tested.

In the study by Digard and colleagues (2012) of Swedish snus users who also occasionally smoked, use of snus products resulted in higher geometric mean $AUC_{(0\text{-}120\ min)}$ than smoking a cigarette (*Lucky Strike Red*), and was, as seen for the C_{max}, correlated with the total nicotine content of a portion (the products had similar pH). The authors noted that the relationship was sub-proportional. Whether the snus product was loose or pouched had no influence: Use of a 1-g portion of loose snus (*Granit)* or pouched snus(*Lucky Strike)* with similar nicotine content and pH resulted in similar AUC and C_{max} values. Smoking a cigarette resulted in a similar C_{max} but slightly lower AUC compared to a pouched snus (*Lucky Strike)* with the same total nicotine content. Cobb and colleagues (2010) did not provide AUC measures.

While Lunell and Lunell (2005) did not measure nicotine PK parameters following smoking, the authors stated in the discussion of their study results that "*Catch Dry Mini* once hourly produced blood levels similar to the lower end of cigarette smoking (7–10 cigarettes/ day), whereas *Catch Licorice* and *Catch Mini* once hourly showed blood levels similar to moderate cigarette smoking (15–20 cigarettes/day). Only *General* once hourly produced steady-state levels around 30 ng/ml, similar to the upper end of cigarette smoking (25–40 cigarettes/day) […]" [italics added].

- C_{max} and AUC values observed for snus users were in the same range of those following smoking and were dependent on total nicotine content of the product and product pH, but not on whether the snus was pouched or loose.

PK parameters (nicotine C_{max} and AUC) - snus use versus NRT use

Four-mg nicotine gum use resulted in C_{max} values similar to, but slightly lower than 1-g snus portions (Digard et al. 2012; Lunell and Curvall 2011), while use of nicotine lozenges (2 and 4 mg) had lower average C_{max} values (Cobb et al. 2010; Kotlyar et al. 2007). The latter were similar to C_{max} values seen with a new product marketed as snus (2010).

In the study by Lunell and Curvall (2011), the mean $AUC_{(0\text{-inf})}$ in users of two snus brands was in a similar range but slightly lower than those measured for users of the 4-mg nicotine gum (no analysis of statistical significance was provided). The authors stated that "The lower C_{max} of NP [nicotine polacrilex] gum compared with Swedish snus in spite of a larger AUC may be explained by a slower and more prolonged absorption from the chewing gum".

Lunell and Curvall (2011) noted that the slightly lower AUC values observed following snus compared to gum use correlated with the - in this study - observed lower nicotine extraction rate from the tested snus products as compared to that from the gum. The authors hypothesized that the less efficient nicotine extraction might be due to the fact that the test persons were regular smokers and naïve (not experienced) snus user.

In fact, in their study of regular snus users, who also occasionally smoked, Digard and colleagues (2012) observed that use of all snus products caused higher AUC and C_{max} values than what was seen after 4-mg nicotine gum use. The AUC and C_{max} values resulting from gum use were also slightly lower than those seen with smoking a cigarette in the same study.

In their previous study, Lunell and Lunell (2005) observed that controlled snus use over a 12-hour period in regular snus users produced two or more times the systemic nicotine exposure ($AUC_{(0\text{-}12\ hrs)}$) than chewing a 2-mg nicotine gum. Use of a novel brand of traditional Swedish snus (*Catch Dry Mini*), with lower pH and moisture than traditional Swedish snus, resulted in a similar internal nicotine exposure ($AUC_{(0\text{-}12\ hrs)}$) similar to use of 2-mg nicotine gum.

In the study by Kotlyar and colleagues (2007), use of 4-mg nicotine lozenges by regular STP users resulted in approximately half of the systemic nicotine exposure ($AUC_{(0\text{-}90\ min)}$) seen with a traditional US moist snuff product (*Copenhagen*).

- Snus use resulted in similar, but slightly higher C_{max} values than use of 4 mg-nicotine gum and approximately two times higher C_{max} values than use of 2 mg-nicotine gum. Use of a novel brand of traditional Swedish snus, *Catch Dry Mini*, resulted in a similar, but slightly lower C_{max} value than 2-mg nicotine gum
- Depending on the prior experience with snus, the AUC values were either slightly lower (naïve snus users) or higher (experienced snus users) than those observed for use of 4-mg nicotine gum. The AUC values from snus use were higher than those from 2-mg

nicotine gum use. Use of a novel brand of traditional Swedish snus*, Catch Dry Mini*, resulted in a similar AUC value as 2-mg nicotine gum**.**

Subjective Effects

Subjective effects - snus use versus smoking

With respect to data related to the products' abuse liability/addictive potential, Digard and colleagues (2012) did not provide a comparison for self-reported sensory perceptions between use of snus products and smoking. Cobb and colleagues (2010) concluded that the noncombustible products they tested, including new products marketed as snus, "deliver less nicotine than own brand cigarettes and fail to suppress tobacco abstinence symptoms effectively." By contrast, Lunell and Curvall (2011) noted, although they did not measure nicotine plasma concentrations during smoking, that the steep rise (relative to what was seen with nicotine gum) they observed with snus "may have an impact on the smoker's satisfaction with respect to cigarette-like "head rush" and withdrawal reduction." In a study with regular Swedish snus users and smokers, Holm and colleagues (1992) concluded that based "[on] questionnaire measures of dependence, there was no difference between smokers and snuffers [Swedish snus users] in self-assessed addiction, craving for tobacco, or difficulty in giving up […]".

- The limited data available suggest that subjective effects associated with abuse liability seen for traditional Swedish snus use appear to be similar to those seen for smoking.

Subjective effects - snus use versus NRT use

Lunell and Curvall (2011) noted that together with the faster absorption of nicotine from snus, the higher "head rush" scores and heart rate increase observed with snus suggested "a faster onset of pharmacological effects in general for Swedish snus compared with [4-mg] nicotine gum". Digard and colleagues (2012) did not provide a comparison for self-reported sensory perceptions between snus products and the nicotine gum. By comparison, in the study by Cobb and colleagues (2010), both the two new products marketed as snus and 2-mg nicotine lozenge "failed to suppress tobacco abstinence symptoms effectively". Kotlyar and colleagues (2007) concluded that use of the 4-mg nicotine lozenge resulted in higher nicotine delivery and reduced subjective measures of craving and withdrawal similarly as or better than the three low-nicotine potentially reduced exposure products (PREPs) (which did not include Swedish snus) tested.

- The limited data available suggest that subjective effects associated with abuse liability seen with traditional Swedish snus use were more pronounced than those seen with NRT use.
- The few data on new products marketed as snus indicates that these products were less likely to reduce negative subjective effects of nicotine dependence, such as withdrawal symptoms.

A III 3.1.2 Nicotine Biomarkers

Details on the significance of different nicotine biomarkers are described in Section 3.1.2 of the main report.

A III 3.1.2.1 Biomarkers of Nicotine Exposure in Users of Snus, New Products Marketed as Snus, Select STPs, NRT and Smokers

Biomarker of nicotine exposure from traditional Swedish snus, alone or in comparison with other US STPs (traditional and new products), NRT, and cigarette smoking have been investigated in several studies by US and Swedish university researchers, and researchers from Swedish Match (Andersson et al. 1994; Andersson et al. 1995; Bolinder et al. 1997b; Bolinder 1997; Bolinder et al. 1997a; Bolinder and de Faire 1998; Eliasson et al. 1991; Eliasson et al. 1995; Ellingsen et al. 2009; Gray et al. 2008; Hatsukami et al. 2004; Holm et al. 1992; Joksic et al. 2011; Post et al. 2005; Wennmalm et al. 1991). This data is supplemented by studies of new products marketed as snus and traditional STPs in smokers and STP users (Blank and Eissenberg 2010; Kotlyar et al. 2011; Naufal et al. 2011; Sarkar et al. 2010). Nicotine biomarker levels from the above studies are provided in **Table A III-2**.

Ratio of Cotinine to Nicotine in Plasma

Nicotine biomarker levels - snus use versus smoking

Cotinine levels were 11 and 8 times higher than nicotine levels in plasma from regular snus users and smokers, respectively, when both were measured briefly after product consumption (Study 2) (Holm et al. 1992). At that time plasma nicotine levels were equal for both snus users and smokers. The difference in cotinine levels between snus users and smokers was not statistically significant after adjusting for age and sex (see next subsection below).

In the study by Sarkar and colleagues (2010), the cotinine $AUC_{(0\text{-}12.25\ hrs)}$ values were 20, 15, and 18.5 times higher than the nicotine $AUC_{(0\text{-}12.25\ hrs)}$ values in plasma from *Marlboro Snus* users, dual users, and those who continued smoking, respectively.

- Results from two studies indicate that plasma cotinine to nicotine ratios measured briefly after product consumption as single time point measurement or as AUC over a 12-hour time of use show a slight tendency to be greater for snus users than for smokers (see Section 3.1.2 for additional discussion).

Nicotine and Cotinine in Plasma/Serum, Saliva, and Urine

To assess internal nicotine exposure, many studies discussed here have measured cotinine in plasma or serum (µg/L), but some studies alternatively analyzed saliva cotinine or urinary cotinine, total cotinine, nicotine, or nicotine equivalents.

Nicotine biomarker levels - snus use versus smoking

In study 2 by Holm and colleagues (1992), under regular use conditions nicotine levels in plasma from smokers (17 cigarettes/day) one minute after smoking and in snus users (21.7 g/day) five to 15 minutes after discarding the snus were almost equal. At that time, cotinine levels were higher in plasma from the snus users than the smokers. However, the authors noted that “this effect was no longer statistically different after controlling for age and sex”.

In studies of Swedish firemen and individuals from the general Swedish population from the 1990s, in which mean or median snus consumption ranged from 21 to 32 g/day, cotinine levels in plasma from snus users (N=21-92) were on average either slightly (Bolinder et al. 1997a; Bolinder and de Faire 1998) or significantly (Bolinder et al. 1997b; Bolinder 1997; Eliasson et al. 1991; Eliasson et al. 1995) higher than those in plasma from smokers (N=19-124) that had a mean or median cigarette consumption of 15 to 19 cigarettes/day.

One study compared cotinine levels in saliva from Swedish adolescent tobacco users (Post et al. 2005). In this study, the median saliva level was significantly higher (~ 4 times) in 28 snus users (4.4 pinches/day) compared to 69 smokers (6.7 cigs/day). No studies were located where saliva levels from adult snus users and smokers were compared. The median cotinine level in saliva from adolescent snus users was approximately half of the average level measured in saliva from adult snus users of a lower nicotine snus (Brand B, 15 g/day) and approximately ¼ of those levels measured in saliva from adult snus users of higher nicotine snus (Brand A, 16.4 g/day) reported by Andersson and colleagues (1995) as well as of adult users of loose (20.8 g/day) and pouched snus (14.4 g/day) reported by the same authors (Andersson et al. 1994).

In a study of Norwegian blue collar tobacco users, geometric mean serum cotinine levels as well as urinary nicotine and cotinine levels (µg/mmol creatinine) were similar in both 11 snus users and in 38 smokers who also occasionally used snus. Total tobacco use for both groups was comparable (10.7 g snus/day and 11 g tobacco in cigarettes/day + 2.6 g snus/week, respectively) (Ellingsen et al. 2009).

In a Swedish study, the median cotinine level measured in urine (µg/L) in 127 snus users was slightly, but not statistically significantly, lower than the cotinine level measured in urine of 43 smokers (12 cigs/day) (Wennmalm et al. 1991). The snus users in this study used, on average, more snus (25 g/d) than those from the Norwegian Study described above (Ellingsen et al. 2009).

In an analysis of NHANES data from 1999-2008, 368 STP users had significantly higher serum cotinine levels than 5040 smokers (serum cotinine levels[1] were 50% higher/levels of smokers were ~70% of those of STP users) (Naufal et al. 2011). The amount of tobacco consumed was not provided in the publication (2011) and instead serum cotinine levels were used as surrogate for tobacco exposure.

The geometric mean cotinine level in serum from these US STP users was higher than from Norwegian snus users in the study by Ellingsen and colleagues (2009) where the average snus consumption was similar to what has been reported as average for pouched snus (Digard et al. 2009). On the other hand, the geometric mean cotinine serum level reported based on NHANES data for US STP users was lower than those reported in studies with snus from the 1990s (as described above), where the average snus consumption was similar to what has been reported for loose snus (Digard et al. 2009).

The geometric mean serum cotinine level in the smokers reported based on NHANES data was also considerably lower than those reported for smokers in the studies from the 1990s. A cut

[1] It is unclear if the serum cotinine levels provided were based on unadjusted or adjusted geometric means.

point of <10 ng/mL serum cotinine has been used by the CDC to define nonsmokers using the NHANES data base (Bernert et al. 2010).

- Nicotine biomarker (plasma or serum cotinine, saliva cotinine, or urinary cotinine or nicotine) levels, measured in regular traditional Swedish snus users were similar to or higher than those in regular smokers. These differences appear only in part to be attributable to differences in amount of tobacco used (means or medians range for adult users, 11-32 g/day snus vs. 11-19 cigs/day; means for adolescent users, 4.4 pinches vs. 7 cigs/day).

Nicotine biomarker levels - snus use versus not using tobacco products

Mean or median plasma cotinine in snus users were more than 100 times higher than those measured in non-tobacco users (Bolinder et al. 1997b; Bolinder 1997; Bolinder et al. 1997a; Bolinder and de Faire 1998; Eliasson et al. 1991; Eliasson et al. 1995; Ellingsen et al. 2009). Urinary nicotine and cotinine levels were between more than 70 and more than 200 times higher in snus users compared to non-tobacco users (Ellingsen et al. 2009; Wennmalm et al. 1991).

- Nicotine biomarker levels measured in regular traditional Swedish snus users were 70-200 times higher than those detected in non-tobacco users.

Changes in nicotine biomarker levels - smokers who switch to using snus

In a Serbian study, smokers (averages, 26-28 cigs/day) willing to quit who used snus as a cessation aid had a decreased (to approximately 68% of baseline level) mean serum cotinine level at the end of the study at week 48, when complete smoking cessation was supposed to have been accomplished (self-reported <10 cigs/day) (Joksic et al. 2011). The group had some residual nicotine intake from smoking at the study end (reflected in mean exhaled breath carbon monoxide level of approximately 12 ppm).

In a study by Hatsukami and colleagues (2004), conventional STP users at week 4 after switching to pouched *General* snus (~13 g/day at week 4) had a similar mean urinary total cotinine (cotinine and its glucuronide in µg/L) level to those means measured in two groups of smokers at baseline of the same study (22 cigs/day). Snus consumption at week 4 was similar to the average consumption in regular snus users as reported in the survey by Digard and colleagues (2009).

Researchers of the Hatsukami working group, Kotlyar and colleagues (2011), analyzed biomarkers in 130 smokers interested in cessation by switching to new products for 4 weeks; after this treatment period the smokers were tapering off all tobacco use. Fifty-one smokers (19.7 cigs/day) switched to a new product marketed as snus (*Camel Snus*; 6.9 pouches/day). At week 4, these users had geometric mean urinary cotinine levels (µg/L) that were reduced to approximately $^1/_5$ of those measured at baseline. In this group, 9.1% of subjects continued to smoke on average more than 3 cigarettes per day, but the geometric mean exhaled carbon monoxide level was below 5 ppm. The authors noted that the product was modified in June 2008, when tobacco and nicotine amount per pouch increased, so the *Camel Snus* nicotine concentrations per pouch may not have been consistent for all study subjects.

In a cross-over study that used a Latin square design with 21 smokers, to adapt methods to measure toxicant exposure and abstinence symptom suppression, subjects switched to two different PREPs, including *Camel Snus,* stopped all tobacco use, or continued to smoke for four 5-day periods interrupted by weekend washout periods (Blank and Eissenberg 2010). Smokers (≥15 cigs/day) who switched to *Camel Snus* had urinary cotinine levels (µg/L) on day 5 that were similar to those measured at baseline; they were slightly lower than cotinine levels measured in urine of smokers who continued smoking (mean collapsed across the day factor 21.9 cigs/day). *Camel Snus* was provided on day 1 through 4 for the upcoming 24 hours. Snus use over each study period averaged 46.6 pouches (approximately 11.7 pouches/24 hours). The mean carbon monoxide level decreased from 23.7 ppm to 6.1 ppm (both collapsed across days) in the *Camel Snus* group.

Blank and Eissenberg (2010) also investigated subjective symptoms in the study participants relative to the regular brand of cigarettes. The authors concluded that "these PREPs were unable to suppress fully tobacco abstinence symptoms and were considered less enjoyable than participants' own brand of cigarette".

In an 8-day study with 115 smokers total, the group of 15 smokers (mean, 17.8 cigs/day) who switched to *Marlboro Snus* had urinary nicotine equivalent levels (mg/24 hrs) and plasma nicotine and cotinine $AUC_{(0\text{-}12.25\ hrs)}$ values at post-baseline that were reduced to almost $^1/_4$ to $^1/_6$ of those measured at baseline (a 71-76% decrease from baseline) and to approximately $^1/_3$ to $^1/_4$ of those measured in smokers who continued to smoke (means before and after, 16.7 and 15.6 cigs/day, respectively) (Sarkar et al. 2010). Mean nicotine biomarker levels were decreased at the end of the study period even for smokers who continued to smoke. It should be noted that *Marlboro Snus* products formerly had relatively low nicotine content[2] (see also Appendix II, Section A II 2.3.3). The authors noted that in this study the study participants chose to use relatively few pouches per day (means, 3.5 pouches/day, 54 min/pouch), but for longer periods of time (up to two hours). By comparison, the mean urinary nicotine equivalent in smokers at baseline was comparable to what has been measured in snus users by Andersson and colleagues (1994; 1995).

- Nicotine biomarker (urinary cotinine and total cotinine) levels in US STP users who switched to traditional Swedish snus were similar to those in smokers measured at baseline in the same study. Also, urinary cotinine levels in smokers switching to a new product marketed as snus were similar to those measured at their baseline (average tobacco consumption baseline vs. end of the switching periods, 22 cigs/day vs. 13/g/day snus or ≥15 cigs/day vs. 19 pouches/day new product marketed as snus)
- When smokers were interested in cessation and switched to traditional Swedish snus or new products marketed as snus, nicotine biomarker (i.e., plasma nicotine and cotinine, serum cotinine, urinary cotinine and nicotine equivalents) levels decreased by amounts ranging from 16% to 68% of baseline (average tobacco consumption baseline vs. end of the switching periods, 26-28 cigs/day vs. unspecified g/day snus or 18-20 cigs/day vs. 3.5-7 pouches/day new products marketed as snus).

[2] Nicotine content was given as 1.52-2.9%, moisture 9.58-13.22%, pH 6.8-7.19 (Sarkar et al. 2010).

Nicotine biomarker levels - using snus in conjunction with smoking: dual use versus smoking; changes in nicotine biomarkers levels - smokers who switch to dual use

In a Swedish study, 187 dual users (7.8 cigs/day + 27 g/day snus) had higher median urinary cotinine level (µg/L) than 43 smokers (12 cigs/day) (Wennmalm et al. 1991). Snus users (N=127; average 25 g/d) had the lowest level; however, the authors noted that there was no statistically significant difference among the tobacco groups.

Similarly, in a Swedish study of adolescent tobacco users, 16 dual users (9.4 pinches or cigs/day) had the highest median saliva cotinine levels (Post et al. 2005). They were significantly higher (almost 7 times) than those in measured in smokers (6.7 cigs/day) and snus only users (4.4 pinches/day).

In the 8-day study by Sarkar and colleagues (2010), for smokers (mean, 17.6 cigs/day) that reduced their smoking by more than 50% and used *Marlboro Snus* in addition, mean biomarker levels of internal nicotine exposure (urinary nicotine equivalents (mg/24 hrs) and plasma nicotine and cotinine $AUC_{(0\text{-}12.25\ hrs)}$) were decreased to approximately 70% of baseline levels as wells as of post-baseline levels of those who continued to smoke (15.6 cigs/day), but the difference did not appear to be statistically significant. The mean tobacco use in the dual use group was 8.4 cigarettes per day and 2.2 pouches/day (61 min usage time/pouch).

- Regular traditional Swedish snus users who also smoked had higher nicotine biomarker levels than smokers or snus only users, consistent with the higher amount of tobacco consumed by the dual users.
- Nicotine biomarker levels in smokers that switched to dual use reducing their smoking by 50% and instead using a new product marketed as snus were slightly but not significantly decreased compared with baseline levels.

Nicotine biomarker levels - smokers switching to snus versus quitting the use of tobacco products

In the Serbian study, smokers willing to quit (averages, 26-28 cigs/day) with either snus or placebo as cessation aid had similarly decreased (to approximately 68% of baseline levels) mean serum cotinine levels at the end of the study at week 48, when complete smoking cessation was supposed to have been accomplished (self-reported <10 cigs/day) (Joksic et al. 2011). Both groups still had some similar residual nicotine intake from smoking (reflected in mean exhaled breath carbon monoxide level of approximately 12 ppm), implying that the serum cotinine levels measured in the snus group was a result of snus use plus some residual smoking, while in the placebo group only residual smoking contributed. However, the authors noted that at week 24 the reduction of average daily cigarette consumption was significantly stronger in the snus group compared to placebo.

In the cross-over study by Gray and colleagues (2008), the average urinary cotinine level (ng/mL) of STP users on day 5 on a non-tobacco placebo had decreased to approximately $^1/_8$ of the day-1 level. Switching to snus resulted in almost eight times higher levels than quitting (they were similar to levels on day 1).

In the cross-over study by Blank and Eissenberg (2010), the average urinary cotinine level (ng/mL) of smokers on day 5 of not using tobacco had decreased to approximately $^{1}/_{25}$ of the day 1 level. Switching to *Camel Snus* resulted in approximately 20 times higher levels than quitting. The average exhaled carbon monoxide level in both the placebo group and the *Camel Snus* group was around 5 ppm.

In the 8-day study by Sarkar and colleagues (2010), the mean urinary nicotine equivalent level (mg/24 hrs) at post-baseline in smokers that stopped using tobacco had decreased to approximately $^{1}/_{225}$ of the baseline level. Switching to *Marlboro Snus* resulted in almost 70 times higher levels than quitting.

- In summary, smokers who switched to snus or new products marketed as snus, continued to have measurable cotinine levels compared to not using a tobacco product at all.

Nicotine biomarker levels - smokers switching to snus versus using NRT

In the study by Hatsukami and colleagues (2004), conventional STP users who switched to pouched *General* snus (~13 g/day at week 4) had a two times higher mean urinary total cotinine (cotinine and its glucuronide in µg/L) level at week 4 of the switch than those that switched to the 21-mg nicotine patch.

In the study by Kotlyar and colleagues (2011) smokers that switched to a new product marketed as snus (*Camel Snus)* had the same geometric mean urinary cotinine (µg/L) level at week 4 as those that switched to the 4-mg nicotine gum or lozenge.

Kotlyar and colleagues (2011) also measured subjective effects and saw stronger withdrawal symptoms with the PREPs tested (including *Camel Snus*) than with NRT use at the end of the 4-week treatment period. They noted that ”the “modified risk” STPs tested were not superior to medicinal nicotine in decreasing subjective measures (i.e., craving, withdrawal) […]”.

- Switching to traditional Swedish snus from US STPs or to a new product marketed as snus resulted in higher or similar nicotine biomarker levels, respectively. This was likely impacted by differences in nicotine content and delivery kinetics of the different NRT products used in the two studies available.
- The limited data indicates that subjective effects related to withdrawal seen with the new product marketed as snus appeared to be no different from those observed with NRT.

A III 3.1.3 Summary of Biomarkers of Exposure to Nicotine

Nicotine Pharmacokinetics

Five studies provided information on PK parameters measured in blood/plasma of snus users or users of new products marketed as snus in comparison with those seen in smokers and NRT users. Two studies of US STP users were also analyzed. Some of these studies also reported on subjective effects experienced by the users.

PK parameters and reported subjective effects - snus use versus smoking

- The nicotine t_{max} for snus users was dependent on the amount of usage time following placement in the mouth, but not nicotine content or portion size. The t_{max} for smokers ranged between 5 and 7 minutes.
- Snus use resulted in a less steep overall rise and – after the end of use period – decrease of the nicotine plasma concentration compared with smoking, although the increase in concentration within the first minutes of use was somewhat comparable to smoking.
- C_{max} and AUC values observed for snus users were in the same range of those following smoking and were dependent on total nicotine content of the product and product pH, but not on whether the snus was pouched or loose.
- The limited data available suggest that subjective effects related to abuse liability seen for traditional Swedish snus use were at least in part similar to those seen for smoking.

PK parameters and reported subjective effects - snus use versus NRT use

- Unlike what was observed for snus use, the t_{max} for nicotine gum and lozenges appear less dependent on usage time and ranged from 30 to 45 minutes.
- Snus use tended to result in faster rise of the nicotine plasma concentration than use of nicotine gum, although this difference was not significant.
- Snus use resulted in slightly higher C_{max} values than use of 4 mg-nicotine gum and approximately two times higher C_{max} values than use of 2 mg-nicotine gum. Use of a novel brand of traditional Swedish snus, *Catch Dry Mini*, resulted in a similar, but slightly lower C_{max} value than 2-mg nicotine gum.
- Depending on the experience of the snus users, the AUC values were either slightly lower (naïve snus users) or higher (experienced snus users) than those observed for use of 4-mg nicotine gum. The AUC values from snus use were higher than those from 2-mg nicotine gum use. Use of a novel brand of traditional Swedish snus, *Catch Dry Mini*, resulted in a similar AUC value as 2-mg nicotine gum.
- The limited data available suggest that subjective effects associated with dependence seen with traditional Swedish snus use were more pronounced than those seen with NRT use.

Nicotine Biomarkers

Seventeen studies informed on biomarkers of nicotine exposure (i.e., as plasma or serum cotinine and nicotine, saliva cotinine, or urinary cotinine, nicotine, total cotinine, or nicotine equivalents) measured in users of traditional Swedish snus or new products marketed as snus in comparison with those in smokers or NRT users.

Nicotine biomarker levels - snus use versus smoking

- Results from two studies indicate that plasma cotinine to nicotine ratios measured briefly after product consumption as single time point measurement or as AUC over a 12-hour time of use show a slight tendency to be greater for snus users than for smokers.
- Nicotine biomarker levels measured in regular traditional Swedish snus users were similar to or higher than those in regular smokers. These differences appear only in part to be

attributable to differences in amount of tobacco used (mean or median ranges for adult users, 11-32 g/day snus vs. 11-19 cigs/day; means for adolescent users, 4.4 pinches vs. 7 cigs/day).

Nicotine biomarker levels - snus use versus not using tobacco products

- Nicotine biomarker levels measured in regular traditional Swedish snus users were 70-200 times higher than those detected in non-tobacco users.

Changes in nicotine biomarker levels - smokers who switch to using snus

- Urinary nicotine biomarker levels in US STP users who switched to traditional Swedish snus were similar to those in smokers at measured at baseline in the same study. Also, urinary nicotine biomarker levels in smokers switching to a new product marketed as snus were similar to those measured at their baseline (average tobacco consumption baseline vs. end of the switching periods, 22 cigs/day vs. 13/g/day snus or ≥15 cigs/day vs. 19 pouches/day new product marketed as snus).
- When smokers were interested in cessation and switched to traditional Swedish snus or new products marketed as snus, nicotine biomarker levels decreased between 16% to 68% of baseline (average tobacco consumption baseline vs. end of the switching periods, 26-28 cigs/day vs. unspecified g/day snus or 18-20 cigs/day vs. 3.5-7 pouches/day new products marketed as snus).

Nicotine biomarker levels - using snus in conjunction with smoking: dual use versus smoking; changes in nicotine biomarkers levels - smokers who switch to dual use

- Regular traditional Swedish snus users who also smoked had higher nicotine biomarker levels than smokers or snus only users, consistent with the higher amount of tobacco consumed by the dual users.
- Nicotine biomarker levels in smokers that switched to dual use reducing their smoking by 50% and instead using a new product marketed as snus were slightly but not significantly decreased compared with baseline levels.

Nicotine biomarker levels - smokers switching to snus versus quitting the use of tobacco products

- Smokers who switched to snus or new products marketed as snus continued to have measurable cotinine levels compared to not using a tobacco product at all.

Nicotine biomarker levels - smokers switching to snus versus using NRT

- Switching to traditional Swedish snus from US STPs or to a new product marketed as snus resulted in higher or similar nicotine biomarker levels, respectively. This was likely impacted by differences in nicotine content and delivery kinetics of the different NRT products used in the two studies available.
- The limited data indicates that subjective effects related to withdrawal seen with a new product marketed as snus appeared to be no different from those observed with NRT.

Table A III-1: Nicotine Pharmacokinetic Parameters Measured for Swedish Snus, New Products Marketed as Snus, NRT Products*, Moist Snuff*, and Cigarettes* under Experimental Conditions

Citation	Study Design	Product			Plasma Nicotine		
		Name (Number of Administrations)	Nicotine Content	pH	t_{max} (min)	C_{max} (ng/mL)	AUC (ng*min/mL) {Time}
Traditional Swedish Snus (Including Novel Brands)							
Digard et al. 2012	Snus users who occasionally smoked (≤40 cigarettes/week) after 12 hrs of abstinence from tobacco and subsequent 60 min snus consumption	*Granit* Loose snus (Fiedler & Lundgren)	10.8 mg per 1-g portion	8-8.3	60 (45-90)	10.8 (34.4) [2]	960 {0-120 min} [16 (31.2)[2] ng*hrs/mL]
		Granit Loose snus (Fiedler & Lundgren)	27.1 mg per 2.5-g portion	8-8.3	60 (45-90)	17.9 (22.8) [2]	1,614 {0-120 min} [26.9 (23.8)[2] ng*hrs/mL]
		Lucky Strike Original, Brown (Fiedler & Lundgren)	10.7mg per 1-g portion	8.0-8.2	60 (20-90)	10.8 (41.4) [2]	1008 {0-120 min} [16.8 (39.6)[2] ng*hrs/mL]
		Lucky Strike Bold(Fiedler & Lundgren)	14.7 mg per 1-g portion	7.9-8.1	60 (45-90)	13.4 (39)[2]	1,224 {0-120 min} [20.4 (37.6) [2] ng*hrs/mL]
Lunell and Curvall 2011	Smokers after 12 hrs of abstinence from smoking and subsequent 30 min snus consumption	*General Onyx*	9.92 mg per 1-g portion	8.7	37.1 ±10.2 (24 – 60)	14.8 ±3.3	3,062 ±1,002 {0-inf}
		General White Large	8.65 mg per 1-g portion	8.7	37.1 ±10.2 (24 - 60)	13.7 ±3.7	2,829 ±1,037 {0-inf}
Lunell and Lunell 2005	Snus users after overnight abstinence from any nicotine use and subsequent 12 hrs snus consumption	*General* (*12x)*	8.84 mg per 1-g portion	8.4	30	29 ±8.53	1,570 {0-720 min} [26.16 ±3.36 ng*hrs/mL]
		Catch Licorice (*12x)*	7.04 mg per 1-g portion	8.5	30	23.79 ±8.6	1,294 {0-720 min} [21.57 ±8.82 ng*hrs/mL]
		Catch Mini (*12x)*	4.53 mg per 0.5-g portion	8.4	30	20.95 ±6.9	1,141 {0-720 min} [19.02 ±6.69 ng*hrs/mL]
		Catch Dry Mini (*12x)*	4.32 mg per 0.3-g portion	7.3	30	10.85 ±5.65	588.6 {0-720 min} [9.81 ±5.12 ng*hrs/mL]
Holm et al. 1992	Snus users after overnight abstinence and subsequent 30 min snus consumption	*Ettan*	2-g portion		35.5	17 ± 5.6	747.4 ±243 {0-60 min}

Table A III-1: Nicotine Pharmacokinetic Parameters Measured for Swedish Snus, New Products Marketed as Snus, NRT Products*, Moist Snuff*, and Cigarettes* under Experimental Conditions

Citation	Study Design	Product			Plasma Nicotine		
		Name (Number of Administrations)	Nicotine Content	pH	t_{max} (min)	C_{max} (ng/mL)	AUC (ng*min/mL) {Time}
New Products Marketed as Snus							
Cobb et al. 2010	Smokers after overnight abstinence from tobacco and subsequent 2 cycles of 15 min use + 30 min observation separated by 15 min break	*Marlboro Snus* (2x)	12.8 mg/g dry weight & 0.35 mg/g unprotonated (Portion=0.2 g)	NR	~15-30 (after 2nd admin)	~3.5	NR
		Camel Snus (2x)	2006 version: 28.2 mg/g dry weight & 6.1 mg/g unprotonated (Portion=0.4 g) 2008 version: not available	NR	15 (after 2nd admin)	7.6	NR
NRT Products							
Digard et al. 2012	Snus users who occasionally smoked (≤40 cigarettes/week) after 12 hrs of abstinence from tobacco and subsequent 30 min gum use	*Nicorette* 4-mg nicotine gum	4.2 mg per piece	NR	45 (20-90)	9.1 (28.6) [2]	786 {0-120 min} [13.1 (28.3) [2] ng*hrs/mL]
Lunell and Curvall 2011	Smokers after 12 hrs of abstinence from smoking and subsequent 30 min of gum use	Nicotine Polacrilex 4-mg nicotine gum	3.8 mg nicotine per portion	NR	46.1 ±16.2 (30 - 90)	12.8 ±2.96	3,190 ±1,310 {0-inf}
Lunell and Lunell 2005	Snus users after overnight abstinence from any nicotine use and subsequent 30 min gum use for 12 hrs (with 30 min breaks)	*Nicorette* 2-mg nicotine gum (12x)	1.91 mg nicotine per portion	N/A	30	12.75 ±4.67	693 {0-720 min} [11.55 ±4.52 ng*hrs/mL]

Table A III-1: Nicotine Pharmacokinetic Parameters Measured for Swedish Snus, New Products Marketed as Snus, NRT Products*, Moist Snuff*, and Cigarettes* under Experimental Conditions							
Citation	**Study Design**	**Product**			**Plasma Nicotine**		
		Name (Number of Administrations)	**Nicotine Content**	**pH**	**t_{max} (min)**	**C_{max} (ng/mL)**	**AUC (ng*min/mL) {Time}**
Cobb et al. 2010	Smokers after overnight abstinence from tobacco and subsequent 2 cycles (separated by 15-min break) of use until dissolve with 45 min observation	*Commit* 2-mg lozenge (2x)	~ 2 mg/portion	NR	30	~6 (after 2nd admin.)	NR
Kotlyar et al. 2007	STP users after overnight/12 hrs abstinence from any nicotine use and subsequent 30 min product use	*Commit* 4-mg lozenge	~4 mg nicotine per portion		~30	7.3 (5.5-9.8)	467 (361-604) {0-90 min}
Traditional Moist Snuff							
Kotlyar et al. 2007	STP users after overnight/12 hrs abstinence from any nicotine use and subsequent 30 min product use	*Copenhagen* 2 g	NR	NR	~30	16.1 (12.1-21.5)	1038 (806-1336) {0-90 min}
Fant et al. 1999	STP users after 3 hrs of abstinence 30 min snuff use	*Copenhagen* 2 g	11.4 mg/g nicotine	8.6	~35	19.5	530.4 {0-30 min}
		Moist Snuff *(Copenhagen, Skoal Long Cut Cherry, Skoal Original Wintergreen)* 2 g	10.4-11.4 mg/g nicotine	7.5-8.6	~22-35	14.9-19.5	208.0-530.4 {0-30 min}

Table A III-1: Nicotine Pharmacokinetic Parameters Measured for Swedish Snus, New Products Marketed as Snus, NRT Products*, Moist Snuff*, and Cigarettes* under Experimental Conditions

Citation	Study Design	Product			Plasma Nicotine		
		Name (Number of Administrations)	Nicotine Content	pH	t_{max} (min)	C_{max} (ng/mL)	AUC (ng*min/mL) {Time}
Smoking							
Digard et al. 2012	Snus users who occasionally smoked (≤40 cigarettes/week) after 12 hrs of abstinence from tobacco and subsequent 5 min of smoking	*Lucky Strike Red* Cigarette	14.6 mg nicotine per cigarette	N/A	7 (5-31)	12.8 (41.3) [2]	888 {0-120 min} [14.8 (30.4) [2] ng*hrs/mL]
Cobb et al. 2010	Smokers after overnight abstinence from tobacco and subsequent 2 cycles (separated by 15-min break) of smoking a cigarette with 45 min observation	Cigarettes (2x)	Average cigarette=1.1 mg	NR	~ 5	20.7	NR

* Select studies only (data for smokers and NRT users were taken from available comparative studies or if these were not available, other representative studies that reported biomarkers levels for smokers or NRT users were used.
NR: Not reported

Table A III-2: Nicotine Biomarker Data as Reported in Studies of Users of Swedish Snus, New Products Marketed as Snus, STPs*, NRT*, and Smokers* from the Literature under Regular Use Conditions

Biomarker	Matrix	Nonsmokers/ Non-Users of Tobacco/ Smokers that tried to quit	NRT Users	Snus/*STP* Users/ Smokers that switched to snus or a new product marketed as snus	Dual Use	Smokers
Nicotine	Plasma (ng/mLxhr))	- [f]		(225.55 →) 40.31 [f]	(202.02 →) 132.94 [f]	(195.67 →) 170.34 [f]
Cotinine		- [f]		(3617.91 →) 781.87 [f #]	(3142.33 →) 2008 [f]	(3566.71 →) 3139.96 [f]
	Plasma or Serum (ng/mL)	2-4 [a, §] LOD [d] 0.05 [¬, e] (101.2 →) 69.1 [m] NA [q]		**326-359** [a, §] **399.2** [c] **137** [d] *188* [¬, e] (98.9 →) **66.1** [m]	**308** [b]	213-258 [a, §] 306.3 [c] 110 [d] 127 [¬, e] 184 [q]
	Saliva (ng/mL)			**80** [n] **342.9; 326.6** [o] **336; 153-159** [p]	**135** [n]	20 [n]
	Urine (μg/L)	5.7 [g] (~1100 →) 143 [h] (~1250 →) ~50 [i] 2.03 [r, ¬]	(~3200 →) ~700 [j]	**1210** [g] (~*1000* →) **~1000** [h] (~1000 →) ~*1000* [i] (~3500 →) 700 [j] **[1908]** [d]	**1773** [g]	1560 [g] (~1000 →) ~1500 [i] [1932] [d] 1043 [r, ¬]
	Urine (μg/mmol creatinine)	1.2 [d]		**159** [d]		161 [d]
Nicotine		0.4 [d]		**29** [d]		41 [d]
Total Cotinine[1]	Urine (μg/L)		(*5759* →) 3204; (6364 →) 3437 [k]	(*6193* →) **5926** [k]		(4412 →) 4450 [k]
Nicotine equivalents[2]	Urine (mg/24 hrs)	(18 →) 0.08 [f] NA [q]		(21.47 →) 5.53 [f, #] **34.5; 35.6** [o] **25.2; 14.3-14.4** [p]	(17.79 →) *11.3* [f]	(20.76 →) 17.77 [f] 13.3 [q]

* Select studies only (data for smokers and NRT users were taken from available comparative studies or if these were not available, other representative studies that reported biomarkers levels for smokers or NRT users were used. Where no data was available for users of traditional Swedish snus or new products marketed as snus, data were supplemented with studies on US STP users.

Bolded results signify those of Swedish snus users; Italics indicate US STP users. Results in [..] indicates calculated results in a unit not provided in the study.

[1] cotinine + glucs;

[2] Nicotine and its metabolites: nicotine-N-glucuronide, cotinine, cotinine-N-glucuronide, trans-3' hydroxycotinine, trans-3' hydroxycotinine glucuronide, nicotine-N' –oxide, cotinine-N' –oxide (Andersson et al. (1994) measured nicotine + 7 while Sarkar et al. (2010) measured nicotine +5)

Table A III-2: Nicotine Biomarker Data as Reported in Studies of Users of Swedish Snus, New Products Marketed as Snus, STPs*, NRT*, and Smokers* from the Literature under Regular Use Conditions

LOD: Limit of detection;
[§] range of means or medians; ¬ Geometric mean (95% Confidence Interval);
[#] significantly ↓ compared to smokers; *significantly different from controls
[a] Bolinder et al. 1997a,b; Bolinder 1997; Bolinder & de Faire 1998; Eliasson et al. 1991; 1995; (N=21-92) snus consumption 21-32 g/day, cigarette consumption 15-19 cigs/day
[b] Eliasson et al. 1995; dual use (N=38): 10.1 cigs/day + 2.5 cans/week (28.2 g/day total)
[c] Holm et al. 1992; 1 min after end of smoking (N=35) or 5-15 min after end of snus consumption during regular use (N=27); 21 g/day snus or 17 cigs/day
[d] Ellingsen et al. 2009; snus consumption 10.7 g/day (N=11), cigarette consumption 17 cigs/day (N=38), N=49 non-tobacco users
[e] Naufal et al. 2011; 368 STP users not specified (included snuff and chewing tobacco users); 16,443 non-tobacco users included NRT users, 5,040 smokers (consumption data was not available); unadjusted geometric means; statistical differences presented are based on adjusted regression model results
[f] Sarkar et al. 2010; 115 Smokers at baseline (grey) and post-baseline after switching (8-day study): Cigarettes only→*Marlboro Snus* only (N=15) 17.8 cigs/day→3.5 g/day, Continued Cigarette consumption (N=30) 16.7→15.6 cigs/day; Cigarettes only→dual use (N=59): 17.6 cigs/day→8.4 cigs/day + 2.2 g/day; nicotine equivalents are nicotine + 5 metabolites
[g] Wennmalm et al. 1991; snus consumption 25 g/day (N=127), cigarette consumption 12.2 cigs/day (N=43), dual use 7.8 cigs/day + 27 g/day snus (N=187)
[h] Gray et al. 2008; Study 2; 19 STP users on day 1 (baseline) and day 5 after switching; approximate average values based on figures: *ad libitum* STP→45 g General snus
[i] Blank & Eissenberg et al. 2010; 21 Smokers on day 1 (baseline) and day 5 after switching; approximate average values based on figures: Cigarettes→Ariva (12.3 tablets/24 hrs), Camel snus (11.7 pouches/24 hours), continued smoking (21.9 cigs/24 hrs)
[j] Kotlyar et al. 2011; Smokers before (grey) and 4 weeks after switching: Smoking→NRT (23.6 cigs/day→7.4 pieces/day) (N=27), Smoking→Camel snus (19.7 cigs/day→6.9 pouches/day) (N=51)
[k] Hatsukami et al. 2004; 41 STP users at baseline and 4 weeks after switching to snus or nicotine patch; 38 smokers after switching to Omni cigarettes or nicotine patch: STP→Patch (2.9 tins/week→NA), STP→Snus (3.1→3.7 tins/week), Cigarettes→Patch (22 cigs/d→NA), Cigarettes→Omni (21.7→26.0 cigs/d)
[m] Joksic et al. 2011: Among daily users of snus the mean amount per day ranged from 3.5 to 4.7 g per day, and was relatively stable over time. Cigarette: On average, 319 participants had smoked 27 cigarettes per day during the past year.
[n] Post et al. 2005; medians based on box-plots provided in figure 1 in Swedish adolescent tobacco users: 28 Snus users (31 pinches/week), 16 Dual users (66 cigs or pinches/week), 69 Smokers (47 cigs/week)
[o] Andersson et al. 1994; means for 23 pouched (14.4 g/day) and 22 loose (20.8 g/day) snus users, respectively; nicotine equivalents are nicotine + 7 metabolites
[p] Andersson et al. 1995; means for pouched snus, higher nicotine (16.4 g/day) and lower nicotine products (18.6 g/day after switch, 15 g/day in regular users) (N=24)
[q] Roethig et al. 2009; 3,585 smokers, 1,077 nonsmokers; weighted mean: number of butts returned/24 hr: 16.0 (0.2)
[r] Goniewicz et al. 2011; 228 Nonsmokers were passive smokers exposed to environmental tobacco smoke. 373 Smokers; Mean cigarettes/day smoked from 3 active smoker groups that were combined: 18.4±8.2, 15.0±8.4, 6.9±7.1

A III 3.2 Biomarkers of Exposure to Trace Level Components

A III 3.2.1 *N*-Nitroso Compounds: TSNAs Biomarkers

Details on the significance of TSNA biomarkers are described in Sections 3.2.1.1-3.2.1.3 of the main report. Available studies that compared TSNA biomarkers (urinary total NNAL, total NNN, and TSNA adducts) in traditional Swedish snus users with smokers and NRT users were supplemented with studies of users in new products marketed as snus and select studies of conventional US STP users (despite limitations) and are discussed below. No comparative data for TSNA in saliva were identified.

A III 3.2.1.1 & 3.2.1.2 Urinary Total NNAL (Biomarkers of NNK) and Total NNN

Urinary biomarkers of TSNA exposure following use of traditional Swedish snus, in comparison with other US STPs (traditional and new products), NRT, and cigarette smoking have been investigated in two studies by US university researchers (Gray et al. 2008; Hatsukami et al. 2004). This data is supplemented by studies in smokers and STP users following use of new products marketed as snus (Blank and Eissenberg 2010; Kotlyar et al. 2011; Sarkar et al. 2010) or traditional STPs (Hecht et al. 2007; Naufal et al. 2011; Stepanov and Hecht 2005). This section discusses both - results of measurements of urinary levels of total NNAL and, where studies were available, of total NNN. The latter analyte has not been as frequently measured as total NNAL. The respective urinary levels reported in the above studies are provided in **Table A III-3**.

Urinary TSNA biomarker levels - snus use versus smoking

No studies were identified in which urinary total NNAL or total NNN in regular snus users or users of new products marketed as snus were measured.

One study, described below in more detail, measured biomarkers of tobacco exposure in traditional US STP users before and four weeks after switching to snus (Hatsukami et al. 2004). This study indicates that, consistent with the generally lower TSNA concentrations present in traditional Swedish snus compared to most traditional US STPs (see Appendix II, Section A II 2.3.6.1), use of snus reduced urinary total NNAL levels to approximately half of baseline, while nicotine intake, measured as total urinary cotinine, did not change. At baseline, NNAL levels in urine from US STP users (3.1 tins/week, ~15 g/day, assuming 34 g tobacco/tin) were slightly higher (approximately 1.3 times (23%)) than those from smokers of conventional cigarettes (22 cigs/day) investigated in the same study. Following the 4-week snus consumption phase, the levels were lowered to approximately 60% of those from smokers at baseline. Cotinine levels in urine of STP/snus users were higher at baseline and throughout the study than those detected in urine from the smokers (see Section III 3.1.2.1). Note though that this study did not provide a statistical comparison of urinary biomarker levels measured in STP/snus users and smokers.

Below is a summary of studies that examined total NNAL or NNN in regular users of US STPs. While the relevance of these results to regular traditional Swedish snus users is not clear, notably because of product chemistry differences, they are provided here in the absence of more applicable data:

In an analysis of NHANES US national survey data from 1999-2008, researchers from R.J. Reynolds reported that urinary NNAL (pg/mg creatinine; geometric means) in 368 US STP

users were significantly higher than those in 5,040 smokers (Naufal et al. 2011). As discussed in Section A III 3.1.2.1, serum cotinine levels were also significantly higher in the STP users compared with those in smokers as well as in the range of those measured in studies with traditional Swedish snus for loose and pouched snus users. The study by Naufal and colleagues (2011) did not provide the adjusted data based on which statements were made with respect to statistical significance. Another limitation of this study is the lack of information on the specific brands or distribution of STP types used; the study authors combined self-reported snuff and chewing tobacco consumers. Based on US patterns of use, the types of STPs are likely to be predominantly US moist snuff, some chewing tobacco, and a low percentage of new products marketed as snus or Swedish snus (Delnevo et al. 2012).

Comparing the data from the analysis by Naufal and colleagues (2011) with that from other studies that have measured urinary total NNAL in smokers and US STP users indicates that the difference seen between the two groups in the NHANES analysis is larger than in most other studies (Carmella et al. 2002; Carmella et al. 2003; Hatsukami et al. 2004; Hecht et al. 2002; Hecht et al. 2007).

In the largest study available that compared STP users with smokers (aside from the analysis by Naufal and colleagues (2011)), the geometric mean of total NNAL (pmol/mg creatinine) in urine of the 180 STP users (4.2 tins/week, ~20 g/day, assuming 34 g tobacco/tin) was similar, although slightly higher than that detected in urine of the 420 smokers (mean, 25.8 cigs/day), while the urinary cotinine levels were significantly higher in the STP users (Hecht et al. 2007). After adjusting for age and gender though, the urinary total NNAL levels reported were significantly higher for STP users compared to smokers (Hecht et al. 2007). The majority of STP users in this study used the traditional US STPs *Kodiak, Copenhagen*, and *Skoal*. The authors concluded that exposure to NNK was similar for STP users and smokers.

Based on studies of predominantly US STP users, the CDC (2012[3]) stated that "Urinary total NNAL levels are similar or slightly higher in users of smokeless tobacco products compared to active smokers, indicative of the higher levels of TSNA and NNK that may be present in smokeless tobacco" (CDC 2012). As stated above and described in Appendix II, Section A II 2.3.6.1, TSNA concentrations in traditional Swedish snus are generally lower compared to most traditional US STPs.

Note that differences in the routes of uptake may also be responsible for differences seen in urinary NNAL levels in STP users versus smokers, but this has not been clearly established. As described in Section 3.2.1.1 of the main report, a higher urinary NNAL level may, aside from arising from a higher internal NNK dose, also indicate decreased metabolic activation to reactive metabolites.

No study was available analyzing total NNN in regular snus users. In a study that analyzed urinary total NNN in 14 smokers and 11 US STP users (not further specified), NNN levels were almost 4 times higher in the STP users (Stepanov and Hecht 2005). The authors stated that "this is consistent with the relatively high levels of tobacco-specific nitrosamines in smokeless tobacco products". However, compared with the difference seen for total NNAL levels these

[3] CDC 2012. http://www.cdc.gov/biomonitoring/NNAL_BiomonitoringSummary.html, accessed April 2013.

authors saw a greater difference for total NNN levels between STP users and smokers. They hypothesized that NNN (as well as NAT, and NAB) could form in the stomach from the respective alkaloids.

- While no studies of regular snus users were available, one study in which US STP users were switched to traditional Swedish snus observed an almost 60% lower mean total NNAL level in urine from the snus users compared with baseline levels in urine from smokers investigated in the same study. No data for total NNN measured in urine of regular traditional Swedish snus users were available.

TSNA biomarker levels - snus use versus not using tobacco products

No studies that directly compared regular snus users with non-tobacco users were identified. Studies with smokers and traditional US STP users indicate that these tobacco users have approximately 200 or almost 1000 times higher urinary NNAL levels than non-tobacco users (Naufal et al. 2011) or approximately 60 or 80 times higher urinary total NNAL levels than nonsmokers exposed to environmental tobacco smoke (Carmella et al. 2003).

- No data that directly compared total NNAL or total NNN in urine of regular traditional Swedish snus users or new products marketed as snus with non-tobacco users were available.

Changes in urinary TSNA biomarker levels - smokers who switch to using snus

No studies could be located that measured NNAL in smokers switching directly to traditional Swedish snus. As described above, in the study by Hatsukami and colleagues (2004) four weeks after switching to pouched *General* snus from conventional US STPs, the mean total urinary NNAL level (pmol/g creatinine) in 19 users was approximately 60% of the mean level measured in 38 smokers in the same study at baseline (smoking their regular cigarettes). With the mean cigarette consumption at baseline of 22 cigarettes/day, the mean total NNAL level measured in these smokers was similar to what has been reported in other studies in smokers of a similar number of cigarettes (e.g., Hecht et al. 2007; Joseph et al. 2005). At the same time, the STP users who had switched to snus had almost unchanged urinary total cotinine levels, indicating that nicotine intake remained unchanged. The mean snus use was similar in tins/week (3.7 tins/week, with 24 1-g portions per tin = ~13 g/d) at week 4 to the previous use of US STP and also similar to what has been observed in regular snus users in Sweden (Digard et al. 2009). Hatsukami and colleagues (2004) concluded that "switching to reduced-exposure tobacco products or medicinal nicotine can decrease levels of tobacco-associated carcinogens, […]".

In addition to the limited data for traditional Swedish snus, data from a study by the same researchers was available for smokers who switched to a new product marketed as snus. In the study of 130 smokers interested in cessation, the average total NNAL level (pmol/g creatinine) decreased significantly by week 4 to less than half of baseline levels in the 51 smokers (19.7 cigs/day) who switched to *Camel Snus* (6.9 pouches/day) (2011). In this group, 9% of subjects continued to smoke more than three cigarettes/day. Urinary cotinine levels were also reduced

to almost $^1/_5$ of those at baseline (smoking) at the end of the 4-week treatment time, probably reflecting the overall reduced tobacco intake due to the subjects' interest in quitting.

A direct extrapolation or comparison of the reduction in NNAL levels seen as a result of switching from smoking to *Camel Snus* to the use of *General* snus is difficult due to differences in product formulations, pouch sizes, nicotine biomarkers measured (see above), and study participant characteristics:

One study that compared components in tobacco products by portion size indicates that the free nicotine concentration in *Camel Snus* was approximately $^1/_4$ (25%) of that in *General* snus per portion size. Because Kotlyar and colleagues (2011) measured only urinary cotinine, while the previous study by the same researchers measured total cotinine in urine (Hatsukami et al. 2004), comparing the nicotine intake between the two studies based on the nicotine biomarker levels is difficult. Per mg free and total nicotine, the *Camel Snus* contained approximately 70% and 30% of the NNK concentration detected in *General*, respectively (Stepanov et al. 2008) (see also Appendix II, Section 2.3.6.1.1). It should be noted though that TSNA concentrations in traditional Swedish snus have continued to decrease over recent years, while nicotine concentrations have been kept constant.

Further, the later study investigated smokers interested in cessation. The smokers who switched to *Camel Snus* consumed less pouches per day (Kotlyar et al. 2011) than those participants in the previous study who were STP users switching to *General* (Hatsukami et al. 2004). In addition, baseline levels in the study participants were considerably different: The mean total NNAL level in smokers at baseline was approximately $^1/_3$ of that in the previous study although the mean cigarette consumption was similar (20 versus 22 cigs/day, respectively). A possible explanation could be product differences leading to reduced NNK exposure of smokers in the later study due to changes in tobacco product formulations (See also Ashley et al. 2010).

Kotlyar and colleagues (2011) also analyzed urinary total NNN levels, which at week 4 of *Camel Snus* use, instead of smoking, were decreased to half (50%) of the baseline geometric mean level. No published data on total NNN levels after switching to traditional Swedish snus were available.

In a study by Blank and Eisenberg (2010), who used the same cross-over study design as Gray and colleagues (2008) (researchers from the same study group) 21 smokers switched to two potentially reduced exposure products, including *Camel Snus*, stopped tobacco use or continued smoking. Similar to their previous study, in which conventional STP users switched to loose *General* snus, no change in total NNAL levels (pg/mL urine), was detected on day 5 of switching from smoking to *Camel Snus.* There was also no change in urinary cotinine levels. The baseline cigarette consumption was reported to be greater than 15 cigarettes/day, and reported average urinary cotinine levels were similar to those reported in other active smokers (e.g., Goniewicz et al. 2011). By comparison, in the study by Kotlyar and colleagues (2011), the average baseline urinary cotinine level of smokers was approximately three times higher. The reason for this difference is not clear.

One important limitation of the study design used by Blank and Eisenberg is the short duration (5 days) of the switches, considering the terminal half-life of NNAL and its metabolites. The brief switching periods were accompanied by weekend washout periods that allowed users to consume their regular tobacco products. In addition, while non-smoking compliance during the treatment week was verified by reduced carbon monoxide breath levels, use of other STPs that might contribute to TSNA intake could not be ruled out.

In the study by Sarkar and colleagues (2010), the effect of a complete or partial switch from smoking 10 to 40 cigarettes/day (range of group means, 16.7-18.5 cigs/day) to *Marlboro Snus* on exposure biomarkers was investigated and compared with continued smoking or complete cessation in a total number of 115 smokers. At post-baseline, total NNAL levels (ng/24-hour urine) in the 15 smokers who switched completely to *Marlboro Snus* were decreased to almost $^1/_3$ of baseline levels and to approximately half of levels in 30 smokers who continued to smoke. In the *Marlboro Snus* user group, measures of nicotine exposure such as AUCs of plasma nicotine and cotinine as well as of nicotine equivalents in urine decreased to $^1/_4$ to $^1/_6$ of baseline levels.

In the study by Sarkar and colleagues (2010), smokers who continued to smoke slightly decreased their nicotine intake and that was accompanied by a slight decrease in mean total NNAL level.

Total NNN levels (ng/24 hrs) in the smokers switching fully to *Marlboro Snus* decreased to $^1/_6$ of baseline levels (2010). As noted earlier, a direct extrapolation of biomarker levels observed with use of different products is challenging since especially new products marketed as snus have been subjected to frequent reformulation and changes in portion size (see Appendix II, Section A II 2.3.6.1.1). It should be noted though that in a study that reported components per portion in several STPs including *Marlboro Snus* and *General*, free nicotine was approximately 25 times higher per portion in *General* and NNK concentrations per mg free nicotine were approximately $^1/_3$ of those in *Marlboro Snus* (Stepanov et al. 2008).

- The limited data from one study on total NNAL levels in urine of US STP users four weeks after switching to traditional Swedish snus in comparison with levels in smokers at baseline suggests that switching from smoking to snus might reduce TSNA biomarker levels, while nicotine biomarker levels remained similar.
- Reductions in urinary total NNAL and total NNN levels (30-50% and 17-50% of baseline smoking levels, respectively) were seen for smokers who switched to new products marketed as snus in two studies for eight days and four weeks; however, in these studies nicotine biomarker levels also decreased significantly (17-25% of baseline smoking levels) suggesting a change in tobacco consumption levels. On the other hand, in a cross-over study of smokers switching to a new product marketed as snus for five days, where no change in nicotine biomarker levels were seen, the urinary total NNAL levels were not impacted either.

Changes in urinary TSNA biomarker levels - using snus in conjunction with smoking: smokers who switch to dual use

No studies were identified that measured NNAL levels in smokers who also used traditional Swedish snus (dual use).

In the study by Sarkar and colleagues (2010), in smokers who reduced smoking by 50% and used *Marlboro snus* the mean total NNAL level (ng/24 hrs) after one week was approximately 70% of baseline. It should be noted that this group of smokers also had the lowest mean baseline NNAL level of all treatment groups, which included complete tobacco cessation and complete switch to *Marlboro snus*. At the same time, measures of nicotine exposure were also approximately 60-70% of baseline levels. In this study, the mean urinary total NNN level (ng/24 hrs) decreased to half (50%) of baseline level.

The authors concluded that "After correcting for the residual effect [observed in the non-tobacco group reflecting e.g., the long half-life of NNAL and its glucuronides], a ≥50% reduction in daily cigarette consumption and dual MSNUS [*Marlboro Snus*] usage resulted in a corresponding 50% reduction in most the biomarkers".

- No studies were available that measured urinary total NNAL levels in smokers who also used traditional Swedish snus (dual use) or who switched to dual use.
- Total NNAL and NNN levels in smokers who reduced smoking by at least 50% and in addition used a new product marketed as snus were reduced (70 and 50% of baseline smoking levels).

Urinary TSNA biomarker levels - smokers switching to snus versus quitting the use of tobacco products

No study comparing smokers that switched to traditional Swedish snus with those who quit tobacco was identified in the literature.

The cross-over study by Gray and colleagues (2008) indicates that at day 5 of stopping tobacco use with a placebo product, the average urinary NNAL (pg/mL) and urinary cotinine decreased to approximately $^1/_3$ and $^1/_7$ of baseline use in the traditional US STPs users. Switching to loose *General* snus, on the other hand, resulted in approximately two times and seven times higher average total NNAL and cotinine levels, respectively, than quitting. The limitations of the study design are discussed above.

In the study by Blank and Eisenberg (2010) that used the same study design as Gray and colleagues (2008) but investigated smokers, smoking cessation resulted in reduced average urinary total NNAL (pg/mL) and urinary cotinine levels to almost $^1/_3$ and $^1/_{25}$ of the baseline values of smokers by day 5 of cessation. Switching from cigarettes to *Camel Snus*, on the other hand, resulted in approximately 3 and 20 times higher average total NNAL and cotinine levels, respectively, than quitting.

In the 8-day treatment study by Sarkar and colleagues (2010), smokers who quit all tobacco use experienced a decrease in mean urinary total NNAL (ng/24 hrs) and nicotine equivalent levels to

less than $^1/_3$ and $^1/_{25}$ of baseline levels, respectively. Switching to *Marlboro Snus*, on the other hand, resulted in approximately 1.4 and 70 times higher mean total NNAL and cotinine levels, respectively, than quitting.

In the same study, the mean total NNN level in smokers who quit all tobacco decreased to $^1/_{34}$ of baseline, while switching completely to *Marlboro Snus* resulted in a level approximately six times higher compared to quitting.

- No data for smokers who switched to traditional Swedish snus in comparison to those who quit was available.
- Urinary total NNAL levels measured in 5-day cross-over studies for US STP users or smokers who switched to new products marketed as snus were two to three times higher compared with those who quit. At the end of an 8-day study, urinary total NNAL and total NNN levels in smokers who switched to a new product marketed as snus were 1.4 and 6 times higher, respectively, compared with smokers who quit.

Urinary TSNA biomarker levels - smokers switching to snus versus using NRT

The study by Hatsukami and colleagues (2004) also investigated how biomarker levels changed in both smokers and US STP users after switching to 21-mg nicotine patches for four weeks. The mean urinary total NNAL levels declined during the first two weeks of NRT use to $^1/_6$ and $^1/_4$ of the baseline levels in former STP users and smokers, respectively, and then further decreased by another 50%. Total urinary cotinine decreased to approximately half of baseline levels. At the end of the study, switching to *General* snus in this study resulted in approximately five to seven times higher mean total NNAL level and two times higher cotinine level compared to using the nicotine patch. Accordingly, the authors concluded that "switching to reduced-exposure tobacco products or medicinal nicotine can decrease levels of tobacco-associated carcinogens, with greater reductions being observed with medicinal nicotine. Medicinal nicotine is a safer alternative than modified tobacco products".

In the study by Kotlyar and colleagues (2011), the switch to NRT products (4-mg nicotine gum or lozenge) was accompanied by a total decrease to $^1/_5$ of the baseline geometric mean total NNAL level with most of the reduction within the first two weeks of treatment. Similarly, cotinine levels decreased to almost $^1/_5$ of baseline by the end of the 4-week treatment period and were similar to those in the *Camel Snus* group. However, switching to *Camel Snus* resulted in significantly (~2x) higher geometric mean total NNAL level compared to using the NRT products, when analyzing only subjects who were abstinent from cigarettes from weeks 2 to 4. In both groups, a similar percentage of users continued to smoke more than three cigarettes/day.

In the same study, the geometric mean total NNN level (pmol/mg creatinine) decreased significantly in urine of smokers who switched to NRT products. This decrease appeared to be similar to the decrease seen for the switch to *Camel Snus*, however, the latter was not statistically significant (Kotlyar et al. 2011). When compared with NRT users, those switching to *Camel Snus* had slightly higher geometric mean NNN levels at the end of the 4-week study period, but this difference was not statistically significant.

- The limited data from one study on total NNAL levels in urine of US STP users who switched to traditional Swedish snus in comparison with those from US STP user and smokers who switched to NRT suggest that switching to snus results in five to seven times higher total NNAL levels than switching to NRT.
- Data from one study observed that smokers interested in cessation who switched to new products marketed as snus had approximately two times higher NNAL levels than those who switched to NRT product use. Urinary total NNN levels were not significantly different between those groups.

A III 3.2.1.3 Adducts of NNK and NNN

Studies of DNA and hemoglobin adducts of NNK and NNN in animal and human tissues were recently reviewed and analyzed by Nilsson (2011). Only one study has reported adduct levels detected in snus users (Heling et al. 2008; Richter et al. 2009b, as cited in Nilsson 2011). This study and the analysis by Nilsson (2011) are discussed in several sections, below. Nilsson's overall conclusions are presented following the individual sections.

Nilsson (2011) extrapolated DNA and hemoglobin adduct levels measured in tissues of experimental animals after exposure to NNK or NNN to those that could be expected after human intake of NNK and NNN from different tobacco products (Swedish snus, smoking, Sudanese toombak). He compared these calculated adduct levels to measured adduct levels in whole lung, liver, leukocytes, and hemoglobin isolated from human smokers and nonsmokers reported in the literature. The study provided no data of adduct levels in these tissues isolated from snus users. Instead, it provided adduct level data from one study investigating oral tissue from snus users in comparison with smokers and non-tobacco users. However, no animal data for adducts in the oral mucosa were presented that would allow a similar extrapolation to those expected in human oral mucosa.

TSNA adduct levels - snus use versus smoking

In his review, Nilsson (2011) cited a study abstract that reported POB-DNA adduct levels detected in oral mucosa samples of tobacco users (Richter et al. 2009b, as cited in Nilsson 2011). Another abstract was identified that appears to refer to the same study or samples, but includes a smaller number of smokers (N=24 vs. N=90) (Heling et al. 2008). POB-DNA adduct levels in oral mucosa samples of 33 Swedish snus users were approximately twice as high as those detected in those of smokers. Heling and colleagues (2008) concluded that "[h]igher adducts levels in snuff dippers may be explained by prolonged exposure of the mucosa to NNN and NNK. However, they do not correspond to the inherent risk of oral cancer which is considerably lower in Swedish snus users than in smokers." Nilsson concluded that "[t]hese results cast doubt on the involvement of POB-DNA adducts in causing oral cancer, especially from Swedish "snuff" […]".

Nilsson (2011) calculated the NNK and NNN intake from Swedish snus using the assumptions of 20 g/day and 60% absorption of TSNAs from the snus. The TSNA concentrations in snus used for these calculations stemmed from analyses reported in 1980/83 as well as 2004. NNK and NNN concentrations were up to 10 times lower in 2004 compared to the earlier measurements.

Using the TSNA concentrations in snus reported in 2004, the respective extrapolated O^6-methylguanine and 7-methylguanine adducts were several orders of magnitude lower than those actually measured in human tissue samples of whole lung, liver, and leukocytes of nonsmokers and smokers (no results for human tissue samples of snus users were provided). Extrapolated levels of the more specific pyridyloxobutyl (POB)-DNA adducts were also approximately 500 times lower based on TSNA concentrations reported in 2004 for snus samples compared to those actually measured in whole lung tissue from nonsmokers. Similar results were seen for calculated adduct levels for smoking 20 cigarettes/day compared to those detected in human samples of smokers and nonsmokers. It should be noted that the levels of POB-adducts reported for the oral tissue were more than 25 times higher than those reported for whole lung tissue for both smokers and nonsmokers (as reviewed by Nilsson 2011). The calculated DNA adduct levels from snus use for tissues other than oral tissue were either similar or approximately half of those calculated for smoking.

POB hemoglobin adducts levels calculated by Nilsson (2011) for smokers and snus users were similar and in the same range as those measured in one study (also reported by Nilsson 2011) that analyzed these adducts in hemoglobin from users of 22 US oral snuff of unknown purity, as well as in 40 smokers and 21 nonsmokers (Carmella et al. 1990). The extrapolated adduct levels based on TSNA concentrations in snus samples reported in 2004 were approximately 5 times lower than those detected in the US oral snuff users by Carmella and colleagues (1990). The extrapolated level based on smoking was almost two times higher than that detected in smokers the same study.

- Data from one small study measuring POB-DNA adducts levels in oral tissue indicate that levels were approximately twice as high in regular snus users as in smokers. Based on these results and considering the higher risk for developing oral cancer in smokers, Nilsson (2011) concluded that these type of adducts are likely not involved in the development of oral cancer.
- Calculated methylguanine- and POB-DNA adduct levels in whole lung, liver, and leukocytes, as well as calculated POB-hemoglobin adduct levels for snus use were either similar or approximately half of those calculated for smoking..

<u>TSNA adduct levels - snus use versus not using tobacco products</u>

POB-DNA adducts levels detected in oral mucosa samples of 33 Swedish snus users were approximately nine times higher than those in 45 nonsmokers (Heling et al. 2008; Richter et al. 2009b, as cited in Nilsson 2011).

The extrapolated POB hemoglobin level for snus was approximately four times higher than the level detected in samples from nonsmokers by Carmella and colleagues (1990). Extrapolated methylated and POB-DNA adduct levels were lower than those actually detected in lung, liver, and leukocytes of nonsmokers.

Based on these results, Nilsson (2011) concluded that “The high background concentrations of methylated and POB-DNA adducts in “unexposed” humans must be ascribed to other sources than tobacco. An external exposure to TSNA that does not appreciably affect the “normal”

background concentrations of critical pro-mutagenic DNA adducts should be considered as "virtually safe", irrespective of the shape of the dose-response relationship." On the other hand, DNA adduct levels calculated for consumption of Sudanese toombak, which contains more than 1000 times higher TSNA concentrations than the reported in 2004 for Swedish snus samples, were in the same range or higher than those detected in human samples, including smokers. Extrapolated POB hemoglobin adduct levels were more than 250 to 2500 times higher than those measured in smokers and snuff users. Similarly, total NNAL levels detected in urine from toombak users was more than 120 and 430 times higher than those from US STP users and smokers, respectively (Carmella et al. 2002). Therefore, Nilsson concluded that, different from Swedish snus and contemporary American cigarettes, "[b]ased on DNA adduct data extrapolated from rodents, exposure to Sudanese "Toombak" can be expected to result in levels of TSNA-induced DNA lesions that are far above those found in "unexposed" individuals, implying a tangible risk for developing cancer, in agreement with the clinical observations [for toombak]."

- Data from one small study measuring POB-DNA adducts levels in oral tissue indicate that levels were approximately nine times higher for snus users than those for non-tobacco users.
- Calculated methylguanine- and POB-DNA adduct levels in whole lung, liver, and leukocytes for snus use were lower than background levels detected in nonsmokers. Based on these results, Nilsson (2011) concluded that TSNA exposure from snus does not significantly contribute to background adduct levels stemming from unknown origin in those tissues.
- Calculated POB-hemoglobin adduct levels for snus use were approximately four times higher than those detected in nonsmokers in one small study.

A III 3.2.1.4 Summary of TSNA Biomarkers

Two studies were identified that measured TSNA biomarkers from both traditional Swedish snus users and smokers. Three studies were available in smokers who switched to new products marketed as snus. Limitations of the extrapolability of data in other types of tobacco products to traditional Swedish snus use should be noted.

<u>TSNA biomarker levels - snus use versus smoking</u>

- While no studies of regular snus users were available, one study in which US STP users were switched to traditional Swedish snus observed an almost 60% lower mean total NNAL level in urine from the snus users compared to baseline levels in urine from smokers investigated in the same study. No data for total NNN measured in urine of regular traditional Swedish snus users was available.
- Data from one small study measuring POB-DNA adducts levels in oral tissue indicate that levels were approximately twice as high in regular snus users as those in smokers. Based on these results and considering the higher risk for developing oral cancer in smokers, Nilsson (2011) concluded that these type of adducts are likely not involved in the development of oral cancer.

- Calculated methylguanine- and POB-DNA adduct levels in whole lung, liver, and leukocytes, as well as calculated POB-hemoglobin adduct levels for snus use were either similar or approximately half of those calculated for smoking.

TSNA biomarker levels - snus use versus not using tobacco products

- No data that directly compared total NNAL or total NNN in urine of regular traditional Swedish snus users or new products marketed as snus with non-tobacco users were available.
- Data from one small study measuring POB-DNA adducts levels in oral tissue indicate that levels were approximately nine times higher for snus users than those for non-tobacco users.
- Calculated methylguanine- and POB-DNA adduct levels in whole lung, liver, and leukocytes for snus use were lower than background levels detected in nonsmokers. Based on these results, Nilsson (2011) concluded that TSNA exposure from snus does not significantly contribute to background adduct levels stemming from unknown origin in these tissues.
- Calculated POB-hemoglobin adduct levels were approximately four times higher for snus than those detected in nonsmokers in one small study.

Changes in TSNA biomarker levels - smokers who switch to using snus

- The limited data from one study on total NNAL levels in urine of US STP users four weeks after switching to traditional Swedish snus in comparison with levels in smokers at baseline suggests that switching from smoking to snus might reduce TSNA biomarker levels, while nicotine biomarker levels in the two groups and compared to baseline remained similar.
- Reductions in urinary total NNAL and total NNN levels (30-50% and 17-50% of baseline smoking levels, respectively) were seen for smokers who switched to new products marketed as snus in two studies for eight days and four weeks; however, in these studies nicotine biomarker levels also decreased significantly (17-25% of baseline smoking levels). On the other hand, in a cross-over study of smokers switching to a new product marketed as snus for five days, where no change in nicotine biomarker levels were seen, the urinary total NNAL levels were not impacted either.

Changes in TSNA biomarker levels - using snus in conjunction with smoking: smokers who switch to dual use

- No studies were available that measured urinary total NNAL levels in smokers who also used traditional Swedish snus (dual use) or switched to dual use.
- Total NNAL and NNN levels in smokers who reduced smoking by at least 50% and in addition used a new product marketed as snus were reduced (70 and 50% of baseline smoking levels).

TSNA biomarker levels - smokers switching to snus versus quitting the use of tobacco products

- No data for smokers who switched to traditional Swedish snus in comparison to those who quit was available.

- Urinary total NNAL levels measured in 5-day cross-over studies for US STP users or smokers who switched to new products marketed as snus were two to three times higher compared to those who quit. At the end of an 8-day study, urinary total NNAL and total NNN levels in smokers who switched to a new product marketed as snus were 1.4 and six times higher, respectively, compared to smokers who quit.

TSNA biomarker levels - smokers switching to snus versus using NRT

- The limited data from one study on total NNAL levels in urine of US STP users who switched to traditional Swedish snus in comparison with those from US STP user and smokers who switched to NRT suggest that switching to snus results in five to seven times higher total NNAL levels than switching to NRT.
- Data from one study observed that smokers interested in cessation who switched to new products marketed as snus had approximately two times higher NNAL levels than those who switched to NRT use. Urinary total NNN levels were not significantly different between those groups.

Table A III-3: Trace-Level Component Biomarker Data as Reported in Studies of Users of Swedish Snus, New Products Marketed as Snus, STPs*, NRT*, and Smokers* from the Literature: TSNAs						
Biomarker	**Unit**	**Nonsmokers/ Non-Users of Tobacco/ Smokers that tried to quit**	**NRT Users**	**Snus/*STP* Users/ Smokers that switched to snus or a new product marketed as snus**	**Dual Use**	**Smokers**
Urinary Total NNAL	pmol/mg creatinine	0.8 [c] 0.042 [f] [0.005] [#, m]	(*2.8* →) 0.2 [a, #,] (2.4 →) 0.3 [a, #] (~0.8 →)~0.15 [b]	(3.2 →) **1.4** [a] (~0.7 →) ~0.3 [b] *2.54* [¬] / *3.29* [∞ d] *3.547* [e] *3.25* [f] *2.9* [g] *[4.7]* [#, m] *2.73* [n]		(2.2 →) 1.5 [a] 2.1 [c] 2.33 [¬] / 2.82 [∞ d] 2.715 [e] 2.6 [f] [1.00] [#, m] 1.53 [n]
	pg/mg creatinine	1.0 [#, m]		*990* [#, m]		210 [m, ⋆, ¥] 217-290 [r, ¬]
	pmol/mL		0.03 [s]	*3.79/ 4.86* [d] *4.2* [k]		2.18/ 2.84 [d] 1.2 [k]
	pg/mL	(~900 →) ~254.3 [h] (~230 →) ~90 [j] 0.93 [#, m] 5.80 [q, ¬]		*(~700 →)* **~600** [⋆, h] *(~750 →) ~800* [⋆, h] (~230 →) ~230 [j] *1260* [#, m]		(~280 →) ~280 [j] 210 [#, m] 165 [q, ¬]
	ng/24 hrs	(683.61 →) 198.25 [l] NA [p]		(752.85 →) 278.25 [⋆, #, l]	(548.35) →) 370.58 [⋆, #, l]	(693.24 →) 599.95 [⋆, l] 439 [p]
Urinary Total NNN	pmol/mg creatinine		(~0.035 →) ~0.015 [b]	(~0.055 →)~0.027 [b] *0.64* [n]		0.18 [n]
	pmol/mL		0.07 [s]			
	ng/24 hrs	(28.2 →) 0.83 [l]		(26.61 →) 4.52 [⋆, #, l]	(18.44 →) *9.39* [⋆, # l]	(18.92 →) 15.22 [⋆, l]
Saliva NNK	ng/g			**ND–13;** **ND–16** [o]		
Saliva NNN	ng/g			**3–74;** **37–140** [o]		
POB DNA Adducts	pmol HPB/mg DNA	2.00 ±2.31 [t]		**17.61 ±7.10** [t]		7.40 ±3.82 [t]

Table A III-3: Trace-Level Component Biomarker Data as Reported in Studies of Users of Swedish Snus, New Products Marketed as Snus, STPs*, NRT*, and Smokers* from the Literature: TSNAs

	adducts/109 TN	600 ±102 [t]		**5280 ±372** [t]		3222 ±120 [t]

* Select studies only (data for smokers and NRT users were taken from available comparative studies or if these were not available, other representative studies that reported biomarkers levels for smokers were used. Where no data was available for users of traditional Swedish snus or new products marketed as snus, data were supplemented with studies on STP users.

Bolded results signify those of traditional Swedish snus users; Italics indicate US STP users. Results in [..] indicates calculated results in a unit not provided in the study.

NA: Not applicable;

ND: Not detected;

TN: Total normal nucleotides

POB: pyridyloxobutyl

HBP: 4-hydroxy-1-(3-pyridyl)-1-butanone

[#] significantly ↓ compared to smokers; *significantly different from controls

[¯] Geometric mean; [°] Arithmetic mean

[a] Hatsukami et al. 2004; STP users (N=41) at baseline and 4 weeks after switching to snus or nicotine patch; smokers (N=38) after switching to Omni cigarettes or nicotine patch: STP→Patch (2.9 tins/week→NA), STP→Snus (3.1→3.7 tins/week), Cigarettes→Patch (22 cigs/d→NA), Cigarettes→Omni (21.7→26.0 cigs/d)

[b] Kotlyar et al. 2011; Smokers at baseline and 4 weeks after switching: 27 Smokers→NRT (23.6 cigs/day→7.4 pieces/day), 51 Smokers→Camel snus (19.7 cigs/day→6.9 pouches/day)

[c] Joseph et al. 2005; mean; consumption [0-5 cigs/day] (N=40), [15-20 cigs/day] (N=99)

[d] Hecht et al. 2007; consumption smokers (26 cigs/day) (N=420); STP users (4.2 tins/week) (N=182)

[e] Hecht et al. 2002 (data for smokers from Hecht et al. 1999); 13 US STP (11 snuff (3.4 tins/week)), 2 chewing tobacco (2 pouches/week) users, 27 smokers (23.7±6.9 cigarettes/day)

[f] Carmella et al. 2003; 55 US STP users, 41 smokers, 18 nonsmokers exposed to environmental tobacco smoke (Consumption data not provided)

[g] Lemmonds et al. 2005; 54 US STP users (6.1 dips/day, 2.8 tins/week)

[h] Gray et al. 2008; Study 2; 19 STP users on day 1 (baseline) and day 5 after switching; approximate average values based on figures: *ad libitum* STP→45 g General snus

[j] Blank & Eissenberg et al. 2010; 21 Smokers on day 1 (baseline) and day 5 after switching; approximate average values based on figures: Cigarettes→Ariva (12.3 tablets/24 hrs), Camel snus (11.7 pouches/24 hours), continued smoking (21.9 cigs/24 hrs)

[k] Carmella et al. 2002; 10 smokers and 10 snuff users. Consumption data not provided in study.

[l] Sarkar et al. 2010; 115 Smokers at baseline (grey) and post-baseline after switching (8-day study): Cigarettes only→*Marlboro Snus* only (N=15) 17.8 cigs/day→3.5 g/day, Continued Cigarette consumption (N=30) 16.7→15.6 cigs/day; Cigarettes only→dual use (N=59): 17.6 cigs/day→8.4 cigs/day + 2.2 g/day; nicotine equivalents are nicotine + 5 metabolites

[m] Naufal et al. 2011; 368 STP users not specified (included snuff and chewing tobacco users); 16,443 non-tobacco users included NRT users, 5,040 smokers (consumption data was not available); unadjusted geometric means; statistical differences presented are based on adjusted regression model results.

[n] Stepanov and Hecht 2005; 11 US STP users, 14 smokers (consumption data not provided).

[o] Österdahl and Sloerrach 1988; 4 snuff dippers; consumption: pouch (2 g for 30 min), loose (individual's own loose snus used for 30 min)

[p] Roethig et al. 2009; 3,585 smokers, 1,077 nonsmokers; weighted mean: number of butts returned/24 hr: 16.0 (0.2)

[q] Goniewicz et al. 2011; Nonsmokers were passive smokers (N=228) exposed to environmental tobacco smoke. Smokers (N=373); Mean cigarettes/day smoked from 3 active smoker groups that were combined: 18.4±8.2, 15.0±8.4, 6.9±7.1

[r] Ashley et al. 2010; 51 smokers (US-New York: 18.3±0.8 cigs/day; US-Minnesota: 19.7±1.3 cigs/day).

[s] Stepanov et al. 2009; 9 smokers who switched to using a nicotine patch for 28 weeks (22 cigs/day (SD=11)).

[t] Heling et al. 2008 / Richter et al. 2009a as cited in Nilsson 2011: Heling et al. 2008 (values reported in pmol HPN/mg DNA): 45 nonsmokers, 33 Swedish snus users, 24 smokers; Nilsson 2011; Richter et al. 2009b (values reported in adducts/10^9 TN): 45 nonsmokers, 33 Swedish snus users, 90 smokers

A III 3.2.2 PAH Biomarkers

Urinary PAH metabolites reflect recent exposure, and the metabolite profile can vary depending on the PAH source, but has also been shown to differ between individuals even at similar exposure within the same workplace (as reviewed in CDC 2009). Summaries of the significance of the available PAH biomarkers are provided below.

No studies that investigated PAH biomarkers in users of traditional Swedish snus were identified. Therefore, available data on PAH biomarkers in users of new products marketed as snus and select data of unspecified US STP users are provided in **Table A III-4**. Study details are discussed below.

A III 3.2.2.1 Urinary Biomarker of B[a]P: 3-Hydroxybenzo[a]pyrene Glucuronide

A major metabolite of B*[a]*P is 3-hydroxybenzo[a]pyrene (3-HOBaP), excreted as its glucuronide in urine (Hecht et al. 2002). Due to B*[a]*P's relatively low concentration in cigarette smoke compared to lower molecular weight PAHs, its metabolites have been reported to be difficult to quantify in smoker's urine. Therefore, there is only very limited data available in tobacco users.

3-Hydroxybenzo[a]pyrene Glucuronide in Urine of Users of a New Product Marketed as Snus

Because no studies that measured biomarkers of B*[a]*P exposure in urine from traditional Swedish snus users were identified, one study by industry researchers from Altria that compared smokers with users of a new products marketed as snus (*Marlboro Snus*) is discussed below (Sarkar et al. 2010). Studies in which Marlboro Snus and other traditional Swedish snus brands were analyzed indicate that B*[a]*P concentrations on a ng/g basis are comparable in both products[4].

Sarkar and colleagues (2010) investigated differences of various biomarkers, including those of B*[a]*P, in urine of users of a new product marketed as snus in comparison with those of smokers and non-tobacco users. These authors quantified 3-HOBaP (pg/24 hrs) in urine of 115 smokers that either stopped using tobacco, switched to *Marlboro Snus,* or reduced smoking to 50% or less and were allowed to use *Marlboro Snus* ad libitum. The urinary levels from these three groups were measured at baseline and at the end of the study period (8 days) and compared to those of smokers that continued to smoke their usual brand. Mean 3-HOBaP levels declined significantly from baseline levels for all groups. It also slightly decreased for the smokers that continued to smoke, since their cigarette consumption was somewhat lowered. More details of the results from this study are presented below.

[4] The B*[a]*P concentration of the *Marlboro Snus* product used in this study was reported to be 0.37 to 0.67 ng/g. Other published analyses have reported concentration in *Marlboro Snus* brands in similar ranges (not detected-2.1 ng/g dry weight (Stepanov et al. 2008); below the quantitation limit (Stepanov et al. 2010)). B*[a]*P concentrations reported in the traditional Swedish snus brands of *General* and *Catch* ranged from not detected (Stepanov et al. 2008) to 0.3 to 1.6 ng/g dry weight (Borgerding et al. 2012). See also Table A II-3a in Appendix 2.

Changes in B[*a*]P biomarker levels - smokers who switch to using a new product marketed as snus

The mean urinary 3-HOBaP level decreased to less than half of baseline for smokers (17.6 cigs/day) who switched completely to *Marlboro Snus* (3.5 pouches/day).

Changes in B[*a*]P biomarker levels - using a new product marketed as snus in conjunction with smoking: smokers who switch to dual use

At study end, smokers (17.6 cigs/day) who became dual users (smoking to ≤50%; 8.4 cigs/day + 2.2 pouches/day) had urinary 3-HOBaP levels reduced to almost half of baseline and this reduction was similar to that observed for the *Marlboro Snus*-only group. Similarly, for both the *Marlboro Snus*-only group and the dual users, the urinary B[*a*]P metabolite level was approximately 50% less than in the group that continued to smoke. Sarkar and colleagues (2010) concluded that after adjusting for residual levels observed in the non-tobacco group, most biomarkers analyzed in this study were reduced by 50% in smokers who reduced the number of cigarettes by 50% and used *Marlboro Snus* freely.

B[*a*]P biomarker levels - smokers switching to a new product marketed as snus versus quitting the use of tobacco products

Smokers who quit showed a decrease in urinary 3-HOBaP levels to approximately $^1/_3$ of baseline. Switching to *Marlboro Snus* resulted in slightly higher levels (the difference in change from baseline was approximately 10%).

A III 3.2.2.2 Urinary Biomarkers of Exposure to Pyrene, Phenanthrene, Fluorene, and Naphthalene

Widely accepted reliable biomarkers for PAH exposure are urinary metabolites of pyrene, naphthalene, and phenanthrene (EFSA 2008). Together with fluorene metabolites these metabolites are measured under the NHANES program and detected in almost all survey participants' urine samples (CDC 2009; Li et al. 2008, as cited in EFSA 2008; Stepanov et al. 2010).

1-Hydroxypyrene (1-HOP), a urinary metabolite of pyrene, is considered to be the most practical and reliable marker for monitoring individual or population exposures to PAHs, since pyrene is commonly found in PAH mixtures (CDC 2009; Hecht et al. 2010; as cited in IARC 2010; Khariwala et al. 2012; Stepanov et al. 2010). 1-HOP is also considered a biomarker of the particulate phase constituents of tobacco smoke and of incomplete combustion products (as reviewed in Hecht et al. 2010). 1-HOP is not tobacco-specific, but smokers have generally approximately two to four times higher urinary levels of this metabolite than nonsmokers (as reviewed in CDC 2009; Hecht et al. 2010). A recent study observed increased urinary 1-HOP levels in smokers with head and neck cancers compared to their matched controls (Khariwala et al. 2012). Depending on environmental or occupational exposure, there can be considerable variation in levels (Khariwala et al. 2012).

1-, 2-, 3-, and 4-hydroxyphenanthrene are urinary metabolites of phenanthrene. Smokers have increased levels of 2-, 3-, and 4-hydroxyphenanthrene (as reviewed in CDC 2009).

Urinary 1- and 2-hydroxynaphthalene (naphthol) are metabolites of naphthalene and typically two to three times higher in smokers than in nonsmokers (as reviewed in CDC 2009).

Urinary 2-, 3-, and 9-hydroxyfluorene are metabolites of fluorene. Levels of 2-hydroxyfluorene or all of these fluorene metabolites have been associated with smoking status in some studies, but results are not consistent (as reviewed in CDC 2009; Li et al. 2008, as cited in EFSA 2008).

Urinary Metabolites of Pyrene, Phenanthrene, Fluorene, and Naphthalene in US STP Users

No studies that measured biomarkers of exposure to these or other PAHs in urine from traditional Swedish snus users were identified. Therefore, one study by industry researchers from Reynolds that compared smokers with users of various unspecified US STPs (including snuff and chewing tobacco) is discussed (Naufal et al. 2011). This study investigated various biomarkers, including those of PAHs, using data from the NHANES 1999-2008 US national survey. Levels of 10 urinary metabolites of pyrene, phenanthrene, fluorene, and naphthalene of 16,443 nontobacco users, 368 STP users (unspecified for the type of smokeless tobacco used), and 5,040 cigarette smokers were compared. Results from this study are presented below.

Because of differences in manufacturing, PAH concentrations in US STPs are generally higher than those of traditional Swedish snus (see Section 2.1 of the main report). Data for traditional Swedish snus, published in the scientific literature, on concentrations of phenanthrene and pyrene and additional data for new products marketed as snus for these components as well as for fluorene, indicates that concentrations in both traditional Swedish snus and new products marketed as snus are considerably lower than those in US moist snuff[5]. One study that analyzed naphthalene concentrations detected similar concentrations in new products marketed as snus ("spit-free tobacco products") and in US moist snuff, but no data was available for concentrations in traditional Swedish snus (see also Appendix II, A II 2.3.6.2).

PAH biomarker levels - US STP use versus smoking

Compared to smokers, urinary levels of 8 of the 10 metabolites measured (1-hydroxypyrene, 2- and 3-hydroxyfluorene, 2-, 3-, and 4-hydroxyphenanthrene, as well as 1- and 2-hydroxynaphthalene) were significantly lower in STP users (Naufal et al. 2011). Urinary levels of 9-hydroxyfluorene were similar in STP users and smokers. Urinary levels of 1-hydroxyphenanthrene were described to be similar to those in smokers as well.

PAH biomarker levels - US STP use versus not using tobacco products

In STP users, urinary levels of 6 of the 10 PAH metabolites measured (1-hydroxypyrene, 2-, 3-, and 9-hydroxyfluorene, and 2- and 3-hydroxyphenanthrene) were significantly higher than in non-consumers (approximately 1.3-1.9 times based on unadjusted geometric means), but significantly lower than in smokers, with the exception of 9-hydroxyfluorene (Naufal et al. 2011).

[5] Data from one study reported concentrations of phenanthrene and pyrene in traditional Swedish snus as 55 and 30 ng/g dry weight versus in US moist snuff as 528-3920 and 323-1060 ng/g dry weight, respectively (Stepanov et al. 2008). Similarly, average concentrations in new products marketed as snus were 41 and 27 ng/g dry weight versus in US moist snuff 4700 and 1290 ng/g dry weight, respectively (Stepanov et al. 2010). In the same study, fluorene was also analyzed and the average concentrations in new products marketed as snus versus US moist snuff were 6 and 827 ng/g dry weight, respectively.

Urinary levels of 1-hydroxyphenanthrene tended to be higher among STP users compared to non-consumers of tobacco, but this difference was not statistically significant.

STP users and non-tobacco consumers had similar urinary levels of both naphthalene metabolites as well as of 4-hydroxyphenanthrene.

Smokers had significantly higher urinary levels of all 10 metabolites of pyrene, phenanthrene, fluorene, and naphthalene than non-consumers of tobacco.

A III 3.2.2.3 Summary of PAH Biomarkers

No studies were available that investigated biomarkers of exposure to PAHs in traditional Swedish snus users. Two studies of smokers switching to *Marlboro Snus* and unspecified US STP users are potentially relevant and are discussed here. Biomarkers analyzed in these studies were urinary metabolites of B*[a]*P, pyrene, phenanthrene, fluorene, and naphthalene. Note that due to the differences in manufacturing methods of traditional Swedish snus and conventional US STPs, data for users of the latter products have only limited applicability to snus users.

PAH biomarker levels - US STP use versus smoking

- In the NHANES data analysis, 8 of 10 PAH metabolites measured were significantly lower in STP users than in smokers (exceptions were 9-hydroxyfluorene and 1-hydroxyphenanthrene).

PAH biomarker levels - US STP use versus not using tobacco products

- Levels of 4-hydroxyphenanthrene and both naphthalene metabolites were similar in STP users to those in non-tobacco users, despite the relatively high concentrations of naphthalene detected in tobacco products (including US moist snuff) as reported in one study.

Changes in PAH biomarker levels - smokers who switch to using a new product marketed as snus

- After switching from smoking to a new product marketed as snus for one week, urinary levels of a metabolite of B*[a]*P decreased to less than half.

Changes in PAH biomarker levels - using a new product marketed as snus in conjunction with smoking: smokers who switch to dual use

- Switching to a new product marketed as snus for one week together with a more than 50% reduction of smoking resulted in a decrease of urinary levels of a metabolite of B[*a*]P to almost half.

PAH biomarker levels - smokers switching to a new product marketed as snus versus quitting the use of tobacco products

- Switching to a new product marketed as snus for one week resulted in slightly less decreased urinary levels of a metabolite of B*[a]*P than quitting for the same period.

In conclusion, in comparison with smoking, any STP use was associated with generally lower urinary levels of PAH metabolites, but the levels were higher than those of non-tobacco users. Note that none of these studies investigated Swedish snus. Limitations of using data derived from these studies are as follows: The study with *Marlboro Snus* had limited follow-up (7-8 days after switching from smoking); the subjects used a small number of pouches per day (3.5 pouches/day vs. reported use of traditional Swedish snus of 13 g (1 g/pouches) or 29 g loose snus (Digard et al. 2009)) and pouches contained less tobacco product than traditional Swedish snus (0.3 g/pouch vs. 0.3-1-pouches incl. novel brands). The study with US STP users likely overestimates PAH biomarkers levels based on data from chemical analyses of PAH concentrations in US STPs and traditional Swedish snus.

Table A III-4: Trace-Level Component Biomarker Data in Studies of Users of New Products Marketed as Snus, STPs*, and Smokers* from the Literature: PAHs

PAH	Biomarker in Urine (μg/g creatinine)	Nonsmokers/ Non-Users of Tobacco	New Products Marketed as Snus or *STP* Users	Smokers
B[*a*]P	3-Hydroxybenzo[a]pyrene	162.70 → 55.93 pg/24 hrs [a]	192.27 → 78.89 pg/24 hrs [a]	193.1 → 155.03* pg/24 hrs [a]
Fluorene	2-Hydroxyfluorene	196.4 (18.3, 212.7)	*301.9* *(237.5, 387.6)* [#, ¥]	962.9 (880.1, 1053.6) *
	3-Hydroxyfluorene	71.5 (66.0, 77.5)	*135.6* *(98.5, 186.8)* [#, ¥]	555.6 (502.7, 614.0) *
	9-Hydroxyfluorene	214.9 (192.5, 237.5)	*387.6* *(235.1, 632.7)* [#]	411.6 (368.7, 464.1) *
Naphthalene	1-hydroxynaphthalene	1636 (1510, 1772)	*1339* *(1012, 1772)* [¥]	7187 (6438, 8022) *
	2-hydroxynaphthalene	1808 (1669, 1959)	*1881* *(1603, 2208)* [¥]	8955 (8184, 9897) *
Phenanthrene	1-hydroxyphenanthrene	129.0 (120.3, 138.4)	*148.4* *(120.3, 181.3)*	190.6 (175.9, 204.4) *
	2-hydroxyphenanthrene	47.0 (42.9, 51.4)	*60.9* *(50.4, 73.7)* [#, ¥]	85.6 (79.0, 92.8) *
	3-hydroxyphenanthrene	83.1 (76.7, 90.0)	*108.9* *(83.1, 142.6)* [#, ¥]	177.7 (160.8, 196.4) *
	4-hydroxyphenanthrene	19.3 (16.9, 21.8)	*19.1* *(10.8, 33.8)* [¥]	39.6 (35.5, 44.7) *
Pyrene	1-hydroxypyrene	43.8 (40.4, 47.5)	*67.4* *(55.7, 81.5)* [#, ¥]	122.78 (111.1, 134.3)*

Notes:

* Select studies only (data for smokers were taken from available comparative studies or if these were not available, other representative studies that reported biomarkers levels for smokers were used. Where no data was available for users of traditional Swedish snus or new products marketed as snus, data were supplemented with studies on STP users.

Data from Naufal et al. 2011, unless otherwise noted; 368 STP users not specified (included snuff and chewing tobacco users); 16,443 non-tobacco users included NRT users, 5,040 smokers (consumption data was not available); unadjusted geometric means; statistical differences presented are based on adjusted regression model results:

* significant difference smokers vs. nonusers,

[#] significant difference STP users vs. nonusers,

[¥] significantly ↓ in STP users vs. smokers or smokers vs. STP users

[a] Sarkar et al. 2010; 115 Smokers at baseline (grey) and post-baseline after switching (8-day study): Cigarettes only→*Marlboro Snus* only (N=15) 17.8 cigs/day→3.5 g/day, Continued Cigarette consumption (N=30) 16.7→15.6 cigs/day; Cigarettes only→dual use (N=59): 17.6 cigs/day→8.4 cigs/day + 2.2 g/day.

A III 3.2.3 Aldehydes Biomarkers

No studies were identified in which biomarkers of exposure to aldehydes were measured in users of snus, new products marketed as snus, or other STPs.

A III 3.2.4 Metals and Metalloids Biomarkers

No studies that analyzed chromium, nickel and barium in blood or urine samples of users of snus, new products marketed as snus, or other STPs were identified.

Data for Swedish snus users in comparison with smokers and nonsmokers was available for cadmium and selenium biomarkers (Ellingsen et al. 2009; Wennberg et al. 2006). In addition, data were available for biomarkers of cadmium, selenium, arsenic, cobalt, lead, and mercury exposure from an analysis of NHANES 1999-2008 US national survey data by researchers from Reynolds (Naufal et al. 2011). Using the NHANES data, blood and urine levels from cigarette smokers, non-tobacco users, and users of various unspecified US STPs (including snuff and chewing tobacco) were compared. Two subsequent analyses of NHANES data from 1999-2006 and 2003-2008 by researchers from the same group provided additional comparative data for arsenic and cadmium levels, respectively (Marano et al. 2012b; Marano et al. 2012a).

Naufal and colleagues (2011) also analyzed urinary beryllium, but noted that more than 40% of samples from all three groups had levels below the detection limit. Therefore, they did not present the results.

Data from all available studies that investigated metal or metalloid biomarkers in users of snus, new products marketed as snus and select data of unspecified US STPs users are provided in **Table A III-5**. Study details are discussed below.

A III 3.2.4.1 Biomarkers of Exposure to Cadmium

Summaries of the significance of cadmium biomarkers are provided in Section 3.2.4.1 of the main report.

Cadmium in Blood and Urine of Snus Users

Two studies from Norway and Sweden are available that reported cadmium levels in blood of snus users, smokers, and nonsmokers (Ellingsen et al. 2009; Wennberg et al. 2006). This data is supplemented with results of comparative analyses of NHANES data for US STP users, smokers, and nonsmokers (Marano et al. 2012a; Naufal et al. 2011; Tellez-Plaza et al. 2012).

Cadmium biomarker levels - snus use versus smoking

In the Norwegian study, mean blood cadmium levels (nmol/L) in 11 snuff users were less than $^1/_5$ of those measured in 38 smokers; on a weight basis tobacco consumption (g/week) was similar in both groups (Ellingsen et al. 2009). In a time-trend study in the population of northern Sweden, the median cadmium concentrations in erythrocytes (µg/L) in 28 male never-smoking snuff users were approximately $^1/_{10}$ of those reported for 123 male and female smokers and half of those reported for 80 ex-smokers (Wennberg et al. 2006).

These results are similar to those of the analysis of the NHANES data from 1999-2008 (Naufal et al. 2011). After adjusting for multiple factors[6] in regression models, STP users (N=360 and 122, respectively) had significantly lower geometric mean blood and urinary cadmium levels (ng/mL, µg/g creatinine) than smokers (N=4830 and 1,574, respectively). These levels were approximately $^1/_3$ or half of those in smokers based on unadjusted geometric means, respectively. In a subsequent analysis of NHANES data from 1999-2006, researchers from the same group presented adjusted[7] data of blood and urinary cadmium levels (Marano et al. 2012a). Geometric mean cadmium levels in blood and urine from STP users (N=272 and 87, respectively) were approximately half of those from smokers (N=3,679 and 1,180, respectively).

In both analyses, these differences were similar to those seen between smokers and controls (Marano et al. 2012a; Naufal et al. 2011), and this was in agreement with results of the NHANES analysis by Tellez-Plaza et al. (2012). In their first analysis, Naufal and colleagues (2011) saw a positive association of urinary cadmium levels with serum cotinine levels for both, smokers and STP users. In their subsequent analysis, however, Marano and colleagues (2012a) saw a statistical relationship between both urinary and blood cadmium with serum cotinine only for smokers, but not for STP users.

Cadmium biomarker levels - snus use versus not using tobacco products

In both the Norwegian and Swedish studies, mean blood cadmium levels or median cadmium concentrations in erythrocytes were similar in 11 snuff users/ 28 male never-smoking snuff users and in 49 non/ 110 never-smoking controls (Ellingsen et al. 2009; Wennberg et al. 2006).

Consistent with the above observations, analyses of NHANES data showed that cadmium levels in blood and urine of STP users (N=272-360 and 87-122, respectively) were similar to those of non-users of tobacco (N=12,454-15811 and 4,110-5,282, respectively) (Marano et al. 2012a; Naufal et al. 2011).

These results suggest that cadmium body burden in snus users is similar to that of nonsmokers, including US STP users.

Summary of cadmium biomarker levels

- Cadmium levels measured in blood and urine from traditional Swedish snus users were lower than in smokers.
- Cadmium levels measured in blood and urine from traditional Swedish snus users were similar to those in non-tobacco users.
- These results are consistent with what has been observed for US STP users.
- In conclusion, the available biomarker data indicate that cadmium intake from snus use does not add a significant cadmium burden above that contributed by diet and other environmental factors.

[6] Gender, race/ ethnicity, age, body mass index, poverty income ratio, survey year, urinary creatinine, and tobacco consumption category

[7] Gender, race/ ethnicity, age, body mass index, poverty income ratio, survey year, urinary creatinine, and tobacco consumption category

A III 3.2.4.2 Biomarkers of Exposure to Selenium

Summaries of the significance of selenium biomarkers are provided in Section 3.2.4.2 of the main report.

Selenium and Glutathione Peroxidase Activity in Blood/Serum of Snus Users

One study analyzed selenium-associated biomarkers in blood/serum of snus users. Ellingsen and colleagues (2009) investigated the impact of tobacco consumption on selenium status and impact on glutathione peroxidase (GPX) and a time trend of selenium status in Norway. This data is supplemented with results of an analysis of NHANES data (Naufal et al. 2011).

Selenium biomarker levels - snus use versus smoking

Mean selenium levels in blood and serum from 11 Norwegian snuff users from a former chlor-alkali worker cohort were significantly higher than in smokers (Ellingsen et al. 2009). For smokers these levels were correlated with cotinine serum levels. The mean GPX activity in serum of the snuff users was similar to those in smokers, but only in smokers it was significantly lower than in non-tobacco users.

In the analysis of the US population data by Naufal and colleagues (2011) STP users had slightly higher selenium serum levels than smokers based on unadjusted geometric means, but this difference was described as not statistically significant in the adjusted regression model analysis.

Selenium biomarker levels - snus use versus not using tobacco products

Mean blood and serum selenium levels in the Norwegian snuff users were similar to those of 49 non-users of tobacco (Ellingsen et al. 2009). The mean GPX activity in serum of the snuff users was slightly but not statistically significantly lower than in non-users of tobacco.

The selenium levels among non-tobacco users in this Norwegian study were similar to those reported for the US population in 1988-1998 (ATSDR 2003). Further, the unadjusted geometric mean of selenium serum levels in non-users of tobacco as reported in an analysis of the NHANES data from 1999-2008 (Naufal et al. 2011) was in the same range, although slightly higher. Similar to the Norwegian study, the analysis of the US population data by Naufal and colleagues (2011) did not yield any significant differences between non-tobacco users and STP users.

Summary of selenium biomarker levels

- Selenium levels measured in blood and serum from traditional Swedish snus users were higher than in smokers.
- Selenium levels measured in blood and serum from traditional Swedish snus users were similar to those in non-tobacco users.
- These results are consistent with what has been observed for US STP users.
- In conclusion, the available biomarker data indicate that selenium intake from traditional Swedish snus use does not add a significant selenium burden above that

contributed by diet and other environmental factors and also does not significantly deplete selenium levels, unlike to what is seen for smoking.

A III 3.2.4.3 Biomarkers of Exposure to Arsenic

Arsenic is rapidly cleared from the blood, and urinary arsenic levels are generally accepted to be reflective of recent exposures and moderately to highly correlated with intakes from drinking water and dietary sources (ATSDR 2007a; CDC 2009; Pappas 2011).

Inorganic arsenic metabolite concentrations in urine generally range from 5 to 20 μg/L (WHO 2001, as cited in IARC 2012). Smoking was not correlated with urinary arsenic content (Gebel et al. 1998a, as cited in IARC 2012).

Arsenic in Urine of US STP Users

Because no studies investigating arsenic levels in urine of Swedish snus users were identified, two available analyses of NHANES US population data that compared arsenic levels in urine (μg/g creatinine) from users of various unspecified US STPs (including snuff and chewing tobacco) with those from smokers and non-tobacco users are discussed below (Marano et al. 2012b; Naufal et al. 2011).

In addition to data for total arsenic presented in the analysis from NHANES 1999-2008 US national surveys, the analysis of NHANES data from 2003-2008, also provided adjusted[8] dimethylarsinic acid, and arsenobetaine (Marano et al. 2012b). Other arsenic species measured in urine under the NHANES program during those years were below the limit of detection in more than 40% of samples from all three groups and were therefore not further used in the analysis by Marano and colleagues (2012b).

The analytical data available for arsenic concentrations in Swedish snus products indicates that they are generally in the range of those detected in US STPs (including moist snuffs and chewing tobacco)[9] (See Appendix II Section 2.3.6.4).

Arsenic biomarker levels - US STP use versus smoking

The analysis of NHANES data from 1999-2008 indicated that after adjusting for multiple factors[10] in regression models, the geometric mean total arsenic levels in urine (μg/mg creatinine) from STP user (N=87) and smokers (N=958) were not significantly different (Naufal et al. 2011). There was a slight negative association of arsenic levels with cotinine levels for smokers, but for STP users no correlation was detected. Marano and colleagues (2012b) concluded that "this provides additional evidence of no relationship between arsenic and (frequency or intensity of) tobacco consumption".

In the subsequent analysis of NHANES data from 2003-2008, adjusted geometric mean total arsenic, dimethylarsinic acid, and arsenobetaine levels were also similar in urine of STP users

[8] Gender, race/ ethnicity, age, body mass index, poverty income ratio, survey year, urinary creatinine, and tobacco consumption category

[9] on a per gram dry or wet weight basis

[10] Gender, race/ ethnicity, age, body mass index, poverty income ratio, survey year, urinary creatinine, and tobacco consumption category

(N=90) and smokers (N=991) (Marano et al. 2012b). These authors stated that levels measured were lowest for the STP users.

Arsenic biomarker levels - US STP use versus not using tobacco products

In the analysis of NHANES data from 1999-2008 after adjusting for multiple factors[11] in regression models, arsenic levels in urine from STP users (N=87) were significantly lower than in non-tobacco consumers (N=3263) (Naufal et al. 2011). Consistent with previous studies, the geometric mean level in smokers were not significantly different from those in non-users of tobacco, although the unadjusted geometric mean was slightly lower.

In their subsequent analysis of NHANES data from 2003-2008, the authors also concluded that adjusted geometric mean urinary total arsenic, dimethylarsinic acid, and arsenobetaine levels were "similar among the three consumer groups, although consistently highest in non-consumers of tobacco [(N=3,385)] and lowest in SLT consumers [STP users; (N=90)]" (Marano et al. 2012b).

Summary of arsenic biomarker levels

- Arsenic levels measured in urine from US STP users and smokers were similar.
- Arsenic levels measured in urine from US STP users were similar to or lower than in non-tobacco users.
- In conclusion, the available biomarker data for US STP users suggest that arsenic intake from use of these products does not add a significant arsenic burden above that contributed by diet and other environmental factors. This is likely also the case for use of traditional Swedish snus; the published analytical data for arsenic concentrations in traditional Swedish snus shows that these are generally in the same range as those reported for US STPs, such as moist snuff and chewing tobacco. Limitations of this extrapolation include possible differences in product use behavior.

A III 3.2.4.4 Biomarkers of Exposure to Cobalt

Urinary cobalt levels reflect recent exposure and decline within 24 hours after exposure ends (CDC 2009). Based on NHANES data from 2007-2008, the geometric mean cobalt urinary levels in the US population 20 years and older was 0.343 μg/L (0.366 μg/g creatinine). Corrected for creatinine, levels in females were higher than in males (CDC 2009).

Cobalt in Urine of US STP Users

Because no studies investigating cobalt levels in urine of snus users were identified, available data from an analysis of NHANES data from 1999-2008 are discussed below (Naufal et al. 2011). No published analytical data for cobalt concentrations in traditional Swedish snus were identified (See Appendix II Section 2.3.6.4).

[11] Gender, race/ ethnicity, age, body mass index, poverty income ratio, survey year, urinary creatinine, and tobacco consumption category

Cobalt biomarker levels - US STP use versus smoking

Naufal and colleagues (2011) did not detect significant differences between cobalt levels in urine from US STP users and smokers after adjusting for multiple factors[12] in regression models. These authors did, however, report a positive correlation of urinary cobalt levels with serum cotinine levels for both US STP users and smokers.

Cobalt biomarker levels - US STP use versus not using tobacco products

In their analysis, Naufal and colleagues (2011) did not detect significant differences between cobalt levels in urine from US STP users and non-tobacco users.

Summary of cobalt biomarker levels

- The limited biomarker data reported for US STP users indicate that cobalt intake from these STPs does not result in a significant cobalt burden above that from diet and other environmental factors. Use of traditional Swedish snus would not be expected to lead to greater cobalt exposures than those from US STPs; however, no published analytical data for cobalt concentrations in traditional Swedish snus alone or in comparison with other STPs were available that would allow to confirm this extrapolation. Other limitations include possible differences in product use behavior.

A III 3.2.4.5 Biomarkers of Exposure to Lead

Blood lead levels reflect both recent exposure and equilibration with lead stored in tissues. Absorbed lead is bound to erythrocytes and then distributed to soft tissues and bone. Urinary lead levels reflect recent exposure, but show greater individual variation and potential for contamination (CDC 2009).

Based on NHANES data from 2007-2008, the geometric mean blood and urinary lead levels in the US-population 20 years and older were 1.38 μg/dL (13.8 μg/L) and 0.512 μg/L (0.545 μg/g creatinine) (CDC 2009). These levels have declined consistently with time since 1999. Blood lead levels in males were higher than in females (CDC 2009). Blood lead levels also increased with exposure to tobacco smoke (Bonanno et al. 2001, as cited in ATSDR 2007b).

Lead in Blood and Urine of US STP Users

Because no studies investigating lead levels in blood or urine of snus users were identified, available data from an analysis of NHANES data from 1999-2008 are discussed below (Naufal et al. 2011).

The newer analytical data available for lead concentrations in traditional Swedish snus products indicate that they are on the lower end of the range of concentrations detected in US STPs (including moist snuffs and chewing tobacco)[13] (See Appendix II, Section A II 2.3.6.4).

[12] Gender, race/ ethnicity, age, body mass index, poverty income ratio, survey year, urinary creatinine, and tobacco consumption category

[13] on a per gram wet and dry weight basis

Lead biomarker levels - US STP use versus smoking

Based on the unadjusted and adjusted data presented in tables and figures by Naufal and colleagues (2011), it appears that blood lead levels were similar in both STP users and smokers, while urinary lead levels were lower in STP users than in smokers. However, in the publication, the authors state that blood lead levels were significantly lower in STP users and urinary lead levels were not different compared to smokers. In either instance, the lead levels were not higher among STP users than among smokers.

Lead biomarker levels - US STP use versus not using tobacco products

In the same population-based analysis, lead levels in blood from US STP users were significantly higher than in non-consumers, whereas lead levels were similar in urine (Naufal et al. 2011). Smokers had significantly higher blood and urinary lead levels than non-consumers and both biomarkers were positively associated with serum cotinine levels.

In a study of the Northern Swedish population, significantly higher erythrocyte lead levels were observed among smokers compared to nonsmokers (Wennberg et al. 2006). The investigators reported a comparison between never-smoking snuff users and non-tobacco users for erythrocyte cadmium concentrations (see Section 3.2.4.1), however, no results that allowed the same comparison with respect to erythrocyte lead concentrations were provided in the study publication.

Summary of lead biomarker levels

- Lead levels measured in blood and urine from US STP users were similar to or lower than in smokers.
- Lead levels measured in blood from US STP users were significantly higher than in non-tobacco users, while lead levels in urine were similar in the two groups.
- In conclusion, the available biomarker data for US STP users suggest that lead intake from US STP use is similar to or lower than that from smoking. The available product chemistry data indicates that lead concentrations in traditional Swedish snus products are on the lower end of the range reported for US STPs, such as moist snuff and chewing tobacco, suggesting that lead intake from snus use is similar to or lower than that from smoking.

A III 3.2.4.6 Biomarkers of Exposure to Mercury

Total blood mercury levels are mostly associated with dietary intake of organic mercury components and increase with fish consumption. Urinary levels mainly consist of inorganic mercury and are associated with, for example, having mercury-containing amalgam tooth fillings (CDC 2009).

Mean total mercury levels in whole blood and urine were reported to be 1 to 8 μg/L and 4 to 5 μg/L , respectively; a mean blood level in persons who do not eat fish was determined to be 2 μg/L (as reviewed in ATSDR 1999).

The geometric mean total blood and urinary mercury levels in the US population 20 years and older, based on NHANES data from 2007-2008, were 0.944 μg/L and 0.477 μg/L (0.507 μg/g

creatinine) (CDC 2009). Creatinine corrected urinary mercury levels were higher in females than in males.

Mercury in Blood and Urine of US STP Users

Because no studies investigating mercury levels in blood or urine from snus users were identified, available data from an analysis of NHANES data from 1999-2008 are discussed below (Naufal et al. 2011).

Very limited analytical data are available for mercury concentrations in snus from one study, where only a few samples of snus, moist snuff, and chewing tobacco were analyzed. This study included products from the US and Sweden but did not specify the particular products measured. The mercury concentrations measured in these products were similar[14] (See also Appendix II, Section A II 2.3.6.4).

Mercury biomarker levels - US STP use versus smoking

In their analysis of NHANES data from 1999-2008, Naufal and colleagues (2011) detected no differences between mercury levels in blood or urine from STP users and smokers.

Mercury biomarker levels - US STP use versus not using tobacco products

Naufal and colleagues' (2011) population-based analysis showed significantly lower mercury levels in blood from STP users and blood and urine from smokers compared with non-users. The authors hypothesized that dietary differences between tobacco users and non-users may account for this difference. In a study in the northern Swedish population, no association of smoking and erythrocyte mercury levels was observed. While this study differentiated non-smoking snuff users and non-tobacco users for cadmium erythrocyte concentrations, no results that allowed the same differentiation for erythrocyte mercury concentrations were provided in the study publication (Wennberg et al. 2006).

Summary of mercury biomarker levels

- Mercury levels measured in blood and urine from US STP users were similar to those in smokers.
- Mercury levels measured in blood from US STP users were significantly lower than in non-tobacco users, while mercury levels in urine were similar in the two groups.
- In conclusion, the available biomarker data for US STP users suggest that mercury intake from these products does not add a significant mercury burden above that contributed by diet and other environmental factors. Similarly, it is likely this is also the case for traditional Swedish snus, when taking into account the very limited analytical data available that indicate that mercury concentrations in traditional Swedish snus are generally similar to those reported for US STPs.

A III 3.2.4.7 Summary of Metals and Metalloids Biomarkers

Data for cadmium and selenium biomarkers of exposure was available for traditional Swedish snus users in comparison with smokers and non-tobacco users. Due to the lack of biomarker

[14] on a per gram wet weight basis

data of other metal/metalloids for snus users, data for US STP users were analyzed. No studies that analyzed chromium, nickel and barium in blood or urine samples from users of any type of STPs were identified.

Metal/metalloid biomarker levels - snus/US STP use versus smoking

- Cadmium levels measured in blood and urine from traditional Swedish snus users were lower than in smokers.
- Selenium levels measured in blood and serum from traditional Swedish snus users were higher than in smokers.
- Arsenic, cobalt, and mercury levels measured in urine and mercury levels measured in blood from US STP users were similar to those in smokers.
- Lead levels measured in blood and urine from US STP users were similar to or lower than in smokers.

Metal/metalloid biomarker levels - snus/US STP use versus not using tobacco products

- Cadmium levels measured in blood and urine and selenium levels measured in blood and serum from traditional Swedish snus users were similar to those in non-tobacco users.
- Arsenic, cobalt, and mercury levels measured in urine from US STP users were similar to or lower than in non-tobacco users. Mercury levels measured in blood from US STP users were significantly lower than in non-tobacco users.
- Lead levels measured in blood from US STP users were significantly higher than in non-tobacco users, while lead levels in urine were similar in the two groups.

In conclusion, the available biomarker data indicate that cadmium and selenium intakes from traditional Swedish snus use do not add a significant burden of these metals/metalloids above that contributed by diet and other environmental factors. The data also suggests that snus use does not significantly deplete selenium levels; this is unlike to what is seen for smoking. Further, the available biomarker data for US STP users suggests that arsenic, cobalt and mercury intakes from these products do not add a significant burden of these metals above that contributed by diet and other environmental factors. Similarly, taking into account the available product chemistry data, it is likely this is also the case for traditional Swedish snus. The available biomarker data for US STP users suggests that lead intake from US STP use is similar to or lower than from smoking. The available product chemistry data indicates that lead concentrations in traditional Swedish snus products are on the lower end of the range reported for US STPs, such as moist snuff and chewing tobacco, suggesting that lead intake from snus use is similar to or lower than from smoking. Limitations of these extrapolations from US STPs to traditional Swedish snus include possible differences in product use behavior.

Table A III-5: Trace-Level Component Biomarker Data in Studies of Users of Swedish Snus, New Products Marketed as Snus, other STPs*, and Smokers* from the Literature: Metals/Metalloids

Biomarker		Matrix	Nonsmokers/ Non-Users of Tobacco	Snus or *STP* Users	Smokers
Arsenic	Total Arsenic	Urine (μg/g creatinine)	9.58 (8.94, 10.28) [a, §] 9.56 (8.92, 10.27) [i, ¬]	*6.17 (5.05, 7.46)* [a, §, #] *6.14 (4.86, 7.74)* [i, ¬]	8.08 (7.17, 9.12) [a, §] 7.98 (7.08, 9.00) [i, ¬]
	Dimethylarsinic acid	Urine (μg/g creatinine)	4.01 (3.82, 4.22) [i, ¬]	*2.68 (2.22, 3.23)* [i, ¬]	3.53 (3.28, 3.80) [i, ¬]
	Arsenobetaine	Urine (μg/g creatinine)	1.96 (1.75, 2.19) [i, ¬]	*1.10 (0.74, 1.62)* [i, ¬]	1.50 (1.26, 1.78) [i, ¬]
Cadmium		Blood (μg/L)	0.4-1 [b] 0.37 (0.10-1.23) [c] 0.30 (0.29, 0.31) [a, §] 0.31 [h, ¬]	**0.33 (LOD-0.83)** [c] *0.28 (0.25, 0.30)* [a, §, ¥] *0.39* [h, ¬]	1.4-4 [b] 1.78 (0.51-4.02) [c] 0.90 (0.87, 0.93) [a, §, *] 0.95 [h, ¬]
		Erythrocyte (μg/L)	0.26 [d]	**0.24** [d]	2.3 (M), 2.5 (F) [d]
		Urine (μg/g creatinine)	0.24 (0.23, 0.25) [a, §] 0.25 [h, ¬] 0.15-0.904 μg/24 hrs [e] 0.19 [f, ¬]	*0.16 (0.13, 0.20)* [a, §, ¥] *0.25* [h, ¬]	0.34 (0.32, 0.37) [a, §, *] 0.44 [h, ¬] 0.26-1.44 μg/24 hrs [e] 0.41 [f, ¬]
Cobalt		Urine (μg/g creatinine)	0.34 (0.33, 0.35) [a, §]	*0.26 (0.24, 0.28)* [a, §]	0.33 (0.31, 0.34) [a, §]
Lead		Blood (μg/L)	18.1 -without ETS, 20.6 - with ETS exposure [g] 13.9 (13.6, 14.3) [a, §]	*19.2 (17.6, 20.9)* [a, §, #, ¥]	28.5 [g] 18.6 (18.0, 19.2) [a, §, *]
		Urine (μg/g creatinine)	0.59 (0.58, 0.61) [a, §]	*0.58 (0.50, 0.68)* [a, §, ¥]	0.72 (0.69, 0.75) [a, §, *]
Mercury		Blood (μg/L)	1.06 (0.99, 1.13) [a, §]	*0.78 (0.69, 0.87)* [a, §, #]	0.81 (0.75, 0.87) [a, §, *]
		Urine (μg/g creatinine)	0.54 (0.52, 0.57) [a, §]	*0.36 (0.30, 0.43)* [a, §, #]	0.42 (0.38, 0.46) [a, §, *]

Table A III-5: Trace-Level Component Biomarker Data in Studies of Users of Swedish Snus, New Products Marketed as Snus, other STPs*, and Smokers* from the Literature: Metals/Metalloids

Selenium	Blood or Serum (μg/L)	120.0 (79.0-181.6) (B), 121.6 (71.1-213.2) (S) [c] 137.0 (134.3, 139.8) (S) [a, §]	**118.4 (102.6-150.0) (B), 122.4 (86.9-165.8) (S)** [c] *137.0 (130.3, 145.5)* (S) [a, §]	109.0 (71.1-165.8) (B), 105.8 (55.3-157.9) (S) [c, #] 130.3 (129.0, 133.0) (S) [a, §, *]
GSH-peroxidase activity	Serum (U/L)	146 (105-203) [c]	**140 (106-182)** [c]	137 (103-201) [c, #]

* Select studies only (data for smokers were taken from available comparative studies or if these were not available, other representative studies that reported biomarkers levels for smokers were used. Where no data was available for users of traditional Swedish snus or new products marketed as snus, data were supplemented with studies on STP users.

Bolded results signify those of Swedish snus users.

LOD: Limit of detection;

[§] Unadjusted geometric mean (95% Confidence Interval);

[¯] Geometric mean;

[a] Naufal et al. 2011; 368 STP users not specified (included snuff and chewing tobacco users); 16,443 non-tobacco users included NRT users, 5,040 smokers (consumption data was not available); unadjusted geometric means; statistical differences presented are based on adjusted regression model results:

[*] significant difference smokers vs. nonusers,

[#] significant difference STP users vs. nonusers,

[¥] significantly ↓ in STP users vs. smokers or smokers vs. STP users

[b] IARC Mono 100C 2012

[c] Ellingsen et al. 2009, cadmium levels were converted from nmol/L to μg/L. 38 smokers (77 g/week), 11 snuff users (75 g/week), 49 non-tobacco users.

[d] Wennberg et al. 2006; 110 nontobacco users (men), 80 ex-smokers (male and female, 123 smokers (male and female), 28 snuff users (men).

[e] as reviewed in IOM 2011 and Hecht et al. 2010

[f] Tellez-Plaza et al. 2012 (NHANES data from 2003-2008); 10,107 never-smokers, 5,128 current smokers.

[g] Bonanno et al. 2001, as cited in ATSDR 2007b

[h] Marano et al. 2012a (adjusted data); Urine: 87 STP users, 1,180 smokers, 4,110 non-tobacco users. Blood: 272 STP users, 3,679 smokers, 12,454 non-tobacco users.

[i] Marano et al. 2012b (adjusted data); 90 STP users, 991 smokers, 3,385 non-tobacco users.

A III 3.2.5 Radionuclides Biomarkers

No studies were identified in which biomarkers of exposure to radionuclides were measured in users of snus, new products marketed as snus, or STPs.

A III 3.2.6 Biomarkers of Other Trace Level Components

No studies were identified in which biomarkers of exposure to ethyl carbamate (urethane), hydrazine, or mycotoxins were measured in users of snus, new products marketed as snus, or other US STPs.

A III 3.2.6.1 Acrylamide Biomarkers

Acrylamide hemoglobin adducts are biomarkers of other trace level components suggested by Hecht and colleagues (2010) (see main report introduction of Chapter 3) and relevant for STP use. Both acrylamide and its metabolite glycidamide can form adducts with hemoglobin, which are measured under the US NHANES program. Adduct levels reported for smokers are considerably higher (3-4 times) than those detected in nonsmokers (CDC 2009).

Data from all available studies that investigated acrylamide biomarkers in users of snus, new products marketed as snus as well as select data reported for unspecified US STPs users are provided in **Table A III-6**.

Acrylamide and Glycidamide Hemoglobin Adducts in US STP Users

Because no studies investigating acrylamide or glycidamide hemoglobin adducts in snus users were identified, available data from an analysis of NHANES data from 1999-2008 are discussed below (Naufal et al. 2011). No published analytical data for acrylamide concentrations in traditional Swedish snus or other STPs were identified.

Acrylamide biomarker levels - US STP use versus smoking

In their analysis of NHANES data from 1999-2008, Naufal and colleagues (2011) reported that both acrylamide and glycidamide adduct levels in STP users were significantly lower than in smokers.

Acrylamide biomarker levels - US STP use versus not using tobacco products

Naufal and colleagues' (2011) population-based analysis showed similar acrylamide and glycidamide adduct levels in STP users and non-users of tobacco.

A III 3.2.6.2 Summary of Biomarkers of Other Trace Level Components

Due to the lack of biomarker data of other trace level components for snus users, data for US STP users was analyzed. Data was available for acrylamide and glycidamide hemoglobin adducts. No data for biomarkers of exposure to ethyl carbamate (urethane), hydrazine, or mycotoxins in blood or urine samples from users of any type of STPs were identified.

Biomarker levels of other trace level components - US STP use versus smoking

- Acrylamide and glycidamide hemoglobin adduct levels in US STP users were significantly lower than in smokers.

Biomarker levels of other trace level components - US STP use versus not using tobacco products

- Acrylamide and glycidamide hemoglobin adduct levels in US STP users were similar to those in non-tobacco users.

In conclusion, the available biomarker data for US STP users suggests that acrylamide intake from these products do not add a significant acrylamide burden above that contributed by diet and other environmental factors. Use of traditional Swedish snus would not be expected to lead to greater acrylamide exposures than those from US STPs; however, no published analytical data for acrylamide concentrations in traditional Swedish snus alone or in comparison with other STPs were available that would allow to confirm this extrapolation. Other limitations include possible differences in product use behavior.

Table A III-6: Trace-Level Component Biomarker Data in Studies of STP* users and Smokers* from the Literature: Acrylamide

Biomarker	Matrix	Nonsmokers/ Non-Users of Tobacco	*STP* Users	Smokers
Acrylamide Adduct	Hemoglobin (pmol/g hemoglobin)	47.9 (46.1, 49.9) [a, §]	*53.5 (40.4, 70.8)* [a, §, ¥]	122.7 (112.2, 134.3) [a, §, *]
Glycidamide Adduct		47.5 (45.2, 49.4) [a, §]	*50.4 (38.9, 65.4)* [a, §, ¥]	101.5 (92.8, 111.1) [a, §, *]

* Select studies only (data for smokers were taken from available comparative studies or if these were not available, other representative studies that reported biomarkers levels for smokers were used. Where no data was available for users of traditional Swedish snus or new products marketed as snus, data were supplemented with studies on STP users.
[§] Unadjusted geometric mean (95% Confidence Interval);
[a] Naufal et al. 2011; 368 STP users not specified (included snuff and chewing tobacco users); 16,443 non-tobacco users included NRT users, 5,040 smokers (consumption data was not available); unadjusted geometric means; statistical differences presented are based on adjusted regression model results:
[*] significant difference smokers vs. nonusers,
[#] significant difference STP users vs. nonusers,
[¥] significantly ↓ in STP users vs. smokers or smokers vs. STP users

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

Reference	Study Objective and Design	Number of Subjects and Products	Results	Comments, Summary & Conclusions
Studies in Snus Users				
Digard et al. 2012	Objective: To determine nicotine absorption for current pouched and loose snus products in comparison with cigarette and an nicotine gum. Randomized, 6-way, crossover study, single administrations with blood samples taken at intervals over 120 min. Each administration was preceded by 12 hrs of abstinence; a least one day gap between product administrations.	Cigarette (*Lucky Strike Red*), Loose snus (*Granit* – 1 g and 2.5 g), Pouched snus (*Lucky Strike* Original, Brown; *Lucky Strike Bold*), Nicotine gum (*Nicorette*) (authors state that the products were typical of cigarette and snus products found in Europe and Scandinavia, respectively) 20 healthy snus who also smoked in Sweden	Nicotine PK parameters in plasma of snus users after 12 h-hrs/overnight abstinence from any nicotine use and subsequent 60 min of snus use, 5 min of smoking, or 30 min of gum use	• The authors noted that nicotine plasma levels from smoking the cigarette rose more rapidly than for the oral products, as expected, however, the total plasma nicotine concentration over the 120 min sampling period was higher for use of all snus products compared to use gum and smoking. • Nicotine pharmacokinetics were similar for the 1-g portions of loose and pouched snus, both containing similar levels of nicotine. • The authors concluded that absorption kinetics were dependent on quantity of tobacco by weight and total nicotine content rather than product form. • No associations between blood pressure, heart rate and nicotine content • No differences in self-sensory perceptions between snus forms • CYP2A6 genotyping classified 12 subjects as extensive metabolizers and eight subjects as intermediate metabolizers. The authors noted that due to the variability of individual exposure levels across test products for all subjects, regardless of metabolic status, the results of the genotyping analysis

Product	Extracted Nicotine (mg/portion)	t_{max} (min)[1]	C_{max}[2] (ng/mL)	$AUC_{(0\text{-}120\ min)}$[2] (ng*hr/mL)
Granit Loose Snus 10.8 mg nicotine/portion (1 g), pH 8.0-8.3	3.45 ±1.42 (32%)	60 (45-90)	10.8 (34.4)	16.0 (31.2)
Granit Loose Snus 27.1 mg/portion (2.5 g), pH 8.0-8.3	6.42 ±2.35 (24%)	60 (45-90)	17.9 (22.8)	26.9 (23.8)
Lucky Strike Original, Brown 10.7 mg nicotine/portion (1 g), pH 8.0-8.2	3.38 ±1.92 (31%)	60 (20-90)	10.8 (41.4)	16.8 (39.6)
Lucky Strike Bold 14.7 mg nicotine/portion (1 g), pH 7.9-8.1	4.53 ±2.09 (31%)	60 (45-90)	13.4 (39)	20.4 (37.6)
Nicorette 4-mg nicotine gum 4.2 mg nicotine/portion	2.62 ±0.36 (63%)	45 (20-90)	9.1 (28.6)	13.1 (28.3)
Lucky Strike Red Cigarette 14.6 mg nicotine/	N/A	7 (5-31)	12.8 (41.3)	14.8 (30.4)

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

<table>
<tr><th>Reference</th><th>Study Objective and Design</th><th>Number of Subjects and Products</th><th>Results</th><th>Comments, Summary & Conclusions</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td>cigarette</td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="5">[1] Median (Range); [2] Geometric mean (GM Coefficient of variation)</td></tr>
</table>
</td><td>were not considered to have any significant impact on the interpretation of the pharmacokinetic data.</td></tr>
<tr><td>Joksic et al. 2011</td><td>Objective: Efficacy of snus to help adult cigarette smokers in Serbia to substantially reduce smoking and eventually completely stop smoking

Investigator-initiated, randomized multicenter, double-blind, parallel-group, placebo-controlled phase 4 clinical trial
Clinical cessation study

Week 1-24: Smoking reduction; week 25-48: complete smoking cessation</td><td>Swedish snus
Placebo

319 healthy smokers in Serbia</td><td>
<table>
<tr><th colspan="4">Smokers of average 27 cig/d that reduced/quit smoking by switching to either snus or placebo</th></tr>
<tr><th>Biomarker</th><th>Week</th><th>Placebo</th><th>Snus</th></tr>
<tr><td rowspan="5">CO in exhaled air (ppm)[§]</td><td>Baseline</td><td>23.5</td><td>23.5</td></tr>
<tr><td>12</td><td>20.2</td><td>20.0</td></tr>
<tr><td>24</td><td>15.8</td><td>16.7</td></tr>
<tr><td>36</td><td>13.2</td><td>13.0</td></tr>
<tr><td>48</td><td>12.1</td><td>11.5</td></tr>
<tr><td rowspan="5">Cotinine in serum (ng/mL)[§]</td><td>Baseline</td><td>101.2</td><td>98.1</td></tr>
<tr><td>12</td><td>70.6</td><td>70.9</td></tr>
<tr><td>24</td><td>71.7</td><td>68.7</td></tr>
<tr><td>36</td><td>69.3</td><td>62.9</td></tr>
<tr><td>48</td><td>69.1</td><td>66.1</td></tr>
<tr><td colspan="4">[§] Mean concentrations</td></tr>
</table>
Cigarette consumption decreased to <10 cigs/d</td><td>• Self-reported cigarette consumption, CO in exhaled air and serum cotinine ↓ similarly over time in snus users and placebo users, but were slightly ↓ in snus users compared to placebo users in week 36 and 48
• The authors noted: “the proportion that reported more extreme reductions (≥ 75%) was statistically significantly higher in the snus group than in the placebo group ($p < 0.01$).”
• They concluded: “The results for biologically verified complete cessation suggested that participants in the snus group were more likely to quit smoking completely than the controls.”</td></tr>
<tr><td>Lunell and Curvall 2011</td><td>Objective: Comparison of extraction and nicotine plasma concentrations and PK parameters from snus and nicotine gum

Open-label, single-dose 3-way crossover study;
12 hr abstinence overnight, drinking coffee or</td><td>Swedish snus (General Onyx, General White Large), nicotine gum

14 smokers, randomly assigned</td><td>
<table>
<tr><th colspan="5">Nicotine PK parameters in plasma of smokers after 12 hrs of abstinence from smoking and subsequent product use for 30 min</th></tr>
<tr><th>Product</th><th>Extracted nicotine/ portion (mg)</th><th>t_{max} (min)</th><th>C_{max} (ng/mL)</th><th>AUC_{inf} (ng*min/mL)</th></tr>
<tr><td>General Onyx 9.92 mg[§] nicotine/portion (1 g), pH</td><td>2.12 ±0.93 (21%)</td><td>37.1 ±10.2 (24 - 60)</td><td>14.8 ±3.3</td><td>3,062 ±1,002</td></tr>
</table>
</td><td>• Use of 1 g portions of Swedish snus produced ↑ nicotine C_{max} in shorter time (↓ t_{max}) and with a quicker onset of “head rush” compared with 4 mg nicotine gum.
• The authors noted that at 8 min after start of administration, mean nicotine plasma concentration was >7 ng/mL for snus use, but only 5 ng/mL for gum use (potential influence on head rush and</td></tr>
</table>

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

Reference	Study Objective and Design	Number of Subjects and Products	Results	Comments, Summary & Conclusions
	carbonated beverages were not allowed; single oral administration on 3 separate occasions, washout periods at least 6 days; blood sampling up to 8 hrs after administration		8.7 |				 *General White Large* 8.65 mg/portion (1 g), pH 8.7 | 2.18 ±0.92 (25%) | 37.1 ±10.2 (24 - 60) | 13.7 ±3.7 | 2,829 ±1,037 Nicotine Polacrilex gum (4 mg) 3.8 mg nicotine /portion, buffered to an alkaline pH | 2.56 ±0.29 (67%) | 46.1 ±16.2 (30 - 90) | 12.8 ±2.96 | 3,190 ±1,310 § Mean ± standard deviation Baseline CO exhaled ≤11 ppm, plasma nicotine concentration <4 ng/mL	withdrawal reduction). The corresponding nicotine plasma concentration after smoking 1 cigarette is 5-14 ng/mL. • Amount of nicotine extracted and AUC for nicotine gum use was ↑ compared to snus use • Authors noted that mean extracted nicotine from snus was ↓ compared to Lunell & Lunell 2005, and hypothesized that this might be due to un-experienced snus-users in the present study compared to experienced snus users in previous study. • The authors noted that snus has an addiction potential. • For details on clinical results see Appendix V: J2
Ellingsen et al. 2009	Objective: Impact of tobacco consumption on selenium status and impact on seleno proteins, such as GSH-peroxidase First morning urine and blood collected on same day Multiple linear regression analysis on dependent variables GSH-peroxidase activity ~ Selenium concentrations (B/S)	Snuff (Norway) former chloralkali workers: 38 smokers & 11 snuff users; 49 Controls	Exposure Parameter | Controls | Snuff | Smokers Tobacco Consumption (g/week) | 0 | 75 (2-200) [10.7 g/d] | 77 (5-150) [11 g/d] + 2.6 (0-50) [0.4 g/d] snuff use Urine Nicotine (µg/mmol creatinine) § | 0.4 | 29 | 41 Cotinine (µg/mmol creatinine) | 1.2 | 159 | 161 Blood (B) or Serum (S) Cotinine (S) (µg/L) § | < DL | 137 | 110	• Cd and Se concentrations similar in controls and snuff users, but significantly ↑ in smokers • Biomarkers of nicotine similar in snuff users and smokers (urinary nicotine slightly ↓ in snuff users compared with smokers, serum cotinine slightly ↑ in snuff users compared to smokers) • GSH peroxidase activity not significantly associated with nicotine-related biomarkers • Authors hypothesized that route of administration of tobacco or

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

<table>
<tr><th>Reference</th><th>Study Objective and Design</th><th>Number of Subjects and Products</th><th>Results</th><th>Comments, Summary & Conclusions</th></tr>
<tr><td></td><td>with independent variables: alcohol consumption, fish meals per week, medication, age, chloralkali plant exposure, smoking</td><td></td><td>
<table>
<tr><td>GSH-peroxidase (S) (U/L)</td><td>146</td><td>140</td><td>137</td></tr>
<tr><td>Selenium (B,S) (µmol/L)</td><td>1.52 (B)
1.54 (S)</td><td>1.50 (B)
1.55 (S)</td><td>1.38 (B)
1.34 (S)</td></tr>
<tr><td>Cadmium (B) (nmol/L)</td><td>3.3</td><td>2.9</td><td>15.8</td></tr>
<tr><td colspan="4">§Geometric means</td></tr>
</table>
Multiple linear regression analysis :
Se (B) ~ fish consumption, Se (B,S) ~ smoking
Se (B) ~ cotinine (S) concentrations in smokers

• Mean GSH peroxidase (S) activity significantly ↓ in smokers versus controls
Average Se (S) concentrations in selected population in Norway ↓ from 1995-2006</td><td>inflammatory processes induced by smoking may influence impact on Se concentrations</td></tr>
<tr><td>Gray et al. 2008</td><td>Objective: Withdrawal suppression and toxicant exposure associated with PREP use in SLT users

Study 1:
4x 4-hr conditions (4x 30 min use of product + 30 min rest period) separated by 48 hrs: STP users abstinent for 10 hrs received catheter followed by 30 min rest, blood sampling, 30 min ad libitum use of 2 g of product (or 1 Stonewall tablet), followed by blood sampling immediately after removal</td><td>General snus Loose, Own brand of STP (Skoal, Copenhagen, Kodiak, Hawken), Stonewall, Bacc-off (Placebo)

Study 1: 13 regular STP users ≥12 months

Study 2: 19 regular STP users</td><td>
<table>
<tr><th rowspan="2">Biomarker</th><th colspan="3">STP users switched to</th><th rowspan="2">STP Own brand</th></tr>
<tr><th>Placebo/ No STP</th><th>Stone-wall</th><th>General Snus</th></tr>
<tr><td colspan="5">Study 1: Plasma immediately after 30 min use of 2 g product or 1 Stonewall tablet (right before ~2-2.6 ng/mL after 10 hrs abstinence)</td></tr>
<tr><td>Nicotine (ng/mL)§</td><td>~2</td><td>~2</td><td>8.7</td><td>16.1</td></tr>
<tr><td colspan="5">Study 2: Urine on day 1 → day 5 of Switch
STPs ad libitum (day 1-4 each 45 g STP or 20 Stonewall tablets available for next 24 hours)</td></tr>
<tr><td>Cotinine (ng/mL)</td><td>~1100 → 143</td><td>~1020 → 628</td><td>~1000 → ~1000</td><td>~1100 → ~1100</td></tr>
<tr><td>NNAL Total (NNAL + NNAL-gluc)</td><td>~900 → 254.3</td><td>~850 → 426</td><td>~700 →</td><td>~750 →</td></tr>
</table>
</td><td>Study 1:
• Plasma nicotine concentration in General snus users (n.s.) ↓ compared to users of own brand of STP

Study 2:
• urinary cotinine concentration in General snus users slightly ↓ compared to users of own brand of STP
• Urinary total NNAL of General snus users significantly (on day 3, but not day 5) ↓ compared to users of own brand STP

• The authors concluded that abstinence symptoms/ withdrawal</td></tr>
</table>

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

Reference	Study Objective and Design	Number of Subjects and Products	Results	Comments, Summary & Conclusions
	of STP Study 2: 4x 5-day use of product (up to 45 g for 24 hrs or 20 *Stonewall* tablets provided on day 1-4) separated by weekend use of own brand STP, Urine sampling on days 1,3,5 Both: visual analogue scales (VAS) – withdrawal & direct effects; questionnaire of smoking urges (QSU); heart rate & blood pressure (not reported)		(pg/mL) | | | ~600 | ~800 § Mean concentrations Study 1: • Craving a dip and QSU factor 1 ↓ after using of all products Study 2: • Anxiousness on the withdrawal scale was rated to be significantly ↓ for all products compared to no STP use • Tobacco strength was rated to be significantly ↓ for *General* snus compared to own brand of STP, but slightly higher than for *Stonewall*	acuity generally did not differ across tobacco conditions
Heling et al. 2008 / Richter et al. 2009b as cited in Nilsson 2011	Objective: To assess the potential relationship between Pyridyloxobutyl (POB) DNA adducts releasing 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) and tobacco use. POB DNA adducts were determined in buccal cells from nonsmokers, smokers and Swedish snus users. Tobacco exposure was determined by questionnaire, cotinine in	Swedish snus Heling et al. 2008: 45 nonsmokers, 33 Swedish snus users, 24 smokers Nilsson 2011; Richter et al. 2009b: 45 nonsmokers, 33 Swedish snus users, 90 smokers	POB DNA adduct levels in oral mucosa Units reported | Nonsmokers | Swedish snus users | Smokers pmol HPB/mg DNA§ | 2.00 ±2.31 | 17.61 ±7.10 | 7.40 ±3.82 adducts/10^9 TN* | 600 ±102 | 5280 ±372 | 3222 ±120 § Values cited in Heling et al. (2008) * Values cited in Nilsson (2011) referring to Richter et al. 2009b	• The authors noted that the levels were lowest in nonsmokers, four-fold higher in smokers, and nine-fold higher in snus users. • The authors also noted that "higher adduct levels in snuff dippers may be explained by prolonged exposure to the mucosa to NNN and NNK. However, they do not correspond to the inherent risk of oral cancer which is considerably lower in Swedish snus users than in smokers."

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

<table>
<tr><th>Reference</th><th>Study Objective and Design</th><th>Number of Subjects and Products</th><th>Results</th><th>Comments, Summary & Conclusions</th></tr>
<tr><td></td><td>saliva, and nicotine in toenails.</td><td></td><td></td><td></td></tr>
<tr><td>Wennberg et al. 2006</td><td>Objective: to assess time trends for the three toxic elements cadmium (Cd), mercury (Hg), and lead (Pb), and to investigate the trends according to lifestyle factors, including tobacco use.

Concentrations in erythrocytes (Ery) were determined after at least four hours of fasting.</td><td>Snuff (Sweden)

600 Swedish men and women from a subsample of the population-based MONICA surveys from 1990, 1994, and 1999. N=28 snuff users (men), 110 non-tobacco users (men), 80 ex-smokers (male and female), 123 smokers (male and female)</td><td>
<table>
<tr><th rowspan="2">Biomarker</th><th colspan="2">Never-Smokers</th><th rowspan="2">Ex-smokers</th><th rowspan="2">Current smokers</th></tr>
<tr><th>No tobacco</th><th>Snuff users (M)</th></tr>
<tr><td colspan="5">Erythrocyte Concentration (µg/L)</td></tr>
<tr><td rowspan="2">Ery-Cadmium§</td><td>0.26</td><td>0.24</td><td rowspan="2">0.47 (M), 0.49 (F)</td><td rowspan="2">2.3 (M), 2.5 (F)</td></tr>
<tr><td colspan="2">0.25 (M), 0.43 (F)</td></tr>
<tr><td>Ery Lead</td><td colspan="2">57 (M), 44 (F))</td><td>67 (M), 37 (F)</td><td>75 (M), 52(F)</td></tr>
<tr><td>Ery-Mercury</td><td colspan="2">2.5 (M), 2.8 (F)</td><td>3.6 (M), 2.6 (F)</td><td>2.7 (M), 3.0 (F)</td></tr>
<tr><td colspan="5">M: males, F: females
§ Median concentrations</td></tr>
</table>
</td><td>• Use of moist snuff had no influence on Ery-Cd among never-smoking snuff-using men.
• Smoking was associated with an increase of Ery-Cd, and there was a positive correlation between number of cigarettes per day and Ery-Cd in daily smokers.
• Smokers had higher Ery-Pb levels than ex-smokers and never-smokers, and there was a significant correlation between Ery-Pb and number of cigarettes smoked per day in daily smokers.
• Ery-Hg decreased in both men and women over the time period and there were no differences by sex or smoking.</td></tr>
<tr><td>Lunell and Lunell 2005</td><td>Objective: Comparison of plasma nicotine levels and relative bioavailability of snus and nicotine gum; in vivo extraction of NaCl from snus

Crossover study – five 12-hr sessions.
Snus users were given 12 hourly repeated doses of 4 different types of snus with 5 days between each session. blood sampling up</td><td>General, Catch licorice, Catch mini, Catch dry mini, Nicorette 2mg-nicotine gum

12 snus users</td><td>
<table>
<tr><th colspan="5">Nicotine PK parameters in plasma of snus users after overnight abstinence from any nicotine use and subsequent 12 hrs of snus</th></tr>
<tr><th>Product</th><th>Extracted nicotine/ portion (mg)</th><th>Median t_{max} (min)§</th><th>C_{max} (ng/mL)</th><th>$AUC_{(0\text{-}12\ hrs)}$ (ng*hr/mL)</th></tr>
<tr><td>General 8.84 mg nicotine/portion (1 g), pH 8.4</td><td>2.74 ± 0.18</td><td>30</td><td>29 ±8.53</td><td>26.16 ±3.36</td></tr>
<tr><td>Catch licorice 7.04 mg/portion (1 g), pH 8.5</td><td>1.55 ± 0.18</td><td>30</td><td>23.79 ±8.6</td><td>21.57 ±8.82</td></tr>
<tr><td>Catch mini</td><td>2 ± 0.11</td><td>30</td><td>20.95</td><td>19.02</td></tr>
</table>
</td><td>• Extracted nicotine from snus portions was ~2->3x ↑ than from 2-mg nicotine gum and from Catch Dry Mini.
• The AUC was ~2-2.7x ↑ after snus use compared to 2-mg nicotine gum and Catch Dry Mini Use.
• The nicotine C_{max} from snus use was ~2-2.7x ↑ compared to that from 2-mg nicotine gum and Catch Dry Mini use.
• Approximate bioavailabilities were calculated to be 40% for General, Catch Mini, and Catch Dry Mini,</td></tr>
</table>

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

<table>
<tr><th>Reference</th><th>Study Objective and Design</th><th>Number of Subjects and Products</th><th>Results</th><th>Comments, Summary & Conclusions</th></tr>
<tr><td></td><td>to 12 hrs after first administration</td><td></td><td>
<table>
<tr><td>4.53 mg nicotine/portion (0.5 g), pH 8.4</td><td></td><td></td><td>±6.9</td><td>±6.69</td></tr>
<tr><td>Catch dry mini 4.82 mg nicotine/portion (0.3 g), pH 7.3</td><td>1.08 ± 0.12</td><td>30</td><td>10.85 ±5.65</td><td>9.81 ±5.12</td></tr>
<tr><td>Nicorette 2-mg nicotine gum 1.91 mg nicotine/portion</td><td>0.84 ± 0.34</td><td>30</td><td>12.75 ±4.67</td><td>11.55 ±4.52</td></tr>
<tr><td colspan="5">§ in last dosing interval</td></tr>
</table>
Nicotine extraction rate was 31, 22, 44, and 22% for the 4 brands of snus, respectively, compared with 44% from Nicorette gum.
</td><td>60% for Catch Licorice, based on reported bioavailability from Nicorette gum and AUC/average extracted dose
The authors noted that a 1-g portion/hr of General produced steady-state nicotine blood levels similar to smoking 25-40 cigs/day, a 1-g portion/day Catch Licorice and a 0.5-g portion/day Catch Mini ≈ 15-20 cigs/day, a 0.3-g portion/hr Catch Dry Mini ≈ 7-10 cigs/day.
For results of in vivo NaCl extraction see Section 2.2.2</td></tr>
<tr><td>Post et al. 2005</td><td>Objective: To validate self-reports of cigarette and snus use in a prospective cohort of adolescents.

Cross-sectional analysis of a cohort sub-sample. Saliva cotinine measured within a median of 2 days of questionnaire to assess questionnaire reliability.</td><td>Snus (Sweden)

520 adolescents (28 snus users, 69 smokers, 16 dual users) in the final grade of junior high school (median age 15.0 years) (BROMS cohort).</td><td>
<table>
<tr><th colspan="4">Median saliva cotinine concentrations according to tobacco group</th></tr>
<tr><th>Exposure Parameter</th><th>Snus</th><th>Dual Users</th><th>Smokers</th></tr>
<tr><td>Tobacco Consumption</td><td>31 pinches/week</td><td>66 cigs or pinches/week</td><td>47 cigs/week</td></tr>
<tr><td>Saliva cotinine (ng/ml)</td><td>~80</td><td>~135</td><td>~20</td></tr>
</table>
</td><td>The authors concluded that there was a high overall correspondence between self-reported tobacco use and use assessed by saliva cotinine. The correlation between the biomarker and the reported patterns of use was very good.
The sensitivity and specificity of the questionnaire compared to the saliva cotinine test were 90% and 93%, respectively, and the overall concordance between the two measures was 93%.</td></tr>
<tr><td>Hatsukami et al. 2004</td><td>Objective: Comparison of toxin exposure from reduced exposure products and medicinal nicotine (patch)</td><td>General snus, Omni Light cigarettes, 21-mg Nicoderm patch

41 US STP users</td><td>
<table>
<tr><th rowspan="2">Exposure Parameter</th><th colspan="2">STP users switched to</th><th colspan="2">Smokers switched to</th></tr>
<tr><th>Patch</th><th>Snus</th><th>Patch</th><th>Omni</th></tr>
<tr><td>Tobacco Consumption</td><td>2.9 tins/week →</td><td>3.1 → 3.7</td><td>22 cigs/d → N/A</td><td>21.7 cigs/d →</td></tr>
</table>
</td><td>Switching to snus for 4 weeks from ad libitum use of widely used brands of STPs in the US resulted in
significant ↓ ~50% in concentrations of urinary total NNAL relative to baseline</td></tr>
</table>

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

<table>
<tr><th>Reference</th><th>Study Objective and Design</th><th>Number of Subjects and Products</th><th>Results</th><th>Comments, Summary & Conclusions</th></tr>
<tr>
<td></td>
<td>After 2 weeks of usual product use, US STP users switched to snus or nicotine patch, smokers switched to Omni cigarettes or nicotine patch for 4 wks.

Urine collected at baseline visits & week 2 & 4</td>
<td>(19 snus, 22 patch) and 38 smokers (22 Omni, 16 patch)</td>
<td>
<table>
<tr><td></td><td>N/A</td><td>tins/week (~12.7 g/d)</td><td></td><td>26.0 cigs/d</td></tr>
<tr><td colspan="5">Urine (at baseline → at week 4)</td></tr>
<tr><td>Total Cotinine (cotinine + glucs) (ng/mL) §</td><td>5759 → 3204</td><td>6193 → 5926</td><td>6364 → 3437</td><td>4412 → 4450</td></tr>
<tr><td>Total NNAL (NNAL -glucs) (pmol/mg creatinine)</td><td>2.8 → 0.2</td><td>3.2 → 1.4</td><td>2.4 → 0.3 #</td><td>2.2 → 1.5</td></tr>
<tr><td>1-OH-Pyr (pmol/mg creatinine)</td><td>Not measured</td><td>Not measured</td><td>1.5 → 0.5 #</td><td>1.4 → 1.1</td></tr>
<tr><td colspan="5">Exhaled air (at baseline → at week 4)</td></tr>
<tr><td>CO (ppm)</td><td>Not measured</td><td>Not measured</td><td>23.5 → 2.0 #</td><td>24.3 → 22.9</td></tr>
<tr><td colspan="5"># anatabine concentration <1 ng/mL urine
§ Mean concentrations</td></tr>
</table>
</td>
<td>concentrations;
• NNAL levels ↓ was greatest in first 2 weeks
• but similar urinary total cotinine concentrations, although there was a significant “overall visit effect”, because the mean cotinine level was decreased at the week-2 visit and increased again at the week-4 visit
While switching to the nicotine patch for 4 weeks of both smokers and STP users resulted in
• ↓ to less than 1/10 of urinary total NNAL relative to baseline concentrations
• While urinary total cotinine concentration ↓ by ~50%
• ↓ to 1/3 of urinary 1-OH-Pyr and ~1/10 of exhaled CO relative to baseline concentrations in smokers
• Authors consider TSNA levels in snus 100x greater that nitrosamine levels in other consumer products, e.g. beer, food</td>
</tr>
<tr>
<td>Bolinder & de Faire 1998</td>
<td>Objective: Comparison of blood pressure and heart rate during daytime and nighttime, among smokeless tobacco users, smokers and nonusers of tobacco.</td>
<td>Snuff (Sweden)

Firemen – 47 smokeless tobacco users (20 ex-smokers), 29 smokers (5 were ex- or current smokeless</td>
<td>
<table>
<tr><th colspan="4">Plasma Concentrations</th></tr>
<tr><th>Exposure Parameter §</th><th>Never Users</th><th>STP Users</th><th>Smokers</th></tr>
<tr><td>Tobacco Consumption</td><td>0</td><td>27 ±15 g/day</td><td>18 ±11 cig/day</td></tr>
</table>
</td>
<td>• The authors concluded that nicotine values were “similar in smokeless tobacco users and smokers,” and that the “higher blood cotinine content in smokeless tobacco users is regarded as an indication of additional nicotine through</td>
</tr>
</table>

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

Reference	Study Objective and Design	Number of Subjects and Products	Results	Comments, Summary & Conclusions
	Cross-sectional study – heart rate, blood pressure and other characteristics of habitual users of tobacco were monitored and compared. Mean plasma nicotine and cotinine concentrations were measured at baseline following >8 h abstention from tobacco use.	users)	<table><tr><td>Nicotine (ng/mL)</td><td>0.2 ±0.3</td><td>4.5 ±5.8</td><td>3.4 ±2.7</td></tr><tr><td>Cotinine (ng/mL)</td><td>3.4 ±2.7</td><td>359 ±173</td><td>258 ±161</td></tr><tr><td colspan="4">§ Mean ± standard deviation</td></tr></table>	gastrointestinal mucosa by swallowing, and not reaching central circulation until inactivated by first pass liver metabolism." • Level of significance not provided for potential differences between nicotine and cotinine levels of smokeless tobacco users and smokers. • For details on clinical results see Appendix V: J1.
Bolinder et al. 1997a	Objective: Comparison of the effects of long-term tobacco use on the atherosclerotic process. Cross-sectional study – Ultrasonographic examination results and other characteristics compared among habitual users of tobacco. Mean plasma nicotine and cotinine concentrations were measured at baseline following >8 h abstention from tobacco use.	Snuff (Sweden) firemen - exclusive (never smoking) 28 smokeless tobacco users, 5 of the 29 smokers were occasional smokeless tobacco users	<table><tr><td colspan="4">Plasma Concentrations</td></tr><tr><td>Exposure Parameter §</td><td>Never-Users</td><td>STP Users</td><td>Smokers</td></tr><tr><td>Tobacco Consumption</td><td>0</td><td>32 ±17 g/day</td><td>18 ±11 cig/day</td></tr><tr><td>Nicotine (ng/mL)</td><td>0.2 ±0.4</td><td>3.7 ±2.5</td><td>5.6 ±4.1</td></tr><tr><td>Cotinine (ng/mL)</td><td>3.8 ±2.5</td><td>338 ±176</td><td>248 ±144</td></tr><tr><td colspan="4">§ Mean ± standard deviation</td></tr></table>	• The authors concluded that smokeless tobacco users are exposed to the same or even higher quantities of nicotine. The higher levels of cotinine found in smokeless tobacco users compared to smokers, although nicotine blood levels were about the same, are in agreement with other studies. • Level of significance not provided for potential differences between nicotine and cotinine levels of smokeless tobacco users and smokers. • For details on clinical results see Appendix V: J1.
Bolinder et al. 1997b; Bolinder 1997	Objective: Comparison of clinical measures of physical fitness and cardiovascular response among long-term	Snuff (Sweden) Firemen - 50 STP users (21 were former smokers), 33	<table><tr><td colspan="4">Blood Concentrations</td></tr><tr><td>Exposure Parameter *</td><td>Non Users</td><td>STP Users</td><td>Smokers</td></tr><tr><td>Tobacco</td><td>0</td><td>21</td><td>15</td></tr></table>	• The authors stated that the plasma concentration of cotinine was significantly higher in smokeless tobacco users than in smokers ($p<0.001$).

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

Reference	Study Objective and Design	Number of Subjects and Products	Results	Comments, Summary & Conclusions
	smokeless tobacco users, smokers and non-users of tobacco. Cross-sectional study – physical characteristics were measured during and after an exercise test. Median blood nicotine and cotinine concentrations presented here were measured at baseline following overnight abstention from tobacco use.	smokers (7 were ex- or current snuff users).	<table><tr><td>Consumption</td><td></td><td>(14-36) g/day</td><td>(10-21) cig/day</td></tr><tr><td>Nicotine (ng/mL)</td><td>0 (0-0.3)</td><td>3.2 (1.6-4.8)</td><td>3.2 (1.7-8.2)</td></tr><tr><td>Cotinine (ng/mL)</td><td>4.0 (0.7-5.8)</td><td>333 (232-421)</td><td>213 (163-359)</td></tr><tr><td colspan="4">*(median (25th – 75th percentile))</td></tr></table>	• Blood nicotine levels were not significantly different. • For details on clinical results see Appendix V: J1, J2.
Andersson et al. 1995	Study 1: After 2 weeks of Brand A use switch to Brand B for 10 weeks Record of consumption, clinical examination of oral mucosa, saliva and urine sampling at end of weeks 1, 2, 4, 8, 12 Study 2: Brand B users Record of consumption, clinical examination of oral mucosa, saliva and urine sampling at end of weeks 1, 2	Swedish snus Brand A (regular type snus with 0.8-0.9% nicotine, pH 8.2-8.5) & Brand B (0.4-0.5% nicotine, pH 7.8-8.2) Study 1: 24 regular Brand-A users Study 2: 18 regular (at least 1 year) Brand-B users	• Brand A users switched to Brand B consumed 2 g more snus than before the switch • Regular Brand B users consumed 3 g less snus than the switched users <table><tr><th>Exposure Parameter *</th><th>Study 1: Brand A snus users switched to Brand B</th><th>Study 2: Regular Brand B snus users</th></tr><tr><td>Tobacco Consumption (g/d)</td><td>16.2 & 16.4 at weeks 1 & 2 → 18.6 at week 12</td><td>15 &15.2 at weeks 1 & 2</td></tr><tr><td>Cotinine in Saliva (ng/ml)</td><td>321 & 352 at weeks 1 & 2 → 150 at week 12</td><td>162 &155 at weeks 1 & 2</td></tr><tr><td>Nicotine equivalents§ in urine</td><td>26.4 & 23.9</td><td>15.2 & 13.5 at</td></tr></table>	Switching from Brand to Brand B resulted • in significant ↓ ~ 50% in cotinine saliva and urinary nicotine equivalent levels to about the same level as detected in regular Brand B users • ↓ in degree of oral mucosal lesions • Authors noted that mucosal changes have been suggested to be related to high pH, but no significant difference in pH between Brand A & B and that only known difference between products was nicotine content. TSNA concentrations were not reported.

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

Reference	Study Objective and Design	Number of Subjects and Products	Results	Comments, Summary & Conclusions
			(mg/24-hr) | at weeks 1 & 2 → 14.9 at week 12 | weeks 1 & 2 Oral mucosal lesions | 46% degree 2 & 37% degree 3 both at week 1 & 2 → 75% degree 2 & 17% degree 1 at week 12 | 45 & 55% degree 2 & 33 & 28% degree 1 at week 1 & 2 § Nicotine equivalents: nicotine and its metabolites in urine (Nicotine, nicotine-GlcA, Cotinine, Cotinine-GlcA, 3' hydroxycotinine, 3' hydroxycotinine-GlcA, Nicotine-N'-oxide and Cotinine-N-oxide) * Mean	
Eliasson et al. 1995	Objective: Comparison of cardiovascular risk factors among regular snuff users and smokers. Cross-sectional study – various characteristics were measured and compared. Mean plasma nicotine and cotinine concentrations were measured at baseline following >1 h abstention from tobacco use.	Snuff (Sweden) 250 men randomly selected from MONICA cohort (N=92 snus users, 124 smokers, 38 dual users)	Plasma Concentrations Exposure Parameter | Snuff Dippers | Dual Users | Smokers Average Tobacco Consumption | 3.2 cans/week (22.9 g/day) | 10.1 cig/day + 2.5 cans/ week (125 g/week or 28.2 g/day) | 16.5 cig/day 16.5 g/day Nicotine* (ng/mL) | 15.5 (9.6-21.4) | 9.5 (5.0-14.1) | 9.8 (7.6-12.1) Cotinine* (ng/mL) | 351 (277-425) | 308 (242-373) | 242 (209-275) *Means and 95% confidence intervals	• Significant differences in plasma cotinine were observed across tobacco groups ($p<0.01$). Significant differences were not observed for plasma nicotine. • The authors noted a higher plasma cotinine concentration found in snuff users compared to smokers and that higher nicotine levels in snuff dippers indicated more recent or greater tobacco exposure. • The authors also noted that cotinine levels correlated with number of cigarettes per day in men, $r=0.38$ ($p=0.013$)and that cotinine concentrations were significantly correlated to the number of snuff cans dipped per week, $r=0.60$ ($p=0.003$).

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

<table>
<tr><th>Reference</th><th>Study Objective and Design</th><th>Number of Subjects and Products</th><th>Results</th><th>Comments, Summary & Conclusions</th></tr>
<tr><td></td><td></td><td></td><td></td><td>• For details on clinical results see Appendix V: J1.</td></tr>
<tr><td>Andersson et al. 1994</td><td>Objective: Uptake and metabolism of nicotine in STP users, correlation of oral mucosal changes to exposure and uptake of tobacco constituents

STP users used own brand for 7 days; 24-hr urine samples & all used STP products collected on day 6; on day 7 saliva collected after 30 min of intake of the STP + 30 min waiting followed by rinsing</td><td>Swedish snus, Chewing tobacco

22 loose snus users , 23 users of portion-bag snus users, 9 users of chewing tobacco</td><td>
<table>
<tr><th>Product & Exposure Parameter</th><th>Portion-bag snus users</th><th>Loose snus users</th><th>Chewing tobacco users</th></tr>
<tr><td>Average Tobacco Consumption (g/day)</td><td>14.4</td><td>20.8 g</td><td>7.2</td></tr>
<tr><td>Product pH</td><td>7.9-8.2</td><td>8.5-8.6</td><td>4.9</td></tr>
<tr><td>Nicotine in product (mg/g)</td><td>9.0-10.3</td><td>8.6-9.1</td><td>21.2</td></tr>
<tr><td>Degree of nicotine extraction (%)</td><td>37.4</td><td>49.1</td><td>54.3</td></tr>
<tr><td>Nicotine extracted (mg/24 hrs)</td><td>47.6</td><td>94.7</td><td>76.4</td></tr>
<tr><td>Cotinine in saliva (ng/ml)</td><td>342.9</td><td>326.6</td><td>470.8</td></tr>
<tr><td>Systemic dose: Nicotine equivalents§ (mg/24-hr)</td><td>34.5</td><td>35.6</td><td>54.1</td></tr>
<tr><td>Total TSNAs in product (μg/g)</td><td>3.7-6.0</td><td>6.1-7.7</td><td>1.8</td></tr>
<tr><td>Degree of TSNA extraction (%)</td><td>55.7</td><td>64.1</td><td>29.7</td></tr>
<tr><td>Total TSNAs extracted (μg/24 hrs)</td><td>44.5</td><td>125.3</td><td>3.3</td></tr>
<tr><td>Oral mucosal lesions</td><td>48% degree 2, 39% degree 3</td><td>23% degree 2, 73% degree 3</td><td>% degree not given</td></tr>
</table>
</td><td>• Amount of nicotine extracted and degree of extraction from portion-bag snus ½ ↓ compared to loose snus and chewing tobacco
• No difference in saliva cotinine concentrations between all 3 groups.
• No difference in systemic dose of nicotine (nicotine and its metabolites in 24-hr urine) between portion bag and loose snus users, but significantly ↑ in chewing tobacco users.
• 3x ↓ amount TSNAs extracted in portion-bag snus users compared to loose snus users. Lower consumption of portion-bag snus, TSNA content ↓ than loose snus, and degree of extraction slightly ↓.
• The authors noted that there was a good correlation between TSNA and nicotine extraction from snus observed. Therefore, the authors stated “”it may be assumed that the amount of TSNA expectorated was proportional to that of nicotine.”
• Based on matched case-control assessments, no correlation between degree of lesions could be found with either total dose of nicotine or consumption factors</td></tr>
</table>

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

<table>
<tr><th>Reference</th><th>Study Objective and Design</th><th>Number of Subjects and Products</th><th>Results</th><th>Comments, Summary & Conclusions</th></tr>
<tr><td></td><td></td><td></td><td>§ Nicotine equivalents: nicotine and its metabolites in urine (nicotine, nicotine glucuronide, cotinine, cotinine glucuronide, 3' hydroxycotinine, 3' hydroxycotinine glucuronide, nicotine-N'-oxide and cotinine-N-oxide)</td><td>• The authors concluded that “The clinical severity of buccal mucosal changes correlated neither with the markers for exposure (i.e. nicotine and TSNA extracted from the smokeless tobacco product), nor with biological makers for uptake of tobacco constituents […]”
• The authors attributed differences in clinical changes to the differences in product pH</td></tr>
<tr><td>Hirsch et al. 1992</td><td>Objective: Comparison of the short-term hemodynamic effects of snuff dipping during rest and dynamic exercise in healthy habitual users of oral snuff.

Placebo-controlled crossover study –

Mean blood nicotine and cotinine concentrations were measured prior to treatment following 24 hour abstinence from snuff dipping.

Mean plasma nicotine and cotinine concentrations were measured 0 (after 24 hours abstaining from</td><td>Commercial Swedish Snuff (unspecified)

9 habitual users of snuff</td><td>
<table>
<tr><th colspan="3">Blood Concentrations in Snuff Users (Pre-treatment)</th></tr>
<tr><th>Exposure Parameter §</th><th>Control Day</th><th>Experimental Day</th></tr>
<tr><td>Tobacco Consumption</td><td>0</td><td>2.5 g</td></tr>
<tr><td>Nicotine (ng/mL)</td><td>0.25 ±0.2</td><td>0.34 ±0.2</td></tr>
<tr><td>Cotinine (ng/mL)</td><td>90.3 ±27.7</td><td>117.1 ±30.3</td></tr>
<tr><td colspan="3">§ Mean ± SEM</td></tr>
</table>
<table>
<tr><th colspan="5">Average plasma concentrations after 2.5 g snuff intake during supine rest at</th></tr>
<tr><th>Biomarker§</th><th>0 min</th><th>15 min</th><th>110 min</th><th>140 min</th></tr>
<tr><td>Nicotine (ng/mL)</td><td>0.3</td><td>7.7</td><td>20.9</td><td>16.9</td></tr>
<tr><td>Cotinine (ng/mL)</td><td>117</td><td>106</td><td>121</td><td>126</td></tr>
</table>
</td><td>• The authors stated that hemodynamic changes were unrelated to nicotine and cotinine concentrations.
• The authors noted that considering the normal exposure time for cigarettes or oral snuff, this will result in a far higher total nicotine exposure after snuff intake compared with that after smoking.
• After snuff intake, plasma nicotine concentrations increased slowly to reach a plateau level at 110 minutes (20 ng/ml). Plasma cotinine increased and reached 126.3 ng/ml at 140 minutes following the first intake of snuff.
• For details on clinical results see Appendix V: J2.</td></tr>
</table>

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

<table>
<tr><th>Reference</th><th>Study Objective and Design</th><th>Number of Subjects and Products</th><th>Results</th><th>Comments, Summary & Conclusions</th></tr>
<tr><td></td><td>snuff), 15, 30, 70, 110 and 140 minutes following intake of 2.5g of snuff.</td><td></td><td>§ Mean</td><td></td></tr>
<tr><td>Holm et al. 1992</td><td>Objective:
Examination of nicotine absorption rate and blood levels in regular snus users; Comparison of dependence and nicotine intakes in snuff users with those in cigarette smokers

Study 1: 2 g of Ettan snus for 30 min after overnight abstinence

Study 2: subjects that used snus or smokers were evaluated on a day of normal use directly after use</td><td>Ettan snus, Swedish snuff

Study 1:
10 regular snus users

Study 2: 27 snus users, 35 smokers</td><td>Plasma Concentrations
<table><tr><th rowspan="2">Exposure Parameter§</th><th>Study 1</th><th colspan="2">Study 2</th></tr><tr><th>Snus Users</th><th>Snus Users</th><th>Smokers</th></tr><tr><td>Tobacco Consumption</td><td>2 g for 30 min after overnight abstinence</td><td>152 g/week [21.7 g/d]</td><td>17 cig/day</td></tr><tr><td>Nicotine (ng/mL)</td><td>-5 min: 0.9
35 min: 15.3
60 min: 12.5</td><td>36.6</td><td>36.7</td></tr><tr><td>Cotinine (ng/mL)</td><td>-5 min: 267.8
60 min: 279</td><td>399.2</td><td>306.3</td></tr></table>§ Mean

PK Parameters in Plasma
Study 1: Snus users after overnight abstinence and subsequent snus consumption for 30 min
<table><tr><th>Biomarker§</th><th>t_{max} (min)</th><th>C_{max} (ng/mL)</th><th>AUC (ng*min/mL)</th></tr><tr><td>Nicotine</td><td>35.5</td><td>17 ± 5.6</td><td>747.4 ± 243 (0-60 min)</td></tr></table>§ Mean</td><td>• In study 2, peak blood nicotine concentrations measured directly after using snus or smoking one cigarette were similar, while cotinine concentrations were higher in snus users than in smokers
• Authors noted that “The higher cotinine levels associated with nicotine absorption by the buccal route reflect the loss of availability due to swallowing and first-pass metabolism, and offer further evidence that it is the systemic availability of nicotine that governs the behavior. However, it is not clear to what extent reinforcement and levels of dependence are determined by steady-state peak levels, trough levels or the rate of nicotine absorption.”</td></tr>
<tr><td>Eliasson et al. 1991</td><td>Objective: Comparison of cardiovascular risk factors among habitual snuff users and smokers.

Cross-sectional study –</td><td>Snuff (Sweden)

Swedish university students and locals recruited using the newspaper - 5 of 21</td><td>Plasma Concentrations
<table><tr><th>Exposure Parameter*</th><th>Non-tobacco</th><th>Snuff</th><th>Smokers</th></tr><tr><td>Tobacco Consumption</td><td>0</td><td>146 (60)</td><td>134 (29)</td></tr></table></td><td>• The authors stated that snuff users showed significantly higher levels of plasma cotinine than smokers. The authors also noted that the low plasma nicotine levels were generally consistent with the fact</td></tr>
</table>

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

Reference	Study Objective and Design	Number of Subjects and Products	Results	Comments, Summary & Conclusions
	various characteristics were measured and compared. Mean plasma nicotine and cotinine concentrations were measured at baseline following 24 hour abstention from tobacco use.	snuff users were ex-smokers, 1 of 19 smokers was a previous snuff user	(g/week) Nicotine (ng/mL): 0.9 (0.5); 3.2 (1.3); 3.3 (2.7) Cotinine (ng/mL): 2.0 (2); 326** (113); 237 (102) *Mean (standard deviation) ** p<0.05 (compared to smokers)	that subjects had abstained from smoking and taking snuff. • The authors noted that the "finding of significantly higher plasma cotinine levels in the morning among snuff-users compared to smokers may reflect a higher steady-state concentration during the previous day." They also noted that, "alternatively, snuff users may swallow nicotine which, on absorption from the gut, is converted to cotinine, thus avoiding entry to the systemic circulation as nicotine." • For details on clinical results see Appendix V: J1.
Wennmalm et al. 1991	Objective: To compare the effect of snuff or cigarette use on the formation of two eicosanoids, thromboxane A2 and prostacyclin. Cross-sectional study – urinary metabolites were measured and compared. Median urinary concentrations were measured at baseline. There was no significant difference among tobacco groups of tobacco abstinence at the time of	Snuff (Sweden) 577 randomly sampled 18-19 year old men screened for enrollment in the Swedish National Defense System. N=127 snuff users, 43 smokers, 187 dual users.	Urinary Concentrations Exposure Parameter: Never Smokers; Snuff; Dual Use; Smokers Tobacco Consumption § : 0; 25 ±1 g/d; 7.8 ±1.3 cig/d + 27 ±3 g/d; 12.2 ±0.8 cig/d Cotinine (µg/L)*: 5.7 (0.6-90); 1210 (3.1-4280); 1773 (840-2800); 1560 (570-3450) § Mean ± SEM * Median (Range)	• Median urinary cotinine concentration of snuff users was slightly ↓ than that of smokers, but the authors stated that there was no significant difference in cotinine concentration among the tobacco groups. • For details on clinical results see Appendix V: J1.

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

Reference	Study Objective and Design	Number of Subjects and Products	Results	Comments, Summary & Conclusions
	urine collection (ranged from 2.2-3.1 hours).			
Österdahl and Slorach 1988	Objective: Correlation of TSNA concentration in product, extraction levels and saliva concentrations Saliva samples were collected before, during 30 min of dipping, and after a single snus use on 2 different days, extraction was determined by difference in TSNA concentrations before and after use	Swedish moist snuff 4 habitual snuff users	See nested table below	• Total TSNA concentrations in the 3 pouches changed between -0.9 - +0.3 μg/g after use. The authors hypothesized *in vivo* TSNA formation in one snuff user. • TSNA concentrations in saliva varied strongly between users and day but were traces to undetectable 20 min after end of use. • Average total TSNA concentration in saliva during 30 min of snuff dipping was calculated to be 15-125 ng/g. • Authors estimated snuff users to be exposed to 0.9-7.5 μg TSNA /hr snuff dipping (adult produces 60 ml saliva/hr).

Component/ Biomarker[§]	TSNAs in snuff sachet/ pouch before use (μg/g)	TSNAs in Saliva at 10-30 min (ng/g)	
		Pouch	Loose
Total TSNAs	9.2		
NNK	1.3	ND - 13	ND - 16
NNN	4.4	3 - 74	37 - 140
NAT	3.5	Trace - 41	17 - 85

[§] Mean

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

<table>
<tr><th>Reference</th><th>Study Objective and Design</th><th>Number of Subjects and Products</th><th>Results</th><th>Comments, Summary & Conclusions</th></tr>
<tr><td colspan="5">Studies of Users of New Products Marketed as Snus and Select Studies of STP Users</td></tr>
<tr>
<td>Kotlyar et al. 2011</td>
<td>Objective: Determination if smokers would be willing to switch to newer products as cessation aid

Baseline observations 2 weeks, smoking + start use 1 week, complete switch at week 4 for 4 weeks (intervention period), then taper off 1 week and follow-up with no tobacco or NRT for 11 weeks

Urine sampling at baseline, week 2 and 4 of intervention period

Abstinence confirmed with CO exhaled <8 ppm, urinary cotinine <35 ng/mL</td>
<td>Camel Snus, Taboka, 4-mg nicotine gum or lozenge

130 smokers interested in cessation, randomized (51 Camel Snus, 52 Taboka, 27 NRT)

Taboka:
0.844-1.26 mg dw free nicotine, 19.1-21.1 mg/g dw total nicotine
Camel Snus:
6.09-9.16 mg/g dw free nicotine, 23.7-28.2 mg/g dw total nicotine</td>
<td>
<table>
<tr><th colspan="4">Average tobacco consumption and associated measured biomarkers</th></tr>
<tr><th rowspan="2">Exposure Parameter</th><th colspan="3">Smokers switched to</th></tr>
<tr><th>NRT</th><th>Taboka</th><th>Camel Snus</th></tr>
<tr><td>Number of participants (N) at study start → at study end/week 16</td><td>27 → 18 (67%)</td><td>52 → 30 (58%)</td><td>51 → 30 (58%)</td></tr>
<tr><td>Mean Tobacco Use</td><td>23.6 cigs/day → 7.4 pieces/day (13.6%)$</td><td>19.8 cigs/day → 5.8 pouches/day (26.8%)$</td><td>19.7 cigs/day → 6.9 pouches/day (9.1%)$</td></tr>
<tr><td colspan="4">Biomarkers** at baseline → at week 4 after switch</td></tr>
<tr><td>CO in exhaled air (ppm)</td><td>~24 → ~4.5*</td><td>~22 → ~6*</td><td>~20 → ~4.5*</td></tr>
<tr><td colspan="4">urine</td></tr>
<tr><td>Cotinine (ng/mL)</td><td>~3200 → ~700*</td><td>~3200 → ~700*</td><td>~3500 → ~700*</td></tr>
<tr><td>Total NNAL (pmol/mg creatinine)</td><td>~0.8 → ~0.15*</td><td>~0.8 → ~0.3*</td><td>~0.7 → ~0.3*</td></tr>
<tr><td>Total NNN (pmol/mg creatinine)</td><td>~0.035 → ~0.015*</td><td>~0.06 → ~0.015*</td><td>~0.055 → ~0.027</td></tr>
<tr><td colspan="4">** averages, based on figures
* significantly different from baseline
$ Subjects who continued to smoke ≥3 cigs/day</td></tr>
</table>
</td>
<td>
• Time dependence: Concentrations of CO, urinary cotinine and total NNAL and NNN were statistically significantly↓ at the end of treatment (intervention week 4) compared with concentrations measured at baseline in all groups, except for urinary NNN in the Camel Snus group (p=0.066).
• Exhaled CO concentration were similar in Camel Snus and NRT users, but slightly ↑ in Taboka users at week 4
• No difference in week 4 urinary cotinine concentrations in all groups
• Total NNAL was similar in Camel Snus and Taboka users, but ↑ compared to NRT users at week 4
• Total NNN was ↑ in Camel Snus users compared to NRT and Taboka users at week 4

• The authors hypothesized that the lack of significant differences in total NNN concentrations was perhaps at least in part due to endogenous NNN production from nicotine
</td>
</tr>
</table>

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

<table>
<tr><th>Reference</th><th>Study Objective and Design</th><th>Number of Subjects and Products</th><th>Results</th><th>Comments, Summary & Conclusions</th></tr>
<tr><td></td><td></td><td></td><td>• Quit-rates and craving for Camel Snus were comparable to those for NRT
• Withdrawal symptoms were significantly higher with both Camel Snus and Taboka, compared to NRT
• During last week of treatment/intervention period, Taboka users smoked significantly more of their usual brand cigarettes than those in the 2 other groups, craving and withdrawal effects were also ↑</td><td></td></tr>
<tr><td>Blank & Eissenberg et al. 2010</td><td>Objective: Adapt methods to measure toxicant and abstinence symptom suppression associated with use of orally administered noncombustible PREPs for smokers

4x 5-day conditions that differed by product used; allowed to use their own brand of cigarettes on the weekends</td><td>Camel Snus, Ariva, own brand cigarettes, or no tobacco (NT)

21 smokers (average ≥15 cigs/day, 1.04 mg nicotine/cig)

Day 1-4 provided with product to use over next 24 hrs</td><td>Average tobacco consumption and associated measured biomarkers
<table>
<tr><th rowspan="2">Exposure Parameter</th><th colspan="3">Smokers switched to</th><th rowspan="2">Smokers continued own brand</th></tr>
<tr><th>No tobacco</th><th>Ariva</th><th>Camel Snus</th></tr>
<tr><td>Tobacco use $</td><td></td><td>12.3 tablets/24 hrs</td><td>11.7 pouches/24 hrs</td><td>21.9 cigs/24 hrs</td></tr>
<tr><td colspan="5">Biomarkers ** at baseline → 5 days after switch (means of 4x)</td></tr>
<tr><td>CO in exhaled air (ppm)</td><td>~25 → ~4</td><td>~25 → ~4
7.2 $</td><td>~21 → ~4
6.1 $</td><td>~23 → ~25
23.7 $</td></tr>
<tr><td colspan="5">Urine</td></tr>
<tr><td>Cotinine (ng/mL)</td><td>~1250 → ~50</td><td>~1250 → ~700</td><td>~1000 → ~1000</td><td>~1000 → ~1500*</td></tr>
<tr><td>Total NNAL (pg/mL)</td><td>~230 → ~90</td><td>~280 → ~150</td><td>~230 → ~230</td><td>~280 → ~280</td></tr>
<tr><td colspan="5">** averages, based on figures
$ collapsed over time</td></tr>
</table></td><td>• Urinary cotinine concentrations on day 1 were slightly ↓ in Camel Snus users and smokers that continued to smoke compared to NT and Ariva users. By day 5, there was no change in Camel Snus users, but cotinine concentrations were ↑ in smokers, ↓ in Ariva users, but only significantly ↓ in NT users compared to smokers
• Total NNAL concentrations were similar on Day 1 for all conditions, but slightly lower for NT and Camel Snus. By day 5, there was no change in total NNAL levels for Camel Snus users or smokers, but a ↓ for Ariva (49.1% d↓) and NT (63.1% ↓).
• Neither PREP suppressed symptoms of tobacco abstinence (e.g., irritability/frustration/anger, urge to smoke) as effectively as smoking
• Authors concluded “noncombustible PREPs for</td></tr>
</table>

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

<table>
<tr><th>Reference</th><th>Study Objective and Design</th><th>Number of Subjects and Products</th><th>Results</th><th>Comments, Summary & Conclusions</th></tr>
<tr><td></td><td></td><td></td><td></td><td>smokers reduce usual exposure to CO and may be able to reduce exposure to other toxicants (e.g., cotinine). Nonetheless, these PREPs were unable to suppress fully tobacco abstinence symptoms and were considered significantly less enjoyable than participants' own brand of cigarette."</td></tr>
<tr><td>Sarkar et al. 2010</td><td>Objective: Comparison of smokers that continued to smoke and those that reduced their number of cigarettes by at least 50% and were allowed to use pouched STP

Day -2 and -1 smokers continued to smoke own brand. On study day 1 randomized into 4 groups for 8 days:
Continued smoking own brand (CS); Dual use (DU) - smoking was reduced to 50% or less of daily cigarette consumption at baseline and use of Marlboro Snus allowed ad libitum; Marlboro Snus (SN) - stopped smoking and allowed to use Marlboro Snus ad libitum. No tobacco (NT) - not allowed to use any</td><td>Marlboro Snus (9.58-13.22% moisture, 1.52-2.9% nicotine, ~9% free nicotine, pH 6.80-7.19, 0.094-0.224 ppm NNK, 0.682-1.117 ppm NNN, 1.375-2.019 ppm total TSNAs, 0.37-0.67 ng/g B[a]P), cigarettes

115 smokers (CS=30, DU=59, SN=15, NT=11)</td><td>
<table>
<tr><th rowspan="2">Exposure Parameter</th><th colspan="3">Smokers switched to</th><th rowspan="2">Smokers continued own brand (CS)</th></tr>
<tr><th>(NT) No tobacco</th><th>Marlboro Snus (SN)</th><th>Dual Use (DU)</th></tr>
<tr><td colspan="5">At baseline → 7-8 days after switch</td></tr>
<tr><td>Cigarettes/day; snus pouches/day</td><td>18.5 → -</td><td>17.8 → -; 3.5</td><td>17.6 → 8.4; 2.2</td><td>16.7 → 15.6</td></tr>
<tr><td colspan="5">Blood/Plasma</td></tr>
<tr><td>CO-Hemoglobin (%)</td><td>5.62 → 1.35</td><td>6.99 → 1.53</td><td>6.13 → 4.74*</td><td>6.96 → 6.37*</td></tr>
<tr><td>Nicotine AUC (ng/mLxhr)</td><td>201.78 → -</td><td>225.55 → 40.31#</td><td>202.02 → 132.94</td><td>195.67 → 170.34</td></tr>
<tr><td>Cotinine AUC (ng/mLxhr)</td><td>3287.14 → -</td><td>3617.91 → 781.87#</td><td>3142.33 → 2008</td><td>3566.71 → 3139.96</td></tr>
<tr><td>trans-3-Hydroxy-cotinine AUC (ng/mLxhr)</td><td>1362.71 → -</td><td>1187.42 → 286.76</td><td>1173.17 → 827.61</td><td>1374.52 → 1273.29</td></tr>
<tr><td colspan="5">Urine</td></tr>
</table>
</td><td>After 1 week, smokers that completely switched to Marlboro Snus had
• 50% ↓ urinary total NNAL levels compared to smokers that continued to smoke, but 1.4x ↑than smokers that quit completely
• 70% ↓ urinary total NNN levels compared to smokers that continued to smoke, but 5.4x ↑than smokers that quit completely
• 50% ↓ urinary hydroxy-benzo[a] pyrene levels compared to smokers that continued to smoke, but 1.4x ↑than smokers that quit completely
• 60% ↓ urinary o-toluidine levels compared to smokers that continued to smoke, but 3x ↑than smokers that quit completely
• Nicotine, cotinine, and trans-3-hydroxycotinine AUCs in plasma ↓ to $^1/_4$ and urinary nicotine equivalents ↓ to $^1/_3$ of those in</td></tr>
</table>

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

Reference	Study Objective and Design	Number of Subjects and Products	Results					Comments, Summary & Conclusions
	tobacco products.		Nicotine equivalents§ (mg/24 hrs)	18 → 0.08	21.47 → 5.53*#	17.79 → 11.3*	20.76 → 17.77*	smokers that continued to smoke • similar COHb, urinary 2-aminonaphthalene, 4-aminobiphenyl, S-phenylmercapturic acid levels, as well as urinary mutagenicity outcomes as smokers that quit completely • The authors concluded that after adjusting for residual levels observed in NT group, smokers that reduced the number of cigarettes by 50% and used *Marlboro Snus* had a corresponding 50% reduction in most in biomarkers (except for urinary nicotine equivalents ~36%, o-toluidine ~24%, and COHb ~29%). • Note: Snus pouches were kept 60 min (up to 2 hrs) in the mouth
			Total NNAL (ng/24 hrs)	683.61 → 198.25	752.85 → 278.25*#	548.35 → 370.58*#	693.24 → 599.95*	
			Total NNN (ng/24 hrs)	28.2 → 0.83	26.61 → 4.52*#	18.44 → 9.39*#	18.92 → 15.22*	
			2-amino-naphthalene (ng/24 hrs)	33.5 → 3.81	38.61 → 2.74	30.7 → 15.55*#	37.76 → 31.85*	
			4-amino-biphenyl (ng/24 hrs)	19.61 → 2.25	23.04 → 2.91	18.72 → 9.88*#	22.13 → 19.22*	
			3-hydroxy-benzo[a] pyrene (pg/24 hrs)	162.70 → 55.93	192.27 → 78.89	132.95 → 78.01	193.1 → 155.03*	
			S-phenyl mercapturic acid (μg/24 hrs)	0.99 → 0.23	1.06 → 0.24	0.98 → 0.58	1.24 → 1.03*	
			o-Toluidine (ng/24 hrs)	192.22 → 54.38	329.51 → 162.82*#	274.07 → 220.59*#	266.43 → 278.26*	
			Urinary mutagenicity (revertants/ 24 hrs)	32,118 → 1,249	36,946 → 1,192	19,863 → 11,416*	25, 929 → 23,810*	
			§ Mean nicotine and 5 metabolites in 24-hr urine: nicotine-N-glucuronide, cotinine, cotinine-N-glucuronide, trans-3' hydroxycotinine, trans-3' hydroxycotinine glucuronide; * significantly different from controls, # significantly ↓ compared to smokers					
Cobb et al. 2010	Objective: Extent to which controlled clinical laboratory methods can be used to	*Marlboro Snus, Camel Snus, Ariva, Commit* 2-mg	Nicotine PK parameters in plasma of smokers after overnight abstinence and subsequent product use (at					• Camel Snus ↑ nicotine plasma concentration slightly more than the NRT Commit, Ariva and

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

<table>
<tr><th>Reference</th><th>Study Objective and Design</th><th>Number of Subjects and Products</th><th>Results</th><th>Comments, Summary & Conclusions</th></tr>
<tr><td></td><td>investigate the acute effects of non-combustible PREPs

Overnight abstinent smokers completed 7 2.5-hr sessions (2x 15 min use +30 min observation separated by 15 mins). Blood sampling every 10-15 min up until 45 min after 2nd administration

Snus products: Participants placed pouch between lip and gum for 15 minutes.

Commit and ARIVA: Participants allowed the product to dissolve in their mouth without chewing or swallowing it.</td><td>nicotine lozenge, own brand cigarettes, Quest cigarettes, sham smoking

28 smokers</td><td>
<table>
<tr><th colspan="4">baseline: 2.4 ng/mL)</th></tr>
<tr><th>Product</th><th>t_{max} (min)</th><th>C_{max} (ng/mL)</th><th>$C_{15\text{-min post 2nd administration}}$ (ng/mL)</th></tr>
<tr><td>Own Cigarette</td><td>~5</td><td>20.7</td><td>~12.5</td></tr>
<tr><td>QUEST</td><td>none</td><td>(2.4)</td><td>(2.4)</td></tr>
<tr><td>SHAM</td><td>none</td><td>(2.4)</td><td>(2.4)</td></tr>
<tr><td>Camel Snus</td><td>~15§</td><td>7.6§</td><td>7.6§</td></tr>
<tr><td>Marlboro Snus</td><td>~30§</td><td>~3.5§</td><td>2.9§</td></tr>
<tr><td>Ariva</td><td>~30§</td><td>~3.5§</td><td>3.4§</td></tr>
<tr><td>COMMIT</td><td>~30§</td><td>~6§</td><td>4.6§</td></tr>
<tr><td colspan="4">§ after 2nd administration</td></tr>
</table>
</td><td>Marlboro Snus only caused very slight ↑ over baseline concentrations
• Camel Snus and Commit after the 2nd application caused plasma concentrations similar to trough concentrations from smoking own brand cigarettes.
• Non-combustible products delivered less nicotine than own brand cigarettes and failed to suppress tobacco abstinence symptoms effectively. Several non-combustible products produced reliable suppression at some time points among several measures of abstinence, though own products always produced greater suppression. Relative to baseline, own brand was associated with significant increases in plasma nicotine levels at almost every time point.</td></tr>
<tr><td>Marano et al. 2012a</td><td>Objective: To investigate whether cadmium has an independent role in diseases associated with tobacco consumption and to analyze biomonitoring data of cadmium in smokers, STP users and non-users of tobacco.

NHANES data (1999-2006)</td><td>Smokeless tobacco products (snuff, chewing tobacco), cigarettes

Urine: 87 STP users, 1,180 smokers, 4,110 non-tobacco users

Blood: 272 STP</td><td>
<table>
<tr><th colspan="4">Adjusted geometric means of biomarkers studied</th></tr>
<tr><th>Biomarker§</th><th>Controls</th><th>US STP Users</th><th>Smokers</th></tr>
<tr><td colspan="4">Blood (B) or Urine (U)</td></tr>
<tr><td>Cadmium (B) (ng/mL)</td><td>0.31</td><td>0.25</td><td>0.44</td></tr>
<tr><td>Cadmium (U) (µg/g creatinine)</td><td>0.25</td><td>0.39</td><td>0.95</td></tr>
<tr><td colspan="4">§ Geometric mean; adjusted for age, gender, race/ethnicity, body mass index, survey year, and tobacco consumption category.</td></tr>
</table>
</td><td>• Cadmium was detected in the blood and urine of all groups: STP users, smokers and non-users of tobacco.
• Mean blood- and urine-cadmium concentrations were higher among smokers compared to STP users, and concentrations among STP users were not significantly different from non-users of tobacco.</td></tr>
</table>

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

<table>
<tr><th>Reference</th><th>Study Objective and Design</th><th>Number of Subjects and Products</th><th>Results</th><th>Comments, Summary & Conclusions</th></tr>
<tr><td></td><td>Individuals aged 20 years and older.</td><td>users, 3,679 smokers, 12,454 non-tobacco users</td><td></td><td></td></tr>
<tr><td>Marano et al. 2012b</td><td>Objective: To investigate whether arsenic has an independent role in diseases associated with tobacco consumption and to analyze biomonitoring data of arsenic in smokers, STP users and non-users of tobacco.

NHANES data (2003-2008) Individuals aged 20 years and older.</td><td>Smokeless tobacco products (snuff, chewing tobacco), cigarettes

90 STP users, 991 smokers, 3,385 non-tobacco users</td><td>Adjusted geometric means of urinary arsenic
<table><tr><th>Biomarker§</th><th>Controls</th><th>US STP Users</th><th>Smokers</th></tr><tr><td>Total arsenic (µg/g creatinine)</td><td>9.56 (8.92, 10.27)</td><td>6.14 (4.86, 7.74)</td><td>7.98 (7.08, 9.00)</td></tr><tr><td>Dimethylarsinic acid(µg/g creatinine)</td><td>4.01 (3.82, 4.22)</td><td>2.68 (2.22, 3.23)</td><td>3.53 (3.28, 3.80)</td></tr><tr><td>Arsenobetaine (µg/g creatinine)</td><td>1.96 (1.75, 2.19)</td><td>1.10 (0.74, 1.62)</td><td>1.50 (1.26, 1.78)</td></tr></table>§ Geometric mean (95% CI); adjusted for age, gender, race/ethnicity, body mass index, urinary arsenobetaine, urinary creatinine, survey year, and tobacco category.</td><td>• Urinary total arsenic, DMA, and arsenobetaine concentrations were similar among the three consumer groups, although consistently highest in non-consumers of tobacco and lowest in SLT consumers.</td></tr>
<tr><td>Naufal et al. 2011</td><td>Objective: Comparison of biomarkers of exposure in smokers, STP users, and non-consumers; correlation of biomarkers with cotinine concentrations

NHANES data (1999-2008) Self-reported non-consumers with serum cotinine >15 ng/mL and multiple product users excluded; unadjusted geometric means calculated</td><td>Smokeless tobacco products (snuff, chewing tobacco), cigarettes

368 STP users (snuff, e.g., Skoal, Skoal Bandits, Copenhagen; chewing tobacco, e.g., Redman, Levi, Garrett, Beechnut. 5040 smokers, 16443 controls</td><td>Unadjusted geometric means of biomarkers studied
<table><tr><th>Biomarker§</th><th>Controls</th><th>US STP Users</th><th>Smokers</th></tr><tr><td colspan="4">Blood (B) or Serum (S)</td></tr><tr><td>Cotinine (S) (ng/mL)</td><td>0.050</td><td>188.7 #</td><td>127.7 *</td></tr><tr><td colspan="4">Heavy Metals (ng/mL)</td></tr><tr><td>Cadmium (B)</td><td>0.30</td><td>0.28 ¥</td><td>0.90 *</td></tr><tr><td>Lead (B)</td><td>13.9</td><td>19.2 #, ¥</td><td>18.6 *</td></tr><tr><td>Mercury (B)</td><td>1.06</td><td>0.78 #</td><td>0.78 *</td></tr><tr><td>Selenium (S)</td><td>137.0</td><td>137.0</td><td>130.3 *</td></tr><tr><td colspan="4">VOCs, aromatic (ng/mL)</td></tr><tr><td>Benzene (B)</td><td>0.031</td><td>0.031 ¥</td><td>0.12 *</td></tr><tr><td>Ethylbenzene (B)</td><td>0.028</td><td>0.029 ¥</td><td>0.063 *</td></tr><tr><td>Styrene (B)</td><td>0.031</td><td>0.033 ¥</td><td>0.067 *</td></tr></table></td><td>• STP users had ↑ serum cotinine, serum heptadichlorobenzofuran, and urinary NNAL concentrations compared to both controls and smokers
• STP users had ↑ blood lead compared to controls, but similar to smokers
• STP users had ↑ concentrations of urinary metabolites of fluorene, phenanthrene, and pyrene compared to controls, but ↓ compared to smokers
• STP users had ↓ blood mercury and urinary arsenic concentrations compared to controls, but similar to smokers
• STP users had similar blood and</td></tr>
</table>

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

Reference	Study Objective and Design	Number of Subjects and Products	Results				Comments, Summary & Conclusions
			Toluene (B)	0.10	0.121 ¥	0.34 *	urinary cadmium, urinary lead, blood VOCs, and acrylamide hemoglobin adduct concentrations compared to controls, but ↓ compared to smokers • STP users had similar serum selenium, 5 out of 6 serum polychlorinated dioxin and furan concentrations compared to controls, but these were ↓ in smokers • All groups had similar urinary cobalt concentrations • In STP users and smokers serum cotinine was associated with 7 biomarkers (urinary Co, urinary Cd, 2 urinary fluorene and 2 urinary phenanthrene metabolites, and urinary NNAL) • In smokers alone serum cotinine was associated with additional 16 biomarkers (blood and urinary Pb, blood Cd, hemoglobin adducts of acrylamide and glycidamide, all VOCs, and all additional PAH metabolites) • The authors hypothesized that differences in Se and Hg might be a result of dietary differences, differences in polychlorinated dioxins and furans due to lower BMI in smokers, NNAL differences due to differences in metabolism based on route of intake
			Xylenes (m-/p-) (B)	0.13	0.15 ¥	0.22 *	
			Acrylamide Hemoglobin Adducts (pmol/g Hb)				
			Acrylamide adducts	47.9	53.5 ¥	122.7 *	
			Glycidamide adducts	47.5	50.4 ¥	101.5 *	
			Polychlorinated Dioxins/Furans (S) (pg/g lipid)				
			Heptachlorodibenzo-p-dioxin	42.1	45.6	18.5 *, ¥	
			Hexachlorodibenzo-p-dioxin	22.4	24.0	17.5 ¥	
			Octachlorodibenzo-p-dioxin	298.9	308 #	200.3 *, ¥	
			Heptachlorodibenzofuran	7.39	10.6 #	7.17 ¥	
			Hexachlorodibenzofuran	4.71	5.00	4.18	
			Pentachlorodibenzofuran	5.21	5.47	4.18 *	
			Urine				
			TSNAs (μg/g creatinine)				
			NNAL	0.0010	0.99 #	0.21 *, ¥	
			Heavy Metals and Metalloids (μg/g creatinine)				
			Arsenic	9.58	6.17 #	8.08	
			Cadmium	0.24	0.16 ¥	0.34 *	
			Cobalt	0.34	0.26	0.33	
			Mercury	0.54	0.36 #	0.42 *	
			Lead	0.59	0.58 ¥	0.72 *	
			PAHs (ng/g creatinine)				
			2-Hydroxyfluorene	196.4	301.9 #, ¥	962.9 *	
			3-Hydroxyfluorene	71.5	135.6 #, ¥	555.6 *	
			9-Hydroxyfluorene	214.9	387.6 #	411.6 *	
			1-hydroxynaphthalene	1636	1339 ¥	7187 *	

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Reference	Study Objective and Design	Number of Subjects and Products	Results	Comments, Summary & Conclusions
			2-hydroxynaphthalene: 1808 / 1881 ¥ / 8955 * 1-hydroxyphenanthrene: 129.0 / 148.4 / 190.6 * 2-hydroxyphenanthrene: 47.0 / 60.9 #, ¥ / 85.6* 3-hydroxyphenanthrene: 83.1 / 108.9 #, ¥ / 177.7 * 4-hydroxyphenanthrene: 19.3 / 19.1 ¥ / 39.6 * 1-hydroxypyrene: 43.8 / 67.4 #, ¥ / 122.78 * based on adjusted regression analysis: * significant difference smokers vs. nonusers, # significant difference STP users vs. nonusers, ¥ significantly ↓ in STP users vs. smokers or smokers vs. STP users § Geometric mean Out of a total of 46 exposure biomarkers evaluated, 13 were excluded, as more than 40% of the samples were below the LOD for all three categories (data not shown).	
Kotlyar et al. 2007	Objective: Comparison of nicotine PK and subjective effects of new PREPs, moist snuff, and nicotine lozenge Randomized crossover study 5 separate sessions of each product once for 30 min; 12 hrs abstinence before, blood sampling and subjective measures (craving, withdrawal, product effects and liking) for 90 min total (during use + 1hr)	*Ariva, Revel, Stonewall, Copenhagen, Commit (4-mg nicotine)* 10 regular STP (*Copenhagen*) users (average use 2.4 (1.5-3.5) tins, 8.1 (3-25) dips/day)	Nicotine PK parameters in plasma of STP users after 12 hrs of abstinence from *Copenhagen* moist snuff use and subsequent product use for 30 min (Geometric means (95% CI)) *Copenhagen* 2 g: t_{max} ~30 min; C_{max} 16.1 (12.1-21.5) ng/mL; $AUC_{(0-90)}$ 1038 (806-1336) ng*min/mL *Commit* 1x 4-mg lozenge: ~30; 7.3 (5.5-9.8); 467 (361-604) *Stonewall* 1 lozenge: ~45; 4.1 (3.1-5.4); 292 (226-376) *Ariva* 1 lozenge: ~25; 2.7 (2.0-3.6); 192 (149-248) *Revel* 1 pouch: ~45; 2.6 (2.0-3.5); 189 (146-244)	• *Copenhagen* use produced a nicotine C_{max} and AUC plasma >2x ↑ compared to *Commit* use, 4x ↑ compared to *Stonewall* use, and 5-6x ↑ compared to *Ariva* and *Revel* use • Commit use produced a nicotine C_{max} and AUC plasma 1.6-2.8x ↑ compared to *Stonewall*, *Ariva* and *Revel* use • New PREPs resulted in lower nicotine concentrations and equivalent or lower reductions in subjective measures compared with medicinal nicotine.

Table A III-7: Studies That Measured Biomarkers in Snus Users, Users of New Products Marketed as Snus, and STP Users*

<table>
<tr><th>Reference</th><th>Study Objective and Design</th><th>Number of Subjects and Products</th><th>Results</th><th>Comments, Summary & Conclusions</th></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Fant et al. 1999</td><td>Objective: Nicotine plasma levels from 4 popular brands of moist snuff; physiological and subjective effects of products in relation to non-tobacco mint snuff

5 sessions:
after 3 hrs of abstinence 30 min of snuff use</td><td>Copenhagen, Skoal Long Cut Cherry, Skoal Original Wintergreen, Skoal Bandits, a non-tobacco mint snuff (either Smokey Mountain Snuff or Oregon Mint Snuff)

10 STP users</td><td>Nicotine PK Parameters in Plasma
2 g of snuff or 1 pouch (0.5 g Skoal Bandits) for 30 min after 3 hrs of abstinence
<table>
<tr><th>US Snuff Product</th><th>t_{max} (min)</th><th>C_{max} (ng/mL)</th><th>$AUC_{(1\text{-}30min)}$ (ng*min/mL)</th></tr>
<tr><td>All Moist snuffs</td><td>~22-35</td><td>14.9-19.5</td><td>208.0-530.4</td></tr>
<tr><td>Copenhagen
11.4 mg/g nicotine, pH 8.6</td><td>~35</td><td>19.5</td><td>530.4</td></tr>
<tr><td>Skoal long cut cherry
11.4 mg/g nicotine, pH 7.5</td><td>~15</td><td>14.9</td><td>333.9</td></tr>
<tr><td>Skoal Original wintergreen
10.4 mg/g nicotine, pH 7.6</td><td>~22</td><td>14.9</td><td>376.3</td></tr>
<tr><td>Skoal bandits</td><td>~30</td><td>4.2</td><td>208.0</td></tr>
<tr><td>Non-tobacco mint snuff</td><td>~22</td><td>0.9</td><td>119.4</td></tr>
</table></td><td>• Amount and rate of nicotine absorption ↑ with ↑ STP product pH
• The authors stated that nicotine delivery was shown to be significantly higher and faster for Copenhagen than for the other two products with lower pH values.
• The authors also noted that subjective effects of moist snuff were also associated with the changes in plasma nicotine concentrations. This was also true for heart rate.</td></tr>
</table>

* Select studies: studies that either had comparative data for traditional Swedish snus/new products marketed as snus users with smokers and/or NRT users, or select studies on US STP users to supplement data

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Appendix IV

Nonclinical Toxicology Studies

Appendix IV-A

Nonclinical Toxicology Studies: In-vitro and Genotoxicity Studies of Traditional Swedish Snus and New Products Marketed as Snus

APPENDIX IV, TABLE A
NONCLINICAL TOXICOLOGY STUDIES: IN-VITRO AND GENOTOXICITY STUDIES OF TRADITIONAL SWEDISH SNUS AND NEW PRODUCTS MARKETED AS SNUS (N=13)

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr><td colspan="5">Traditional Swedish Snus</td></tr>
<tr>
<td>Cederblad et al. 2012</td>
<td>Objective: To evaluate the combined effects of single nucleotide polymorphism (SNPs) with ethanol and tobacco products on cell behavior from healthy individuals

Test system: Peripheral blood mononuclear cells (PBMCs) from 54 health donors, analyzed for SNPs in 30 candidate genes

Endpoints: cell cycle progression, cell death</td>
<td>Ettan snus, Marlboro American Blend cigarettes, ethanol 0.2%, pure nicotine 100 µM

Extracts: Snus - no extraction solvent information provided, smoke extract, incl. particulate phase extracted with ethanol; both were normalized to 100 µM nicotine

Groups: ethanol, nicotine, snus extract, snus extract + ethanol, smoke extract + ethanol

Duration: 3 days</td>
<td>
<table>
<tr><th rowspan="2">Cell status (%)</th><th rowspan="2">Controls</th><th rowspan="2">Nicotine</th><th rowspan="2">Snus</th><th colspan="3">EtOH +</th></tr>
<tr><th>Snus</th><th>smoke</th><th>-</th></tr>
<tr><td>G0/G1 -phase</td><td>93.2</td><td>88.6**</td><td>95.4</td><td>95.0</td><td>96.7</td><td>89.8**</td></tr>
<tr><td>S-phase</td><td>2.6</td><td>7.1**</td><td>3.2</td><td>3.6</td><td>1.9</td><td>6.1**</td></tr>
<tr><td>G2-phase</td><td>1.8</td><td>2.9*</td><td>1.6</td><td>1.3</td><td>1.5</td><td>1.8</td></tr>
<tr><td>Death</td><td>11.1</td><td>13.9</td><td>11.7</td><td>12.2</td><td>19.8**</td><td>12.2</td></tr>
</table>
*p<0.05; ** p<0.005

Normal cell cycle progression in cells under nicotine and ethanol treatment correlated with SNPs in 6 different genes: IL-12RB2, Rad52, ABCA1, XRCC2,CCND3 and TP53

The degree of cell death in ethanol-treated cells was significantly correlated with SNP genotypes in MDM2, ABCA1, GASC1 genes of individual blood donors.

Cell death under snus treatment was correlated to a SNP in the ABCA1 gene[1].
</td>
<td>
• Cell cycle progression and cell death after treatment with snus or snus + ethanol did not differ from those measured in control cells.
• Cigarette smoke extract + ethanol caused significant ↑ in cell death, but no impact on cell cycle.
• Both, pure nicotine at the same concentration as in the snus or smoke extract or pure ethanol significantly ↑ number of cells in stages of cell replication.
• SNPS in 10 out of 30 candidate genes investigated correlated with cell cycling behavior induced by ethanol and/or tobacco products
• The authors concluded that in normal human resting cells, cigarette smoke + ethanol induced massive cell death without any direct influence on cell cycle progression.
• Certain SNPs might predict the
</td>
</tr>
</table>

[1] *ABCA1* gene: encodes in humans for the protein ATP-binding cassette transporter ABCA1 (member 1 of human transporter sub-family ABCA), also known as the cholesterol efflux regulatory protein (CERP). This transporter is a major regulator of cellular cholesterol and phospholipid homeostasis

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr><td></td><td></td><td></td><td></td><td>individual risk of developing diseases and cancer induced by exposure to cigarette smoke and alcohol.</td></tr>
<tr><td>Coggins et al. 2012</td><td>Objective: To determine whether Swedish snus is active in in vitro assays classically used to predict carcinogenicity in humans

Test System: Salmonella typhimurium, mouse lymphoma TK cells, Chinese hamster fibroblasts V79, Balb/c 3T3 mouse fibroblasts

Endpoints: mutagenicity (Ames reverse mutation, mouse lymphoma (ML) forward mutation, micronucleus formation (MN)), cytotoxicity (neutral red cell viability assay)</td><td>General PSOL, Catch Licorice PSWL, Catch Dry Mini PSW, Catch Dry Mini 2 PSW (experimental flavoring), 2S3 reference moist snuff

Extracts: 500 mg/mL for 24 hrs at 37°C water; Catch Dry Mini 2 PSW: water, DMSO

Groups: aqueous extracts of 4 snus and 1 reference products, DMSO extract of experimental snus

Duration: 3-24 hrs</td><td>:
<table>
<tr><th></th><th>General</th><th>Catch Licorice</th><th>Catch Dry Mini</th><th colspan="2">Catch Dry Mini 2 experimental</th><th>2S3</th></tr>
<tr><th></th><th></th><th></th><th></th><th>water</th><th>DMSO</th><th></th></tr>
<tr><td>Extract concentration</td><td colspan="6">Cell Viability after 24 hrs (%)</td></tr>
<tr><td>25 mg/mL</td><td></td><td></td><td>70-90</td><td>70-90</td><td>80</td><td></td></tr>
<tr><td>50 mg/mL</td><td>70-80</td><td>70-80</td><td>0</td><td>0</td><td>44</td><td>70-80</td></tr>
<tr><td>Test System</td><td colspan="6">Genotoxicity</td></tr>
<tr><td colspan="7">Without S9</td></tr>
<tr><td>TA98</td><td></td><td></td><td>+</td><td>+</td><td></td><td></td></tr>
<tr><td>TA100</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>TA1535</td><td>(↑↑)</td><td>(↑↑)</td><td></td><td></td><td></td><td></td></tr>
<tr><td>TA1537</td><td>+</td><td></td><td>++</td><td></td><td></td><td></td></tr>
<tr><td>TA102</td><td>++</td><td></td><td>+</td><td>++</td><td>(+)</td><td>+</td></tr>
<tr><td>MLA 3 hr</td><td></td><td></td><td></td><td></td><td>+</td><td></td></tr>
<tr><td>MLA 24 hr</td><td>+</td><td>(+)</td><td>++</td><td>+++</td><td>+</td><td>+</td></tr>
<tr><td>MN 3 +17hrs*</td><td></td><td></td><td></td><td>++</td><td>+</td><td></td></tr>
<tr><td>MN 20 hrs</td><td></td><td></td><td></td><td>++</td><td></td><td></td></tr>
<tr><td colspan="7">With S9</td></tr>
<tr><td>TA98</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>TA100</td><td></td><td></td><td>+</td><td></td><td>+</td><td></td></tr>
</table></td><td>• Both water extracts of Catch Dry Mini samples were strongly cytotoxic at the highest extract concentrations tested. Therefore, positive results in Ames test, MLA, and MNA at the higher concentration may be explained by overt cytotoxicity
• General and Catch Licorice were mostly negative in both MLA and MNA, except for some instances where only the highest concentrations tested significantly positive with no indication of a dose-response relationship at lower concentrations
• Only without metabolic activation, General and Catch Licorice responses in TA1535 were 2-3 times ↑ compared to solvent control, but only at the highest concentration; General responses in TA102 were also significantly ↑ at the two highest concentrations tested
• After metabolic activation only, 2S3 responses in MNA were significantly and dose-related ↑ at the two highest</td></tr>
</table>

CITATION	STUDY OBJECTIVE & DESIGN	TEST PRODUCTS, DOSAGE, DURATION,& GROUPS	RESULTS	SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS
			TA1535 + TA1537 + TA102 + MLA 3 hr + MN 3+17hrs* + + ++ +++ ++ * 3 hrs treatment + 17 hrs recovery period (↑↑): 2-3x ↑ over solvent control response, but only at highest concentration ++: 2 highest concentrations significantly ↑ compared to solvent control response +: highest concentration significantly ↑ compared to solvent control response (+): random significantly ↑ compared to solvent control response, but no D-R	concentrations tested • The authors concluded that the broadly negative findings add to the large amount of epidemiological data from Scandinavia showing the Swedish snus are associated with considerably lower carcinogenic potential when compared with combusted tobacco products. • Limitations: water extracts only (except for experimental snus)
Laytragoon -Lewin et al. 2011	Objective: To assess the direct effects of test products/ substances on adult normal fibroblasts from the oral cavity and adult endothelial cells Test system: Adult normal human endothelial cells (HSAVEC), normal human fibroblasts (F19),	*Ettan* snus, American blend cigarettes, 0.2% ethanol, pure nicotine Extracts: 7.5 g Snus in medium for 16 hrs, vol not given, Smoke extract - particulate phase extracted with ethanol; Extracts had nicotine concentrations: 12.5-100 µM and up to400 µM for	<u>Cell proliferation after 24-hr treatment</u>: • 0.2% ethanol alone – no effect • Highest nicotine concentration as pure nicotine (400 µM) or snus extract (100 µM) showed a tendency to ↓ DNA synthesis • At lower nicotine concentrations (25-100 µM) both pure nicotine and snus extract had a tendency to ↑ DNA synthesis, addition of ethanol slightly ↑ this tendency • At the same nicotine and ethanol concentrations smoke extract strongly ↓ DNA synthesis <u>Cell morphology and death after 24-hr treatment</u>: • 0.2% ethanol alone – no effect • At 100 µM nicotine both pure nicotine and snus extract with or without ethanol caused prominent cytoplasmic vacuolization, but no cell death At the same nicotine and ethanol concentrations smoke extract significantly induced phenotypic abnormalities, cells lost normal cell integrity and cell-to-cell contact, cell death ↑	• Snus extract containing 100 µM nicotine changed gene expression and caused vacuolization in both fibroblast and endothelial cells and ↓ DNA synthesis in endothelial cells; addition of ethanol only slightly influenced these outcomes • Cigarette smoke extract containing 100 µM nicotine + ethanol changed gene expression less, but caused strong cell abnormalities and ↑ cell death and ↓ DNA synthesis • 100 µM nicotine changed gene expression and caused vacuolization in both fibroblast

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr><td></td><td>non-immortalized[2]

Endpoints: cell proliferation (DNA synthesis), gene expression profiles, cellular morphology & cell death</td><td>nicotine only

Groups: ethanol, nicotine, nicotine + ethanol, snus extract, snus extract + ethanol, smoke extract + ethanol

Duration:
Up to 24 hrs</td><td>(68% endothelia cells and 52% fibroblasts)

Gene Expression Changes
(% cells compared to controls)
after 1 hr-treatment at nicotine concentrations 100 µM
<table><tr><th rowspan="2">Cell Type</th><th rowspan="2">Nicotine</th><th rowspan="2">Snus</th><th colspan="4">EtOH (0.2 +</th></tr><tr><th>Snus</th><th>smoke</th><th>nicotine</th><th>-</th></tr><tr><td>Endothelial cells</td><td>84</td><td>81</td><td>85</td><td>51</td><td>85</td><td>60</td></tr><tr><td>Fibroblasts</td><td>73</td><td>78</td><td>78</td><td>73</td><td>48</td><td>47</td></tr></table></td><td>and endothelial cells, ↑ DNA synthesis; addition of ethanol only slightly influenced these outcomes
• The authors noted that smoking 25 cigarettes per day a smoker accumulates approximately 100 µM nicotine in saliva (Feyerabend et al. 1985)
• The authors concluded that cigarette smoke induced massive cell death and various abnormalities at cellular and molecular levels in surviving endothelial cells and fibroblasts, and genetic alterations together with inflammatory environment may potentially promote tumorigenesis in smokers;
• They also noted that nicotine replacement therapy might induce abnormalities in normal cells
• Limitations: No statistical evaluation was provided</td></tr>
<tr><td>Sandhu et al. 2011</td><td>Objective: To examine the effects of water-</td><td>General snus, nicotine</td><td>• Initial studies with nicotine concentrations seen in snus users (15 ng/mL[3]) showed no effect, therefore levels as seen in smokers (25 ng/mL[4]) were studied</td><td>• Cerebral arteries exposed to snus extract or nicotine altered G-protein-coupled receptor-</td></tr>
</table>

[2] DMEM/Harm's F-12 media with 10% fetal bovine serum
[3] As cited to be reported in Foulds et al. 2003 after one dose of snus use
[4] As cited to be reported in Benowitz et al. 1994 and Foulds et al. 2003

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr>
<td></td>
<td>or lipid (DMSO)-soluble snus and nicotine on the expression of vasocontractile G-protein-coupled receptors (GPCR): Endothelin ET_B (ET) receptor, Serotonin 5-HT_{1B} (5-HT) receptor, Prostanoid TP (TP) receptor

Test system: rat cerebral arteries cultured in serum-free medium

Endpoints: isometric tension/contractile responses to respective receptor agonists (myographic); receptor expression (real-time PCR of</td>
<td>Extract: snus bags dissolved for 1 hr at 37°C in water or DMSO (volume not given). Final nicotine concentrations were 250 µg/mL, diluted 1/10, and diluted to test concentrations in culture of 25 and 250 ng/mL

Groups: water-soluble snus, DMSO-soluble snus, nicotine; all at 25 ng/mL (similar to plasma levels as in smokers), 250 ng/mL nicotine

Duration: 24 hrs</td>
<td>• The lower nicotine concentration did not impact the contraction significantly, but ↓ mRNA levels of all receptors, while the higher nicotine dose ↑ ET and 5-HT–mediated contractions, with no impact on mRNA levels. No effect on protein levels of any receptor.
<table>
<tr><th colspan="7">Vasocontractile GPCR Mediated Changes and Expression</th></tr>
<tr><th>Parameter</th><th colspan="2">Nicotine</th><th colspan="2">Water soluble snus</th><th colspan="2">DMSO-soluble snus</th></tr>
<tr><td>Nicotine concentration (ng/mL)</td><td>25</td><td>250</td><td>25</td><td>250</td><td>25</td><td>250</td></tr>
<tr><td colspan="7">Cerebral artery contraction in response to respective receptor agonists</td></tr>
<tr><td>ET</td><td>(↓)</td><td>↑</td><td>(↓)</td><td>-</td><td>-</td><td>(↑)</td></tr>
<tr><td>5-HT</td><td>(↑)</td><td>↑</td><td>↓</td><td>↑</td><td>↓</td><td>↑</td></tr>
<tr><td>TP</td><td>-</td><td>(↑)</td><td>-</td><td>(↑)</td><td>(↑)</td><td>(↑)</td></tr>
<tr><td colspan="7">Receptor mRNA levels</td></tr>
<tr><td>ET</td><td>↓</td><td>(↓)</td><td>(↓)</td><td>-</td><td>↑</td><td>-</td></tr>
<tr><td>5-HT</td><td>↓</td><td>(↓)</td><td>(↓)</td><td>(↓)</td><td>-</td><td>↑</td></tr>
<tr><td>TP</td><td>↓</td><td>(↓)</td><td>(↑)</td><td>(↑)</td><td>-</td><td>-</td></tr>
<tr><td colspan="7">Receptor protein levels</td></tr>
<tr><td>ET</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>5-HT</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>TP</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
</table>
All responses compared to vehicle control. () slight changes, but not significant.</td>
<td>mediated contractions.
• Snus extracts impacted only the 5-HT receptor-mediated contractions significantly.
• Nicotine only at the higher concentrations impacted ET and 5-HT receptor mediated contractions significantly.
• Only DMSO snus extracts and nicotine at the lower concentration showed significant changes on the transcriptional level (mRNA level) [5]
• The authors suggested that both transcriptional and post-translational mechanisms are responsible for some of the receptor alterations
• The authors hypothesized that alteration of GPCR expression is most likely a molecular mechanism
• The authors concluded that snus and nicotine may have potential impact on cerebral vasculature and on the development of cardiovascular disease</td>
</tr>
</table>

[5] A different study by these investigators (Xie et al. 2010) reported that lipid-soluble cigarette smoke particles and nicotine also altered the expression of some G-protein coupled receptors in the rat artery

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr><td></td><td>mRNA, immunohisto-chemistry)</td><td></td><td>• Water-soluble snus at both nicotine concentrations changed 5-HT-receptor mediated contractions, with no significant impact on mRNA or protein levels of any receptor
• DMSO-soluble snus at both nicotine concentrations changed 5-HT-receptor mediated contractions with ↑ mRNA levels of this receptor at the higher concentration. ET mRNA levels ↑ at the lower nicotine concentration. Protein levels of any receptor were not affected.</td><td></td></tr>
<tr><td>Costea et al 2009</td><td>Objective: To investigate in vitro the biological effects of a toombak extract in comparison with a Swedish snuff extract

Test system: Primary (from healthy human buccal mucosa) keratinocytes (NOK) and fibroblasts (NOF); dysplastic oral keratinocytic cell line (DOK)

Endpoints: cell morphology, cell viability (ELISA), cell cycle (flow cytometry), DNA</td><td>Ettan snus (Gothia Tobak AB), Toombak

Aqueous extracts: 100 g/300 mL phosphate buffer pH 7.2-4, 1 hr, 37°C) at different dilutions

Groups: Controls, Ettan, toombak

Duration: up to 6 days</td><td>
<table>
<tr><th colspan="7">Component Concentrations</th></tr>
<tr><td></td><td colspan="3">Ettan</td><td colspan="3">Toombak</td></tr>
<tr><td>Component</td><td>nicotine</td><td>NNK</td><td>NNN</td><td>nicotine</td><td>NNK</td><td>NNN</td></tr>
<tr><td>Product (µg/g dw)</td><td></td><td></td><td></td><td></td><td>427</td><td>232</td></tr>
<tr><td>Saliva* (µg/mL)</td><td>73-1560</td><td></td><td>0.025 -0.42</td><td>3-620</td><td></td><td>0.5-20</td></tr>
</table>
* data from Idris et al. 1992 and Hoffmann et al. 1981
<table>
<tr><th colspan="5">Cytotoxicity</th></tr>
<tr><td>Parameter</td><td colspan="2">Ettan</td><td colspan="2">Toombak</td></tr>
<tr><td>Extract Dilution</td><td>1/100</td><td>1/10</td><td>1/100</td><td>1/10</td></tr>
<tr><td>Nicotine (µg/mL)</td><td>170</td><td>1700</td><td>300</td><td>3000</td></tr>
<tr><td>NNK (µg/mL)</td><td>0.063</td><td>0.63</td><td>8.3</td><td>83</td></tr>
<tr><td>NNN (µg/mL)</td><td>0.042</td><td>0.42</td><td>4.9</td><td>49</td></tr>
<tr><td>Cell morphology at 24 hrs</td><td>(+) NOK cells</td><td>++</td><td>+</td><td>++</td></tr>
<tr><td>Cell death (%) after 24 hrs</td><td><20</td><td>>70</td><td>≥60</td><td>>80</td></tr>
</table>
• LD_{50} of Ettan snus 2.5-25x higher than for toombak with difference for NOK>DOK>NOF
• 10x higher extract concentration (1/100) of Ettan snus compared to toombak (1/1000) was necessary to cause</td><td>• Compared to toombak extract, 10-100x higher extract concentrations of Ettan snus were necessary to cause similar changes in cell morphology, apoptosis, cell proliferation
• Cell LD_{50}s of Ettan snus were 2.5-25x higher than those of toombak
• The authors concluded that together with the product chemistry and epidemiological data, the results indicate a much higher potential for toombak to induce abnormal development of normal oral mucosal cells than Swedish snuff.
• Limitation:
Nicotine and TSNA levels found in 1/100 extract of Ettan snus are on the very low end of levels found in saliva in snus users, while those in 1/10 extract are on</td></tr>
</table>

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr><td></td><td>double-strand breaks, apoptosis (chromatin condensation, annexin V), plasma membrane permeability, cell growth</td><td></td><td>significant ↑ number of cells in G2 phase (G2/M block) in both NOK and NOF cells
• 10x higher extract concentration (1/10) of Ettan snus compared to toombak (1/100) was necessary to cause significant ↑ in chromatin condensation and Annexin V-detectable membrane changes indicating apoptosis
• 100x higher extract concentration (1/10) of Ettan snus compared to toombak (1/1000) was necessary to cause significant ↑ number of cells with DNA double strand breaks in NOK. Fibroblasts were less sensitive than keratinocytes. Dysplastic keratinocytes were resistant to Ettan snus extract, but not to toombak extract, indicating that they are less prone to further transformation and progression towards malignancy induced by snus but not by toombak
• Cumulative adverse effects over a 6-day treatment: no influence of NNN or nicotine alone at concentrations equivalent to those in 1/10 extract of toombak; 100x higher extract concentration (1/10-1/100) of Ettan snus compared to toombak (1/1000-10000) was necessary to cause significant ↓ number of cells, with sensitivity of NOK ≥NOF≥DOK; 1/1000000 snus extract ↑ number of NOF.</td><td>the very high end. Nicotine and TSNA levels found in 1/100 extract of toombak were rather in the middle of levels detected in toombak users.</td></tr>
<tr><td>Andersson et al. 2006</td><td>Objective: to evaluate the effect of Swedish moist snuff extract in comparison with US moist snuff on PDL fibroblast growth[6]</td><td>Swedish moist snuff (Ettan Gothia Tobak AB) and American snuff (Kentucky reference snuff)

Extracts: 100 g snuff/300 mL distilled water for 1 hr), diluted to 1-100</td><td><table><tr><th colspan="5">Component Concentrations (µg/mL)</th></tr><tr><td></td><td colspan="2">Ettan</td><td colspan="2">Kentucky Ref Snuff</td></tr><tr><td>Component</td><td>nicotine</td><td>TSNAs</td><td>nicotine</td><td>TSNAs</td></tr><tr><td>Extract</td><td>10,600</td><td>0.3</td><td>15,800</td><td>1.5</td></tr><tr><td>10% Extract</td><td>1,600</td><td>0.03</td><td>1,580</td><td>0.15</td></tr><tr><td>3% Extract</td><td>318</td><td>0.009</td><td>474</td><td>0.045</td></tr></table></td><td>• 0.3%-1% extract no effect or ↑ cell number, no effect on alkaline phosphatase production
• 3% extract of both snuffs ↓ cell number and production of alkaline phosphatase
• No differences between Swedish and Kentucky moist</td></tr>
</table>

[6] Cells cultured in DMEM with 1% fetal bovine serum

CITATION	STUDY OBJECTIVE & DESIGN	TEST PRODUCTS, DOSAGE, DURATION,& GROUPS	RESULTS	SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS
	Test System periodontal ligament cells (from 3 healthy volunteers) Endpoints: cell morphology, alkaline phosphatase production (ELISA)	µL/mL Groups: controls, 0.3%, 1 3%, (10% extract) Duration: 24 hrs	• In pre-experiments, 0.3% extracts ↑ alkaline phosphatase production, while 10% snuff extracts caused 100% cell death within 9 hrs	snuff with respect to morphological changes and production of alkaline phosphatase • The authors concluded that smokeless tobacco has biological effects on periodontal tissues, in terms of the two markers measured
Merne et al. 2004	Objective: To investigate the effects of snuff extract on growth and differentiation of oral epithelial tissues. Test system: three-dimensional cell culture consisting of co-culture of HaCaT cells[7] and fibroblasts from primary buccal mucosa in collagen gel	*Ettan* Snus (8 mg/g nicotine) Extract: 1 g/10 mL FAD[8] medium for 2 hrs at 37°C; 10% w/v = 6 mg/mL nicotine, diluted to 1% concentration Groups: 1% extract, controls Duration: 6-18 days treatment	• Mean Ki-67-positive nuclei per field examined ↓ in snus extract-exposed compared to control cells, but only significantly on day 6 of culture. • P53 expression ↓ in several snus extract-exposed cells compared to control cells (HaCaT cells have mutation in *TP53* gene resulting in increased p53 positive cells) • Cytokeratin (Ck) 10 ↓ in snus extract-exposed compared to control cells, indicating disturbances in the differentiation process • Exposure to snuff for more than 12 days resulted in morphologic changes such as cellular damage (intercellular dyskeratosis, cellular vacuolization, lack of basal cell layer, apoptotic cells with nuclear fragmentation and other nuclear abnormalities), impaired cellular adhesions and severe degeneration of the epithelium. . • No differences in matrix components (collagen, fibroblasts)	• Authors considered experimental setting to be similar to wound healing. • In agreement with findings in their previous study of snuff user's lesions (Merne et al. 2002), snus extract did not stimulate cell proliferation, as detected by Ki-67 staining • 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.

[7] Immortalized human keratinocyte cell line
[8] FAD medium contains D-MEM with 25% Ham-F12 medium and 10% fetal bovine serum

CITATION	STUDY OBJECTIVE & DESIGN	TEST PRODUCTS, DOSAGE, DURATION,& GROUPS	RESULTS	SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS
	Endpoints: cell morphology; immunohisto-chemistry of markers of cell proliferation (Ki-67), cell cycle regulators (p53) and epithelial differentiation (cytokeratins, involucrin, filaggrin)			
Hasseus et al. 1997	Objective: To evaluate how commercial Swedish moist snuff and some of its derivatives affects the functional capacity of accessory cells[9] Test system: spleen cells, T-cells, oral mucosa epithelial cells isolated from Lewis rats; T-cell mitogenesis	*Röda Lacket* snus Extracts: snus 10 g/50 mL DMEM at 37°C for 1 hr; alkaloids and TSNAs: all diluted to concentrations as found in snus (100 µg/g anabasine, 10 mg/g nicotine, 0.2 µg/g NAB, 2.5 µg/g NNN, 0.8 µg/g NNK, 0.001 µg/g NDMA) Groups: snus extracts, individual alkaloids & TSNAs	• Spleen cells: 0.8% snus extract concentration significantly ↓ cell proliferation (DNA synthesis); 2% extract = IC_{50}; after pretreatment viable cells were incubated for 72 hour: ≤6% extract – recovery, no recovery with 50% extract • Oral epithelial cells, incl. Langerhans cells and T-cells: 12.5% snus extract concentration significantly ↓ T-cell proliferation (DNA synthesis); 4% extract = IC_{50}; after pretreatment, viable cells were incubated in cross over design (pretreated epithelial cells + untreated T cells and vice versa) ≤6% extract – recovery, no recovery after pretreatment of epithelial/Langerhans cells with 50% extract + untreated T-cells or pretreatment of T-cells with 50% extract + untreated epithelial/Langerhans cells. • Spleen cells: incubation or pretreatment with alkaloids or TSNAs at similar concentrations as found in snus did not have any significant impact on spleen cell proliferation • Oral epithelial cells, incl. Langerhans cells and T-cells:	• While snus extract significantly ↓ spleen cell and T-cell proliferation with IC_{50} = 2-4% extract (4-8 µg snus/mL), the individual alkaloids and TSNAs tested did not change ↓ spleen cell and T-cell proliferation significantly. • The authors concluded, that snus extract can evoke an immunosuppressive effect on mitogen-driven T-cell proliferation using cells from oral epithelium as accessory cells • The authors noted that the local immunosuppression may be induced not only through an

[9] Accessory cell: antigen-presenting cell (APC), a cell that displays foreign antigen complexes with major histocompatibility complex (MHC) on their surfaces. T-cells may recognize these complexes using their T-cell receptors (TCRs). These cells process antigens and present them to T-cells.

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr><td></td><td>stimulated with Concanavalin A (Con A)[10]

Endpoints: Cell viability (Trypan blue exclusion) after pretreatments only; cell proliferation ([^{3}H]-thymidine incorporation into DNA synthesis during S phase)</td><td>Duration:
72 hrs incubation, 4 hrs pretreatment</td><td>incubation or pretreatment with Alkaloids or TSNAs at similar concentrations as found in snus did not have any significant impact on T-cell proliferation, although NNN had a tendency to be stimulatory, while NAB had a tendency to be inhibitory
• None of the components was mitogenic (tested without Con A treatment)
• Nicotine at a similar concentration as found in snus (10 mg/g) resulted in a solution with pH>9 and could not be tested at this concentration; dilution to 1/10000 of normally detected in snus was tested and no impact on cell proliferation</td><td>effect not only on Langerhans cells but also T-cells
• The authors noted that impaired immunosurveillant capacity may open direct or indirect avenues for adverse effects</td></tr>
<tr><td>Liu et al. 1997</td><td>Objective: to investigate expression of O^6-methylguanine-DNA methyl-transferase (MGMT)[11] in specimens of buccal mucosa, and cultures of both normal mucosal epithelial cell and fibroblasts;
To evaluated if</td><td>Snuff unspecified

Extracts: Snuff extracts (from M. Curvall, Swedish Tobacco Company) prepared as in Jansson et al. 1991);
Tobacco, [bidi smoke condensate, betel leaf, areca nut] *
Positive control: mercury chloride</td><td><table>
<tr><th colspan="6">MGMT enzyme activity and Cytotoxicity</th></tr>
<tr><td>Extraction Solvent</td><td colspan="2">Water</td><td colspan="3">Methylene chloride</td></tr>
<tr><td>Product</td><td colspan="2">Snuff</td><td>Snuff</td><td colspan="2">Tobacco</td></tr>
<tr><td>Extract concentration (µg/mL)</td><td>100</td><td>150</td><td>900</td><td>700</td><td>1000</td></tr>
<tr><td>MGMT activity (% of control)</td><td>74 ±16</td><td>62 ±7</td><td>69 ±13</td><td>80 ±3</td><td>66 ±13</td></tr>
<tr><td>Inhibition of MTT reduction (% of control)</td><td>50</td><td>70</td><td>50</td><td>50</td><td>N/A</td></tr>
</table></td><td>• Aqueous and organic snuff extracts as well as organic tobacco extract ↓ MGMT activity at concentrations that also caused cytotoxicity
• With respect to the in vitro studies conducted, the authors concluded that they “indicated the possible inhibition of MGMT by habitual use of tobacco and betel quid. The release and absorption of aqueous or lipid-soluble reactive chemicals from these sources may potentially</td></tr>
</table>

[10] Con A: Concanavalin A is a lectin. In cell culture applications, it has the ability to induce mitogenic activity of T-lymphocytes and to increase synthesis of cellular products (Sigma-Aldrich Product Information).
[11] MGMT: Enzyme that repairs premutagenic O^6-methylguanine lesions induced in DNA by alkylating agents.

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr><td></td><td>complex mixtures from tobacco and similar products can influence MGMT

Test system: human buccal fibroblasts

Endpoints: Cell viability (MTT assay);
MGMT activity</td><td>*data here not presented

Groups: organic extracts of snuff, tobacco;
Aqueous extracts of snuff</td><td></td><td>reduce the number of functional MGMT molecules in buccal mucosa and, therefore, increase the risk of oral cancer associated with the concomitant exposure to alkylating agents.”</td></tr>
<tr><td>Jansson et al. 1991</td><td>Objective: To investigate the potential genotoxicity of Swedish moist oral snuff to provide optimal data for the prediction of carcinogenicity in rodents.

Test system: Salmonella typhimurium, V79 Chines hamster cells, human lymphocytes test, mice/mouse bone marrow cells Drosophila melanogaster</td><td>Swedish snus (no brand provided), regular and unsalted

Extracts: snus regular or salt-free - 100 g/300 mL water, 1 hr; regular snus - 50 g/300 mL methylene chloride, 4 hrs

Groups:
Snus regular aqueous, methylene chloride, snus salt-free aqueous</td><td>
<table>
<tr><th colspan="4">Genotoxicity of Snus Extracts</th></tr>
<tr><th>Extracts/ Endpoint studied</th><th>Snus aqueous</th><th>Snus salt-free aqueous</th><th>Snus methylene chloride</th></tr>
<tr><td>In vivo MNs</td><td>-</td><td>Not tested</td><td>-</td></tr>
<tr><td>In vitro SCEs</td><td>+</td><td>Not tested</td><td>+</td></tr>
<tr><td>Sex-linked lethal mutations</td><td>Not tested</td><td>Not tested</td><td>-</td></tr>
<tr><td colspan="4">-S9</td></tr>
<tr><td>TA98</td><td>-/-</td><td>Not tested</td><td>-/+</td></tr>
<tr><td>TA100</td><td>-/+</td><td>Not tested</td><td>-/+</td></tr>
<tr><td>TA1535</td><td>-/-</td><td>Not tested</td><td>-/-</td></tr>
<tr><td>TA1537</td><td>-/-</td><td>Not tested</td><td>+/-</td></tr>
<tr><td>CAs</td><td>+</td><td>-</td><td>-</td></tr>
<tr><td>HPRT gene mutation</td><td>-/-</td><td>Not tested</td><td>-/-</td></tr>
<tr><td colspan="4">+S9</td></tr>
<tr><td>TA98</td><td>-/-</td><td>Not tested</td><td>+/+</td></tr>
</table>
</td><td>• Methylene chloride extraction was added to estimate impact of local exposure to hydrophobic compounds by direct contact with snus
• The aqueous extract was considered non-mutagenic in the Salmonella typhimurium strains tested, while the methylene extract was mutagenic after metabolic activation;
• Neither snus extract induced gene mutations in mammalian cells at the HPRT locus in vitro
• CAs observed with the aqueous extract without metabolic activation were induced likely due to the high salt content in snus; both snus extracts induced CA after</td></tr>
</table>

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr>
<td></td>
<td>Endpoints:
Ames reverse mutagenicity, chromosome aberrations (CAs), gene mutations (HPRT), sister chromatid exchange (SCE) micronucleus (MN) induction, sex-linked recessive lethal mutations</td>
<td></td>
<td>
<table>
<tr><td>TA100</td><td>-/+</td><td>Not tested</td><td>+/+</td></tr>
<tr><td>TA1535</td><td>-/-</td><td>Not tested</td><td>-/-</td></tr>
<tr><td>TA1537</td><td>-/-</td><td>Not tested</td><td>-/+</td></tr>
<tr><td>CAs</td><td>+</td><td>+</td><td>+</td></tr>
<tr><td>HPRT gene mutation</td><td>-/-</td><td>Not tested</td><td>-/+</td></tr>
</table>
+: significant difference; -: no significance, results from 2 experiments
<u>Aqueous snus extract</u>
• Ames test: (at concentrations corresponding to 220 mg snus/plate) considered to be negative in all strains in two experiments.
• CAs: induced CAs in presence and absence of metabolic activation; salt-free snus aqueous extract induced only in presence of metabolic activation.
• SCE: highly significant and dose-related
• No effect in HPRT assay or in vivo mouse bone marrow cells in MN assay
<u>Methylene chloride snus extract</u>
• Ames test: mutagenic effects, unequivocally with metabolic activation
• CAs: significantly induced CAs in presence of metabolic activation
• SCE: highly significant and dose-related
• No effect in HPRT assay or in vivo mouse bone marrow cells in MN assay, or recessive lethal mutations in Drosophila
</td>
<td>metabolic activation in mammalian cells in vitro, but not MN in vivo in mice
• Both extracts induced SCEs in human lymphocytes in vitro
• The methylene extract did not cause recessive lethal mutations in Drosophila
• The authors concluded that based on their results the carcinogenic potential of Swedish snus should be considered to be low, a conclusion in agreement with the low oral cancer incidence in Sweden.</td>
</tr>
<tr>
<td>Curvall et al. 1987</td>
<td>Objective: To investigate if the urine from snuff users, like that of smokers, exhibits elevated levels of mutagens</td>
<td>Swedish wet snuff (no brand given), cigarettes

Extracts: Urine 24-hr samples filtered over Sep-Pak C_{18} columns, after</td>
<td>• Mutagenic activity of the urine samples was detected only in the presence of S9
• Urinary mutagenicity in samples from smokers was significantly ↑ ($p < 0.001$) compared to controls (and all other groups).
• No difference between urinary mutagenicity in snuff users, abstinent snuff users, and controls
• No difference between urinary nicotine and cotinine levels</td>
<td>• The authors detected no significant difference in mutagenic activity between urine from the snuff users and urine from the non-tobacco users.
• In smokers, normal consumer levels of nicotine were</td>
</tr>
</table>

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr><td></td><td>Test system: Salmonella typhimurium TA 98 with or without S9 activation

Endpoints: reverse mutagenicity (Ames Test)</td><td>washing, eluted with dichloromethane; after washing and solvent removal, urine concentrates were dissolved in DMSO for analysis

Groups: (all male) 8 snuff users, 8 smokers, , 6 non-tobacco users; in addition 6 snuff users collected urine after 1 week of abstinence</td><td>between snuff users and smokers; no difference between controls and abstinent snuff users
<table>
<tr><th colspan="5">Urinary Mutagenicity and Nicotine Uptake (Urinary Nicotine Metabolite Concentrations) in Tobacco Product Users</th></tr>
<tr><td>Urinary Parameter</td><td>Non-Tobacco Users</td><td>1-Week Abstinent Snuff Users</td><td>Snuff Users</td><td>Smokers</td></tr>
<tr><td colspan="5">Mean Urinary Concentration</td></tr>
<tr><td>Nicotine mg/L</td><td>0.005</td><td>0.009</td><td>1.39</td><td>1.67</td></tr>
<tr><td>Nicotine mg/24 hr</td><td>0.008</td><td>0.015</td><td>2.00</td><td>2.04</td></tr>
<tr><td>Cotinine mg/L</td><td>0.006</td><td>0.014</td><td>1.46</td><td>2.44</td></tr>
<tr><td>Cotinine mg/24 hr</td><td>0.008</td><td>0.020</td><td>2.12</td><td>3.15</td></tr>
<tr><td colspan="5">Mean Urinary Mutagenic activity +S9 in T98</td></tr>
<tr><td>Revertants/ mL urine</td><td>0.5 (0.2-0.9)</td><td>0.8 (0.4-1.1)</td><td>0.9 (0.3-1.5)</td><td>6.5* (4.2-12.8)</td></tr>
<tr><td>Revertants/ 24 hrs (x10^{-3})</td><td>0.9 (0.4-2.2)</td><td>1.2 (0.5-2.4)</td><td>1.3 (0.3-2.5)</td><td>8.6* (4.2-17.6)</td></tr>
</table>
* significantly different from controls</td><td>accompanied by elevated urinary levels of mutagens, while no such increase in urinary mutagen was observed when the same nicotine levels are reached in snus users.
• The authors concluded that levels of urinary mutagens are not elevated by habitual usage of Swedish snuff</td></tr>
<tr><td>Hirsch et al. 1984</td><td>Objective: To study the effects of water-extractable compounds from snus on HSV replication in vitro

Test system: Monkey kidney cells (GMK &</td><td>Röda Lacket snus, Ettan snus, Tre Ankare pouched snus (low nitrosamines, 6x lower TSNA concentration than Röda Lacket)

Extracts: 10 g/50 mL Eagles MEM at</td><td>• Röda Lacket extract 1:1 and 1:2 induced morphological signs of toxicity in GMK cells after 5-6 days, 1:4 and lower concentrations did not
• 1:10 volume of Röda Lacket extract 1:1 ↓ cell growth measured by cell count and DNA replication slightly (up to 48 hrs incubation), but not concentrations of 1:5 and less; 1:25 seemed to ↑ cell growth
• HSV infectivity: Undiluted Röda Lacket extract slightly ↓ infectivity of HSV
• HSV attachment to cells: 1:50 Röda Lacket extract significantly ↓ uptake rate of HSV into cells compared with</td><td>• Snus extracts did not inactivate HSV, but inhibited virus DNA replication
• The snus brand with higher TSNA concentration inhibited HSV replication significantly stronger than that with lower concentrations
• Non-toxic nicotine concentrations alone did not cause the same extent of virus</td></tr>
</table>

CITATION	STUDY OBJECTIVE & DESIGN	TEST PRODUCTS, DOSAGE, DURATION,& GROUPS	RESULTS	SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS
	Vero cells) Endpoints: cell growth (cell count, DNA replication ([3H]thymidine)); virus production, HSV DNA replication, HSV adsorption, infected cells (radiolabeled virus);	37°C for 1 hr; – nicotine 0.8 mg/mL *Röda Lacket* extract, 1.2 mg/mL in *Ettan* extract Duration: 6 days	controls; 1:1 completely inhibited HSV attachment to cells • HSV replication: 1:50 *Röda Lacket* extract ↓ number of virus plaques to 43%, 1/10 to 9% • Effect after HSV infection: 1:25 *Röda Lacket* extract inhibited HSV to 15-72% 1:5 inhibited to 65-98%, • Effect on HSV DNA synthesis: 1:10 *Röda Lacket* extract ↓ DNA synthesis • Effect on penetration and transport of HSV to cell nuclei: 1:10 *Röda Lacket* extract ↓ • No effect of flavoring agents on inhibitory effect on HSV production; nicotine alone at higher concentrations than the diluted extracts inhibit HSV but not as completely as the extracts	inhibition as the snus extract • The authors concluded that the snus extracts have inhibitory effects on the production of cytolytic HSV-1 infections. They suggested that an interaction between tobacco products and HSV-1 might be involved in the development of dysplastic lesions in the oral cavity.
New Products Marketed as Snus				
Rickert et al. 2009	Objective: To explore the toxicological properties of contemporary STPs by in vitro assays; (to determine levels of target analytes in STPs sold in Canada [details in product chemistry]; to build a market map of commercially available STPs) Test system: Salmonella typhimurium,	*Du Maurier Freshmint* and *Original* snus, Various STPs available on Canadian market Extracts: 25 g/225 mL DMSO, at 37°C for 21 hrs; also subsets extracted with artificial saliva, dichloromethane	<u>*Cytotoxicity:*</u> • *Du Maurier snus Original* DMSO extract caused less than 50% cytotoxicity at the highest sample concentration tested (20 µg/mL dry weight basis). It was similar to other US-type moist snuff tobacco products. Only the Indian gutkha-type product (Manikchand) DMSO extract caused strong cytotoxicity at extract concentrations ≥4 µg/mL dry weight basis. Dichloromethane or artificial saliva extracts of *Du Maurier* snus were not tested. • The moist snuff and chewing tobacco products tested did not cause any significant cytotoxic dose-response. By contrast the extracts of the gutkha product at extract concentrations >10 and ≥4 µg/mL dry weight basis, respectively, significantly ↑ cytotoxicity. <u>*In vitro* micronuclei assay</u>: • None of the DMSO extracts of STP samples, including of *Du Maurier,* reached the 50% cytotoxicity target and with or without S9 metabolic activation did not exceed 1.4% <u>*Ames Assay:*</u>	• The authors hypothesized that several product components could lead to the weak positive responses seen in the assays: • NNK concentrations in the STPs appeared to correlate with the results of the MN assay. • Authors speculated that high salt content and alkalinity could lead to false positive results; while nitrite/nitrate, mutagenic dicarbonyl compounds and Maillard reaction products may be contributing to weak positive mutagenicity and clastogenicity test results • The authors concluded that "attempts to use bioassays of cytotoxicity, clastogenicity, and

CITATION	STUDY OBJECTIVE & DESIGN	TEST PRODUCTS, DOSAGE, DURATION,& GROUPS	RESULTS	SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS
	Chinese hamster ovary (CHO) cells Cytotoxicity (Neutral red), clastogenicity (micronuclei (MN)), mutagenicity (Ames reverse mutation)		• responses weak and variable; no significant dose-response with most of the DMSO extracts, including *Du Maurier snus* extract; none of the responses was 2x ↑ over background.	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 interference in the products."

Appendix IV-B

Nonclinical Toxicology Studies: Animal Studies of Swedish Snus

APPENDIX IV, TABLE B
NONCLINICAL TOXICOLOGY STUDIES: ANIMAL STUDIES OF SWEDISH SNUS (N=9)

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN (Animal Model & Endpoints)</th><th>TEST PRODUCTS, DOSAGE, DURATION, & GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr>
<td>Schwartz et al. 2010</td>
<td>Objective: To assess long-term changes induced by daily usage of 4 different STPs side by side

Test system: Sprague Dawley rats

Route of administration: surgical lip canal model (starting 3 weeks after surgery)

Endpoints: Histopathology of lip canal tissue; immune-histochemistry: p16, protein present in normal oral keratinocytes even with hyperplasia, but suppressed expression in rodents in moderate and</td>
<td>Ettan Snus, Stonewall, Skoal, Copenhagen

Dosage & Duration: 150-200 mg 2x/day for 12 months (5 days/week)

15 rats/group</td>
<td>
Histopathology in Lip Canal Mucosa in Different STP Treatment Groups and Product Chemistry of STPs
<table>
<tr><th>Parameters measured</th><th>Controls (Cotton)</th><th>Stone-wall</th><th>Ettan Snus</th><th>Copen-hagen</th><th>Skoal</th></tr>
<tr><td colspan="6">Reported Product Chemistry</td></tr>
<tr><td>Moisture (%)</td><td></td><td>12</td><td>53</td><td>53</td><td>53</td></tr>
<tr><td>pH</td><td>-</td><td>7.70</td><td>8.52</td><td>7.86</td><td>8.00</td></tr>
<tr><td>Unprotonated nicotine (%)</td><td></td><td>1.3</td><td>1.9</td><td>3</td><td>3</td></tr>
<tr><td>Total TSNAs (ppm)</td><td></td><td>0.28</td><td>5.1</td><td>37.6</td><td>64.0</td></tr>
<tr><td>NNK (ppm)</td><td></td><td>0.04</td><td>2.8</td><td>2.5</td><td>4.3</td></tr>
<tr><td>NNN (ppm)</td><td></td><td>0.06</td><td>1.12</td><td>15.4</td><td>20.8</td></tr>
<tr><td>NAT (ppm)</td><td></td><td>0.17</td><td>1.05</td><td>18.5</td><td>36.8</td></tr>
<tr><td>NAB (ppm)</td><td></td><td>0.007</td><td>0.09</td><td>1.2</td><td>2.1</td></tr>
<tr><td colspan="6">Histological Changes (Grade #) in Mucosa of Lip Canal Tissue</td></tr>
<tr><td>+</td><td>9</td><td>3</td><td>2</td><td>2</td><td>0</td></tr>
<tr><td>++</td><td>1</td><td>7</td><td>9</td><td>5</td><td>3</td></tr>
<tr><td>+++</td><td>0</td><td>2</td><td>1</td><td>4</td><td>8</td></tr>
<tr><td>P16-positive cells in areas of abnormal epithelial tissue architecture (%)</td><td>~57</td><td>~62</td><td>~52</td><td>~45 *</td><td>~37 *</td></tr>
<tr><td>PCNA-positive cells in regions of positive epithelial cells (%)</td><td>~22</td><td>~20</td><td>~27*</td><td>~32*</td><td>~40*</td></tr>
<tr><td>Mitotic cells per field (10 µm length oral mucosa)</td><td>~0.07</td><td>~0.1</td><td>~0.1</td><td>~0.22*</td><td>~0.2*</td></tr>
</table>
</td>
<td>
• Histopathological changes: significantly ↑ in all STP groups compared to controls; dysplastic changes - Ettan ≈ Stonewall & significantly ↓ compared to Skoal & Copenhagen, correlating with TSNA concentrations (& unprotonated nicotine)
• Changes in cell proliferation: PCNA positive cells significantly ↑ with Ettan compared to controls, but not with Stonewall; changes were highest with Skoal and Copenhagen
• Mitotic figures: Ettan ≈ Stonewall ≈ control, but significantly ↑ with Skoal and Copenhagen
• Loss of p16 expression: amount of p16-positive cells among abnormal tissue cells - Ettan ≈ Stonewall ≈ controls, but significantly ↓ with Skoal and Copenhagen
</td>
</tr>
</table>

CITATION	STUDY OBJECTIVE & DESIGN (Animal Model & Endpoints)	TEST PRODUCTS, DOSAGE, DURATION, & GROUPS	RESULTS	SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS
	severely dysplastic and fully malignant oral epithelium; proliferating cell nuclear antigen (PCNA), marker of cell proliferation associated with dysplasia and oral squamous cell carcinoma (OSCC)		# + abnormal mucosa architecture without dysplasia, variable hyperkeratosis & hyperplasia ++ mild dysplasia characterized by low levels of pleomorphism and hyperchromatism at the stratum basalis and adjacent layers, with hyperkeratosis and hyperplasia also typically observed +++ Moderate to severe dysplasia with high levels of pleomorphism and hyperchromatism with abnormal mitoses in lower 1⁄3 to 2⁄3 of epithelium at sites of rete pegs extending into the stroma * significantly different from controls Histopathological changes: • Controls: (Cotton) slight-extensive hyperplasia, hyperkeratosis, not dysplastic • Ettan snus-treated rats: hyperplasia, hyperkeratosis, basal cells with hyperchromatism, dyskeratosis, slight pleomorphism, limited growth (rete pegs) into stroma (connective tissue), mild dysplasia • The authors noted: All STPs "produced varying degrees of acute, sub-acute and chronic inflammation in the stroma. In rare instances this inflammatory infiltrate occupied the epithelium extending from the stratum basalis to the stratum corneum." No correlation between inflammation and dysplastic changes observed, "but it is reasonable to assume that ST induced inflammation earlier may contribute to the original development of the dysplasia and abnormal epithelial extensions."	• Effects were not reversible in 3 month-follow up (data not presented) • The authors noted that p16 is considered a tumor specific marker, and that a decrease "is not associated with reversible mucosal hyperkeratosis or hyperplasia" (changes that occur with short-term exposure to snuff); "in human OSCC there is some disagreement on when during tumor progression p16 is suppressed." • Authors' conclusion: "While all ST products caused dysplasia, the products with lower levels of TSNAs and unprotonated nicotine caused less, consistent with the model that tobacco with low levels of nitrosamines might potentially induce fewer carcinomas in humans"
Song et al. 2010	Objective: To examine the carcinogenic effects of tobacco product in the	*General* snus, 2R4F reference cigarette	• No impact on body weight, no other significant adverse effects, such as skin sensitivity, loss of hair. • No changes in other organs, e.g., oral mucosa, lungs, bladder, stomach, colon, kidneys compared to controls Pancreatic Histopathology in EI-IL-1β mice:	• No effects in tobacco-treated WT mice on histopathology or other markers measured • Snus and TS caused

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN (Animal Model & Endpoints)</th><th>TEST PRODUCTS, DOSAGE, DURATION, & GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr>
<td></td>
<td>pancreas and their interaction with chronic pancreatitis; both are known independent risk factors for pancreatic cancer

Test system: Transgenic mouse model of chronic pancreatitis[1] (Elastase-IL-1-β mice) in comparison with wild type (C57B/6) mice;

Route of administration: Smoke extract in drinking water, snus in diet

Endpoints: Urinary cotinine & trans-3´-hydroxycotinine (3´-HC) to assess tobacco</td>
<td>Dosage: General snus in diet at 5, 7, 9% (wt/wt) (started on 5% and gradually increased to 9% within 12 weeks); Smoke extract: smoke according to modified FTC standard protocol[2] (40 puffs/mL PBS, diluted to 1/100 in drinking water)

Duration: 15 months

20-30 mice/ group</td>
<td>
• At age 4 months all EI mice (2-3 months treatment) – severe chronic pancreatitis (chronic inflammation, acinar atrophy, tubular complexes, fibrosis)
• At 4 months of treatment with TS extract or 4-5 months with snus flattened pancreatic ductal epithelium in similar incidence, but different severity: in a few main pancreatic ducts and also in segmental ducts in snus-treated; In main pancreatic ducts and >40% of segmental ducts in smoke extract-treated;
• flattened cells had higher proliferative index (Ki-67) than those in controls, but no apoptosis;
• At 4 months of treatment, glandular atrophy severe in TS-treated compared to controls (moderate-marked), later onset, less severe, & significantly lower incidence in snus-treated
• No effect on fibrosis and tubular complexes
<table>
<tr><th colspan="10">Pancreatic Histopathology and Nicotine Intake in Different Treatment Groups</th></tr>
<tr><th rowspan="2">Endpoints measured</th><th colspan="2">Controls PBS Water</th><th colspan="2">Controls SDS diet</th><th colspan="2">General Snus (diet)</th><th colspan="2">Ref Cigarett smoke extract (drinking water)</th></tr>
<tr><th>WT</th><th>EI-IL-1β</th><th>WT</th><th>EI-IL-1β</th><th>WT</th><th>EI-IL-1β</th><th>WT</th><th>EI-IL 1β</th></tr>
<tr><td colspan="10">Tobacco consumption: Urinary nicotine biomarker (ng/mL)</td></tr>
<tr><td>Cotinine</td><td>BLQ</td><td>3.75</td><td>BLQ-1.3</td><td>BLQ-1.0</td><td>36275</td><td>23174</td><td>93.5</td><td>145.</td></tr>
<tr><td>3´-HC</td><td>BLQ-1.1</td><td>BLQ-3.3</td><td>BLQ-8.2</td><td>BLQ-2.1</td><td>114064</td><td>118176</td><td>1103</td><td>194</td></tr>
<tr><td colspan="10">Pancreatic Histopathology</td></tr>
</table>
</td>
<td>similar histopathological changes in the pancreas with different severity
• Snus and TS ↑ COX-2 and TNF-α expression;
• other chronic pancreatitis-associated genes ↑ in TS-treated mice, much less in snus-treated mice
• the authors noted: “Although Snus intake in these mice was comparable with those of Snus users, the amount of tobacco intake in TS-treated mice may be lower than found in human cigarette smokers.”
• Other study limitations: study/observation period length too short; Small number of mice carried out to 15 months
• The authors concluded that the study showed “for the first time the importance of interactions between tobacco and</td>
</tr>
</table>

[1] Elastase-IL-1-β mice moderately express human IL-1-β, gene associated with chronic pancreatitis. Authors note that “The mice develop chronic pancreatitis at an early age that closely mimics that found in human beings. However, no preneoplastic or neoplastic lesions occur after >24 months of observation, suggesting that chronic pancreatitis per se may not be sufficient to induce pancreatic cancer in this model.”

[2] 2 sec/puff, 4 puffs/min, 35 ml/puff

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN (Animal Model & Endpoints)</th><th>TEST PRODUCTS, DOSAGE, DURATION, & GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr><td></td><td>consumption; Histopathology; cell proliferation by Ki67 immunohistochemical staining; apoptosis by TUNEL assay; COX-2 expression; chronic pancreatitis-associated genes (TNF-α, IL-6, TGF-β1, SDF-1)</td><td>starting age 5-8 weeks</td><td>
<table>
<tr><td>Flattening of ductal epithelium</td><td>0</td><td>NR</td><td>0</td><td>NR</td><td>0</td><td>15/29
52%</td><td>0</td><td>16/22
73%</td></tr>
<tr><td>Acinar cell injury</td><td>0</td><td>90%</td><td>0</td><td>90%</td><td>0</td><td>10/29
35%</td><td>0</td><td>14/22
64%</td></tr>
</table>
mRNA levels in EI-IL-1β mice:
• COX-2 expression 2x↑ with snus and TS compared to controls
• TNF-α expression ↑ with snus (transiently 7-9 months) and TS-treatment (4-9 months) compared to controls
• mRNA levels of IL-6, TGF-β1, and SDF-1 were not significantly affected by snus t, but ↑ with TS
</td><td>chronic pancreatitis in altering the biology of the pancreas. The findings support notion that both cigarette smoke and Snus are potentially cytotoxic and carcinogenic to the pancreas.”</td></tr>
<tr><td>Stenström et al. 2007</td><td>Objective: To study if snus is potentially carcinogenic in the stomach; especially in hosts with a high risk for gastric cancer development and concurrent Helicobacter pylori infection

Test system: male[3] wild-type (FVB/N) mice (WT), gastrin</td><td>General
snus

Dosage and duration: diet containing ~5-9% snus for 6 months, starting 6 weeks after H. pylori infection

8-22/group</td><td>
Stomach Wall Histopathology in Different Treatment Groups
<table>
<tr><th rowspan="2">Endpoints measured</th><th colspan="2">Controls</th><th colspan="2">Snus</th><th colspan="2">Snus + H. pylori</th></tr>
<tr><th>Wild-type</th><th>INS-GAS</th><th>Wild-type</th><th>INS-GAS</th><th>Wild-type</th><th>INS-GAS</th></tr>
<tr><td>Snus intake (mean kidney cotinine µg/mL)</td><td>0</td><td>0</td><td>724.6</td><td>836.0</td><td>665.7</td><td>838.8</td></tr>
<tr><td>H. pylori infection rate</td><td>0</td><td>0</td><td>0</td><td>0</td><td>17/20 (85%)</td><td>12/22 (55%)</td></tr>
<tr><td>Number of finally evaluated mice</td><td>11/11</td><td>8/8</td><td>8/8</td><td>8/8</td><td>17/20</td><td>12/22</td></tr>
<tr><td>Carcinoma in situ</td><td>0/11</td><td>2/8</td><td>0/8</td><td>4/8* (50%)</td><td>9/17*# (53%)</td><td>12/12*# (100%)</td></tr>
<tr><td>Dysplasia (Score)</td><td>0</td><td>~2.4</td><td>~0.1</td><td>~2.5</td><td>~1.8*#</td><td>~3.2*#</td></tr>
<tr><td>PCNA</td><td>~11</td><td>~11</td><td>~10</td><td>~45*</td><td>~31*#</td><td>~70*#</td></tr>
</table>
</td><td>• Snus treatment alone in wild-type mice significantly ↑ number of caspase-3 positive cells indicating an ↑ rate of apoptosis but had no impact in INS-GAS mice;
• snus treatment alone ↓ number of ECL cells (based on pancreastatin-staining) in both wild-type and INS-GAS mice
• The authors suggested that snus is a potential gastric carcinogen in mice, and concluded that this study supports the</td></tr>
</table>

[3] Male mice were used because gastric cancer development was shown to be sex dimorphic in gastrin transgenic mice

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN (Animal Model & Endpoints)</th><th>TEST PRODUCTS, DOSAGE, DURATION, & GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr>
<td></td>
<td>transgenic mice FVB (INS-GAS) mice (mouse model of gastric cancer)[4]

Route of administration: Diet;

Concomitant with infection with Helicobacter pylori

Endpoints: carcinoma in situ (histopathology of stomach wall from the squamocolumnar junction through antrum); PCNA, caspase-3, pancreastatin, ghrelin (immunohisto-chemistry); serum gastrin levels (radioimmunoassay); kidney cotinine</td>
<td></td>
<td>
<table>
<tr><td>(number/gland)</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Caspase-3 (number/gland)</td><td>~3</td><td>~12</td><td>~12*</td><td>~13</td><td>~23*#</td><td>~9*#</td></tr>
</table>
* significantly different compared to respective controls, # significantly different compared to snus only treatment
<u>Snus intake</u>:
• cotinine levels in all snus-treated mice similar, slightly ↑ in INS-GAS mice. Serum gastrin-7 was not impacted by treatment or infection.
<u>Histopathology</u>:
• Wild type mice: snus treatment resulted in mild morphologic changes in the stomachs compared to controls. None of the changes were significantly different from controls based on pathological grades of the stomach.
• Wild type mice infected with H. pylori (snus group only) and all INS-GAS mice with or without H. pylori infection and independent of snus use displayed similar pathological grades of intestinal metaplasia, foveolar hyperplasia, oxyntic gland atrophy, epithelial defects, and inflammation; there was however a slight increase of these effects in the order of WT+snus+H. pylori < INS-GAS < INS-GAS +snus < INS-GAS +snus+H. pylori
• Dysplasia: wild type mice - no influence of snus treatment, but significant ↑ with additional H. pylori infection; INS-GAS mice - ↑ over wild type mice, no influence of snus treatment, but significant ↑ with additional H. pylori infection;
• INS-GAS or H. pylori infection in conjunction with snus ↑ carcinoma in situ incidence significantly
<u>Proliferation</u>:
• INS-GAS or H. pylori infection in conjunction with snus ↑ cell proliferation (PCNA) significantly
</td>
<td>hypothesis that snus exposure accelerates gastric cancer development in the setting of hypergastrinemia and/or H. pylori infection.
• Carcinoma in situ observed were associated with ↑ rates of epithelial cell proliferation, apoptosis - common features of gastric carcinogenesis
• The authors noted that the results illustrate the potential co-carcinogenic effect of snus in animal models, which may be relevant for a subset of patients.
• Limitations: no control group of either WT or INS-GAS mice that received only H. pylori infections.</td>
</tr>
</table>

[4] INS-GAS mouse: human gastrin gene under insulin promoter control in pancreatic β-cells, resulting in elevated levels of circulating amidated gastrin and development of spontaneous intestinal metaplasia, dysplasia, and carcinoma in situ; majority of INS-GAS mice develop spontaneous stomach cancer by 20 months of age

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN (Animal Model & Endpoints)</th><th>TEST PRODUCTS, DOSAGE, DURATION, & GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr><td></td><td>levels as indicator of snus intake</td><td></td><td><u>Apoptosis</u>:
• The only parameter significantly ↑ by snus only treatment in wild type mice were caspase-3 levels (apoptotic cells), while snus had no impact on those in INS-GAS mice, which were already ↑. In INS-GAS mice, snus + H. pylori infection ↓, while in wild type mice, the combination ↑ the number of apoptotic cells further.
<u>Cells of stomach lining</u>:
• ↓ number of ECL (enterochromatin-like) cells (based on pancreastatin-staining) by snus treatment in both wild-type and INS-GAS mice, but A-like cells (Ghrelin-producing cells) and mucous cells were not impacted by any of the treatments.</td><td></td></tr>
<tr><td>Sand et al. 2002</td><td>Objective: To investigate the local effects of snus, 4-nitroquinoline-N-oxide (4-NQO), HSV-1 on the amount of subepithelial mast cells in the oral mucosa

Test system: male Sprague Dawley & Lewis rats

Route of administration: surgical lip canal

Endpoints: mast</td><td>Swedish snuff

Dosage and duration: 200 mg snus 2x/day = 12 hrs/day, 5 days/week for 23 months.

Test groups: 10-15/group, final count - SD rats: 8 controls, 12 HSV-1 inoculation (1x/month), 13 snuff, 15</td><td>• 2 rats in HSV-1 group died from encephalitis, another 11 rats from this and other groups suffered from pronounced autolysis and had to be excluded. All animals killed ~23 months.
• Only head and neck region cancers reported: 6 oral SCCs[5] in 6 rats
• Hyperplasia most prevalent in NQO+snus group
• The amount of countable subepithelial mast cells in the oral mucosa was significantly ↓ only in the NQO group.
• Rats with head and neck tumors of all groups had ↓ mean mast cell number (30.8/mm) in the test canal compared to controls, but not statistically significant.

Head and Neck Region Histopathology in Different Treatment Groups
<table><tr><th rowspan="2">Endpoint</th><th colspan="4">SD rats</th><th colspan="2">Lewis Rats</th></tr><tr><th>Controls
I</th><th>HSV-1
III</th><th>Snus
II</th><th>HSV-1 + Snus
IV</th><th>NQO
V</th><th>NQO + Snus
VI</th></tr><tr><td>Cancer</td><td>0</td><td>2</td><td>1</td><td>1</td><td>2</td><td>2</td></tr><tr><td>Dysplasia (in</td><td>0</td><td>1</td><td>2</td><td>1</td><td>0</td><td>0</td></tr></table></td><td>• The authors concluded that Swedish snuff (either alone or with HSV-1) has only minimal effects on subepithelial oral mast cells.
• Only the carcinogenic 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.

Unclear if these are same animals as in Larsson 1989, but tumor/lesion counts is slightly different;</td></tr>
</table>

[5] 3 oral SCCs in close proximity to the entrance of, but not in the test canal, 3 SCCs in crevicular epithelium close to the orifice of lip canal

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN (Animal Model & Endpoints)</th><th>TEST PRODUCTS, DOSAGE, DURATION, & GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr><td></td><td>cell number in mucosa, tumor incidence, preneoplastic lesions and reactive changes</td><td>HSV-1 + snuff; Lewis rats: 12 NQO (1x/week for 5 weeks), 12 NQO + snuff</td><td>
<table>
<tr><td>squamous epithelium on lip or crevicular epithelium)</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Mast cell in mucosa in test canal (mean number/mm2)</td><td>39.4</td><td>33.4</td><td>39.0</td><td>41.3</td><td>23.9*</td><td>30.6</td></tr>
</table>
* different from controls (p<0.01)
</td><td>might be reevaluation</td></tr>
<tr><td>Larsson et al. 1989</td><td>Objective: To evaluate if snuff functions as a tumor promoter in rats initiated with 4-NQO or HSV-1

Test system: male Sprague Dawley & Lewis rats

Route of administration: surgical lip canal

Endpoints: Clinical signs, complete necropsy, tumor incidence, preneoplastic lesions</td><td>Swedish snuff

Dosage and Duration: 200 mg snus 2x/day = 12 hrs/day, 5 days/week for 30 months

Test groups: 15/group, final count - SD rats: 8 controls, 12 HSV-1 inoculation (1x/month), 13 snuff, 15</td><td>
• 2 rats in HSV-1 group died from encephalitis, another 11 rats from this and other groups suffered from pronounced autolysis and had to be excluded. All animals killed at 16-30 months when moribund. No differences in survival between groups, but Lewis rats lived longer than SD rats
• Snus-treated groups had slower body weight gain than the other groups[6]
<table>
<tr><th colspan="7">Lesions/Tumors in Different Treatment Groups</th></tr>
<tr><th rowspan="2">Select Lesions</th><th colspan="4">SD rats</th><th colspan="2">Lewis Rats</th></tr>
<tr><th>Controls VI</th><th>HSV-1 I</th><th>Snus II</th><th>HSV-1 + Snus III</th><th>NQO IV</th><th>NQO + Snus V</th></tr>
<tr><td>Ear duct SCC</td><td></td><td>1</td><td></td><td>1</td><td></td><td></td></tr>
<tr><td>Lip SCC</td><td></td><td>1</td><td></td><td></td><td>2</td><td></td></tr>
<tr><td>Oral cavity SCC[7]</td><td></td><td></td><td>1</td><td></td><td>1</td><td>2</td></tr>
<tr><td>Nose SCC</td><td></td><td></td><td>1</td><td></td><td></td><td></td></tr>
<tr><td>Lip SC papilloma</td><td></td><td></td><td></td><td></td><td></td><td>1</td></tr>
</table>
</td><td>
• No significant differences in incidence of SCCs of head and neck region between groups; no significant difference of tumors in specific organs, but
• Total number of tumor-bearing animals and malignant tumors was ↑ in HSV-1 infected + snus-treated animals compared to controls, HSV-1 only, and snus only.
• No significant difference between in the NQO and NQO + snus groups.
• The authors concluded that snuff does not function as a tumor
</td></tr>
</table>

[6] Authors offered several explanations for the slower weight gain in snus groups: (a) general toxicity due to snus, (b) increased metabolic activity due to nicotine, (c) chronic inflammation of lip region due to snus with soreness and pain preventing maximum food intake

[7] SCCs of the oral cavity were located in the crevicular epithelium close to the orifice of the lip canal

<table>
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<tr><td></td><td></td><td>HSV-1 + snuff; Lewis rats: 12 NQO (1x/week for 5 weeks), 12 NQO + snuff</td><td>
<table>
<tr><td>Forestomach SCC</td><td></td><td></td><td></td><td></td><td></td><td>1</td></tr>
<tr><td>Forestomach SC papilloma</td><td></td><td></td><td></td><td></td><td>1</td><td></td></tr>
<tr><td>Dysplasia in lip, crevicular epithelium, or forestomach</td><td></td><td></td><td></td><td>2</td><td>1</td><td>5</td></tr>
<tr><td>Hyperplasia in lip, crevicular epithelium, or forestomach</td><td>2</td><td>10</td><td>17 + 2 in fore-sto-mach</td><td>29</td><td>14</td><td>15</td></tr>
</table>
• Oral cavity SCC only in snus only-treated rats (1) and in both NQO groups.
• Other tumors in snus only-treated rats that were not seen in other groups: 1 colon adenocarcinoma, 1 skin demoplastic fibroma
• Tumors in HSV-1/snus group not seen in controls, HSV-1 only or snus-only: 2 adenocarcinoma of the breast, 1 pheochromocytoma of adrenal gland, sarcoma of the stomach, salivary gland, and scrotum, adenoma of the breast (2) and adrenal cortex (2), cavernous hemangioma, of the gingival mucosa, fibrous histocytoma of the breast
• Most hyperplastic lesions in HSV-1 + snus group, foreign body giant cell granulomas in connective tissue of lips significantly ↑ in NQO + snus group</td><td>promoter in the oral cavity after initiation with 4-NQO and also did not have any specific promoting effects on the oral cavity after HSV-1 infection.[8]
• The author noted snuff appeared to be a general tumor promoter in combination with HSV-1 infection;
• Rats that were repeatedly infected with HSV-1 in this study exhibited signs of generalized infection.
• Limitations: Combining tumors regardless of tissue site and tumor type appears questionable</td></tr>
<tr><td>Hirsch et al. 1986</td><td>Objective: To evaluate the reversibility of snuff-induced</td><td>Röda Lacket snus

Dosage and</td><td>• Controls: lips - slightly hyperplastic epithelium, thickening of both the stratum granulosum and spinosum; surface covered with thickened orthokeratin layer; no to mild subepithelial connective tissue inflammation.</td><td>• Snus-exposure for 13 months resulted in lesions of the lip and oral cavity similar to those in</td></tr>
</table>

[8] The authors theorized that polyphenols in snuff could function as local inhibitors of cancer development.

<table>
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<tr><td></td><td>lesions in a well-established rat model

Test system: Female Sprague-Dawley rats

Route of administration: surgical lip canal

Endpoints: histopathology of lip, gingival epithelium of lower incisors (crevicular epithelium), tongue, buccal mucosa</td><td>Duration: 200 mg snus 2x/day = 12 hrs/day, 5 days/week for 13 months.

Test groups:10 Controls, 30 snus-treated (10 sacrificed after 13 months treatment, 10 sacrificed 1 month and 10 4 months after end of treatment)</td><td>• Snus - Group 1 (sacrifice after 13 months of treatment): lips - generalized slight-moderate hyperplasia; hyperorthokeratosis; focally vacuolated cells, slight-moderate acanthotic proliferation; mild focal atopia and ulcerations of squamous epithelium; border between the stratum basale and the connective tissue was always well defined; slight-severe inflammation of the connective tissue, prominent fibrosis (100%). Tongue – no difference to controls. Buccal mucosa epithelium - ↑ keratinization, slight-moderate hyperplasia, slight acanthosis. Gingival sulcus epithelium – moderate-severe hyperplasia, ↑ keratinization, atrophy, focal ulcerations
• Snus - Group 2 and 3 (sacrifice 1 or 4 months after cessation of treatment): lips - lesions were ↑atrophic, ↓ acanthotic, ↓ inflammatory, with ↓ atypia of the squamous epithelium, but ↑ incidence of severe subepithelial fibrosis. Tongue & buccal mucosa – mild hyperplasia, hyperkeratotic, mild inflammation in connective tissue. Gingival sulcus epithelium after 4 months – hyperplasia ↓, little-no keratinization, ↓ ulcerations</td><td>other studies
• If exposure was followed by a 1-4 months break, lesions were reversible in part, but lip squamous epithelium stayed atrophic and ulcerations were in part persistent, subepithelial connective tissue stayed fibrotic ….
• The authors concluded that snuff exposure results in hyperplastic, reactive, reversible lesions of the oral mucosal lesions.
• They suggested that snuff and the TSNAs in it may predominantly act as promoter when administered for a relatively short period of time.</td></tr>
<tr><td>Hirsch et al. 1984</td><td>Objective: To compare the pathological changes of rat oral mucosa and the possible</td><td>Röda Lacket snus

Dosage and Duration: 200 mg snus</td><td>• 3 rats of each of the HSV-1 groups were in poor condition after a second application of the virus and died from encephalitis
• 2 oral SCCs[9] in snus + HSV-1 group
<table><tr><th>Select Lesions</th><th>controls</th><th>HSV-1</th><th>Snus</th><th>Snus + HSV-1</th></tr></table></td><td>• 2 oral SCCs in snus + HSV-1 treated animals
• The authors noted that even though the tumors were not detected at the exact site of the</td></tr>
</table>

[9] Ulcerated, one in palatal side of right molar region of upper jaw, one lingual side in molar region, invaded bone; mild to moderate dysplasia in crevicular epithelium (The stratified squamous epithelium lining the inner aspect of the soft tissue wall of the gingival sulcus. Synonym: sulcular epithelium) of the lower incisor. The authors later noticed that all 3 tumors likely originated from the gingival sulcus epithelium and not from the squamous epithelium of the test canal in the lip. Gingival sulcus was thought to be more sensitive, covered with thin unkeratinized epithelium, and more exposed to snus (Hirsch et al. 1986).

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN (Animal Model & Endpoints)</th><th>TEST PRODUCTS, DOSAGE, DURATION, & GROUPS</th><th>RESULTS</th><th>SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS</th></tr>
<tr>
<td></td>
<td>carcinogenic effects of HSV-1 in combination with long-term administration of snuff, HSV-1 alone or snuff alone

Test system: Female Sprague-Dawley rats

Route of administration: surgical lip canal

Endpoints: complete post-mortem examination with histopathology</td>
<td>2x/day = 12 hrs/day, 5 days/week for 18 months.

Test groups: 10 Controls, 10 snus-treated + sham inoculation, 10 snus-treated + HSV-1 inoculation, 10 HSV-1 inoculation</td>
<td>
<table>
<tr><td>Oral squamous epithelium hyperplasia</td><td>5/10 (mild)</td><td>6/7 (mild)</td><td>7/10 (mild-moderate)</td><td>6/7 (mild-severe)</td></tr>
<tr><td>Oral (lip) mucosal dysplasia</td><td>0/10</td><td>0/7</td><td>3/10 (mild)</td><td>4/7 (mild)</td></tr>
<tr><td>Crevicular epithelium of lower incisor dysplasia</td><td></td><td>40%</td><td>10%</td><td>86%</td></tr>
<tr><td>Oral SCC</td><td>0/10</td><td>0/7</td><td>0/10</td><td>2/7</td></tr>
<tr><td>Anal SCC</td><td>0/10</td><td>0/7</td><td>1/10</td><td>0/7</td></tr>
<tr><td>Sarcoma of the retroperitoneum</td><td>0/10</td><td>0/7</td><td>1/10</td><td>1/7</td></tr>
<tr><td>Papillary squamous hyperplasia of forestomach</td><td>0/10</td><td>0/7</td><td>5/10</td><td>2/7</td></tr>
</table>
<u>Histopathology of the oral cavity</u>:
• Controls: mild hyperplastic squamous epithelium, covered with orthokeratin layer; no or slight inflammation, slight-moderate fibrosis
• HSV-1: slight hyperplastic squamous epithelium, areas of atrophy; hyperorthokeratinization of surface layer cells; no-mild inflammation in connective tissue; severe fibrosis
• Snus: mild-moderate hyperplastic squamous epithelium, areas of slight atrophy; hyperorthokeratinization of surface layer cells; vacuolization, acanthosis; mild inflammation; mild dysplasia in 30% of rats; all severe fibrosis
• Snus + HSV-1: moderate hyperplastic squamous epithelium, hyperorthokeratinization of surface layer cells; ulcerated lesions, slight-moderate acanthosis; pronounced inflammation; mild dysplasia in >50% of rats; all severe fibrosis
<u>Extra-oral tumors</u>:
• Additional tumors seen in HSV-1 and snus + HSV-1 group, but not in others: cystic choliangioma of the liver (1 each), ovary adenofibroma (0,1), phaeochromocytoma of the adrenal gland (1,0), desmoplastic fibroma of skin (0,1)
</td>
<td>application of the 2 agents it is reasonable to assume that they were caused by HSV-1 and snus in the saliva
• Their location, the crevicular epithelium, has been reported to have weak protective capacity
• Total number of malignant tumors was statistically significantly ($p<0.05$) higher in rats exposed to Swedish snuff or Swedish snuff + HSV-1 than other groups.
• The authors concluded that HSV-1 in combination with snuff exposure may also be associated with the development of oral SCCs.
• Limitations: Combining tumors regardless of tissue site and tumor type appears questionable</td>
</tr>
</table>

CITATION	STUDY OBJECTIVE & DESIGN (Animal Model & Endpoints)	TEST PRODUCTS, DOSAGE, DURATION, & GROUPS	RESULTS	SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS
Hirsch and Johansson 1983	Objective: To study the long-term effect of snuff exposure on the oral mucosa Test system: Male and female Sprague-Dawley rats Route of administration: surgical lip canal, 10 days after healing Endpoints: complete post-mortem examination with histopathology	*Röda Lacket* snus Dosage and Duration: 200 mg snus 2x/day = 12 hrs/day, 5 days/week for 9-22 months Test groups: 15 Controls, 42 regular pH (pH 8.3) snus-, 10 highly alkaline (pH 9.3) snus-treated	Duration: • control animals: 18 months (controls N=15) • standard snus treated animals: sacrificed after 9 months (N=12), 12 months (N=14), or when moribund after 18-22 months of exposure (N=16); • alkaline snus animals: sacrificed when moribund after 18-22 months of exposure (N=10) Clinical signs: • Physical activity ↓ in snus-treated after 9 months, in controls after 14 months Body weight gain: no significant differences Oral mucosal changes: Controls: mildly hyperplastic epithelium, no to mild inflammation Snus-treated: • 1 single tumor (squamous cell carcinoma)[10] detected after 8.5 months; Mild to moderate squamous epithelium hyperplasia, focal severe hyperplasia, hyperorthokeratosis with vacuolated cells, focal acanthotic proliferation of the epithelium; mild to severe inflammation of the connective tissue (lymphocytes, histiocytes, mast cells); 3/12 (9 months), 2/14 (12 months) mild dysplasia in lip mucosa, • Only slight differences between treated animals exposed for 9-12 and 22 months: In 22-months exposed rats had aside from hyperplastic also atrophic lesions and aside from inflammation a more prominent fibrosis; 4/16 mild dysplasia in lip mucosa, 2/16 rats had severe dysplastic changes in the crevicular epithelium • High pH snus-treated had similar changes to regular snus group at 18-22 months exposure, but fibrosis was less prominent and epithelial lining atrophic and ulcerated, less frequent vacuolization; 2/10 mild dysplasia in lip mucosa Lesions outside the oral cavity: • Snus-treated rats for 18-22 months ↑ incidence (6/26) of	• 1 squamous cell carcinoma in the oral cavity in snus-treated animals • Clear difference in histopathological appearance of the lesions of snus-treated rats vs. controls, with markedly higher frequency of hyperorthokeratinized, athrophic, ulcerated and mildly dysplastic and fibrotic lesions • The authors could not draw any conclusions between duration of exposure and severity of lesions, but high incidence of athrophic lesions presence of dysplasia in 18-22 month groups • The authors noted that in human snuff-dippers fewer athrophic and ulcerated lesions, but more vacuolated cells were observed • Findings outside the oral cavity were rare and the only lesion ↑ in treated

[10] Ulcerated and located in left side of oral cavity, extending from the incisor and involving both upper and lower jaws; invaded bone

CITATION	STUDY OBJECTIVE & DESIGN (Animal Model & Endpoints)	TEST PRODUCTS, DOSAGE, DURATION, & GROUPS	RESULTS	SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS
			squamous papillary hyperplasia of the forestomach, but no overt forestomach tumors compared to controls sacrificed at the same time (0/5)	animals compared to controls was squamous cell hyperplasia of the forestomach • The authors concluded that exposure of rats to snus for most of their lifetime resulted in lesions mainly restricted to the epithelium and the underlying connective tissue of the test canal • They noted that spontaneous tumors of the oral mucosa are extremely rare in SD rats, and therefore the possibility that the tumor was induced by snuff cannot be completely ruled out.
Hirsch and Thilander 1981	Objective: Modification of an existing animal model to create an environment similar to the buccal cavity in man in order to study the influence of snuff on the oral mucosa Test system: male	*Röda Lacket* snus Dosage and Duration: 200 mg snus 2x/day = 12 hrs/day, 5 days/week for 9 months Test groups: 2 controls, 4 snus-treated	Clinical signs: ↑ activity in snus-treated animals after injection and throughout test period, slightly lower body weight gain. Snuff exposure: ~1 g/kg/day (~5x > human exposure) Saliva pH: pH 8-9 in test canals and oral cavity Nicotine blood concentration: • Controls: 12 ng/mL; • Snus-treated: 83-250 ng/mL Histopathology: • after 14 days healing mucosa in test canal and on skins side of lip showed complete epithelialization • Controls after 12 months: slightly hyperplastic epithelium with thickening; somewhat thicker orthokeratin layer; mild inflammation in subepithelial connective tissue	• First study to introduce the surgical lip canal model to study snuff with limited number of animals • No tumors after 9 months of snus treatment in 4 rats • The authors noted that the histological snuff lesions in rats correlated with those reported from human studies, with higher incidence in animals than humans for

CITATION	STUDY OBJECTIVE & DESIGN (Animal Model & Endpoints)	TEST PRODUCTS, DOSAGE, DURATION, & GROUPS	RESULTS	SUMMARY, COMMENTS, & AUTHORS CONCLUSIONS
	and female Sprague-Dawley rats Route of administration: surgical lip canal, Endpoints: snuff exposure, saliva pH, nicotine blood levels, histopathology of lower lip/test canal		• Snus-treated after 9 months: mild-moderate hyperplastic epithelium, foci of marked hyperorthokeratosis, in some areas looser with focally vacuolated cells; thickening of epithelium due to keratinization, but also by widening and/or thickening of stratum granulosum and stratum spinosum (acanthosis); mild to severe inflammation in the connective tissue	hyperkeratosis, hyperkeratotic and slight dysplastic lesions. This could be explained by higher amount of snuff used, retention time and species differences. • They noted that the physiological conditions, including presence of saliva, were similar in the test canal and the oral cavity and that the model can be used to induce lesion in the oral mucosa similar to those observed in man. • The authors concluded that this experimental model seemed to fulfill the main requirements for studying the effects of snuff on the oral mucosa.

Appendix V

Summary Tables of Epidemiology Studies

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=12)

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 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 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 factor with the highest relative risk for the development of Snuff dipper's lesion was the package form (loose vs. portion-bag) (RR=3.39, $p < 0.010$). Number of sites where quid was placed, hours of daily use and grams of snuff daily were also significantly associated with Snuff dipper's lesion, while years with regular snuff habit, or age were not.	**The authors concluded that clinical changes of the gingival margin are less pronounced among those who use portion-bag snuff (and a lower frequency of gingival recessions) than among those who use loose snuff. Portion-bag snuff is associated with less severe clinical oral mucosal lesions compared with such lesions among users of loose snuff.** Relative risks do not appear to be controlled for any confounding factors. A limitation of this study is its cross-sectional study design. Causality cannot be determined since outcome and exposure are assessed simultaneously.

APPENDIX A-1
DESCRIPTIVE STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS
(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. Users of loose snuff consumed a greater daily amount of snuff and had used snuff for a considerably longer period. 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). "Snuff" is defined as loose or portion-bag packed Swedish moist snuff in this paper.		

APPENDIX A-1
DESCRIPTIVE STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (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 the use of Swedish snus and periodontal bone loss (as assessed by bone height).	Cross-sectional study Subjects were 84 apparently healthy men (ages 26 to 54) who were recruited among employees of the Swedish Armed Forces. The study was carried out from November 2002 through December 2003. 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. Subjects provided information on tobacco habits via a structured questionnaire as 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). "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.** The authors 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.) One limitation of this study is its cross-sectional study design. Causality cannot be determined since outcome and exposure are assessed simultaneously. Another limitation is the small number of participants.

APPENDIX A-1
DESCRIPTIVE STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (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 study 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. Examiners were carefully calibrated with each other before the study. "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; $p < 0.01$). 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 noted that snuff use was correlated significantly with increased incisal and occlusal wear.** The variables considered in the step-wise multiple linear regression analysis include number of teeth, sex, bruxism, age, snuffer, and 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. One limitation of this study is its cross-sectional study design. Causality cannot be determined since outcome and exposure are assessed simultaneously.

APPENDIX A-1
DESCRIPTIVE STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS
(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 study 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). <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% "Snuff" was not defined.	<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 note that potential confounders such as diet, oral hygiene, and fluoride exposure could explain differences between tobacco users and non-tobacco users. 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. A limitation of this study is its cross-sectional study design. Causality cannot be determined since outcome and exposure are assessed simultaneously.

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

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hugoson and Rolandsson 2011 Jönköping, Sweden This study examined the relationship between current smoking and the use of snus with periodontal health compared with non-tobacco users.	Cross-sectional study The study population consisted of three cohorts. In 1983, 1993 and 2003, a stratified random sample was invited to take part in a dental health exam; n=130 who turned 20, 30, 40, 50, 60 & 70 in these years. 550, 552 and 523 attended the 1983, 1993 & 2003 exams, respectively. Participants were examined clinically and radiographically. Diagnostic criteria: number of teeth, plaque, gingival status, probing pocket depth (PPD)≥4 mm, height of the alveolar bone level and classification by periodontal disease experience. Current smokers and snus users were defined as daily smokers (number of cigarettes per day noted) and daily snus users (number of boxes per day). The total numbers of non-tobacco users, smokers and snus users were 1142 (526 males and 616 females), 345 (156 males and 189 females) and 104 (99 males and 5 females), respectively. "Snuff" defined as Swedish moist snuff (snus).	Multiple logistic regression shows, after adjusting for age, gender and sociodemographic variables, that relative to non-tobacco users, cigarette smokers had statistically significantly less gingivitis, a higher frequency of PPD ≥ 4mm and a higher incidence of severe periodontitis. There was no significant association between gingivitis, frequency of PPD ≥ 4mm and periodontal disease experience and snus use.	**The authors concluded that using Swedish moist snuff (snus) did not seem to be a risk factor for periodontal disease.** Odds ratios were adjusted for age, gender, education, employment and marital status. The authors stated that approximately 90% of the individuals in all age groups in all studies were Caucasian and born in Sweden. The results relating to the use of tobacco in the population are in agreement with the results of national Swedish studies. One limitation of this study is its cross-sectional study design. Causality cannot be determined since outcome and exposure are assessed simultaneously. Without being able to track individuals, there is, therefore a lack of information about how long different individuals have used tobacco and whether or not they have stopped using it or switched from one form to another. Another limitation that the authors point out is the small number of snus users, especially in 1983, though the results were similar in the different examination years when the number of snus users was more satisfactory.

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

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hugoson et al. 2012 Jönköping, Sweden This study examined the relationship between smoking and the use of snus with dental caries compared with non-tobacco users.	Cross-sectional study The study population consisted of three cohorts. In 1983, 1993 and 2003, a stratified random sample was invited to take part in a dental health exam; n=130 who turned 20, 30, 40, 50, 60 & 70 in these years. 550, 552 and 523 attended the 1983, 1993 & 2003 exams, respectively. Participants were examined clinically and radiographically. Decayed and filled tooth surfaces (DFS) were recorded. Current smokers and snus users were defined as daily smokers (number of cigarettes per day noted) and daily snus users (number of boxes per day). The total numbers of non-tobacco users, smokers and snus users were 1142 (526 males and 616 females), 345 (156 males and 189 females) and 104 (99 males and 5 females), respectively. "Snuff" was defined as Swedish moist snuff (snus).	Multiple logistic regression shows, after adjusting for age, gender and sociodemographic variables, that relative to non-tobacco users, cigarette smokers had statistically significantly increased prevalence of DFS in 1983. No relationship was observed among snus users. In 1983 there was no significant difference in mean DFS between non-users and smokers (unadjusted), but a statistically significantly higher DFS in non-users and smokers when compared to snus users. With respect to number of teeth, non-users as well as smokers had statistically significantly fewer teeth in 1983 than snus users. In 2003, non-users and snus users had statistically significantly more teeth than smokers.	**The authors concluded that the results of the studies performed in 1993 and 2003 indicate that daily smoking or snus use does not increase the risk of dental caries.** Odds ratios were adjusted for age, gender, education, employment and marital status. The results relating to the use of tobacco in the population are in agreement with the results of national Swedish studies. One limitation of this study is its cross-sectional study design. Causality cannot be determined since outcome and exposure are assessed simultaneously. Without being able to track individuals, there is, therefore a lack of information about how long different individuals have used tobacco and whether or not they have stopped using it or switched from one form to another. Another limitation that the authors point out is the small number of snus users, especially in 1983, though the results were similar in the different examination years when the number of snus users was more satisfactory.

APPENDIX A-1
DESCRIPTIVE STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (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 study 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. Those who had used snuff at least once daily were considered "regular snuff dippers. "Snuff" was not specifically defined in this paper.	Regular use of snuff did not differ between dentate and edentulous men and women. Regularly smoking men and women had significantly higher risk to be edentulous than non-tobacco users.	**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.** The authors caution that the design of this study does not allow any conclusions on causality but merely on covariations between these variables. The study had a high response rate: ~80%.

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

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Julihn et al. 2008 Stockholm, Sweden This study investigated the relationship between various potential risk factors and incipient alveolar bone loss and subgingival calculus.	Cross-sectional study 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. Tobacco habits were described in terms of cigarette smoking and snuff use. The frequency was expressed as never, sometimes, or daily. In the statistical analysis, the categories ''never'' and ''sometimes'' were combined, for both cigarette smoking and snuff use, respectively, and referred to as ''no daily''. "Snuff" was not specifically defined in this paper.	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 (14 cases) was not statistically significant (OR = 1.15, 95% CI: 0.7 – 1.89). The adjusted odds ratio among smokers (29 cases) was also not statistically significant (OR = 1.22, 95% CI: 0.8 – 1.86). The only risk factors that were statistically significantly correlated with incipient bone loss were subgingival calculus and proximal restoration ≥ 1.	**The authors conclude that 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. The authors do not come to a conclusion regarding snuff use.** Odds ratios were adjusted for education level and occupation status for both mother and father. There were few daily smokers and snuff users in this study. The authors note that the power of the variable "smoking" habit was only 40%. Another limitation of this study is its cross-sectional study design. Causality cannot be determined since outcome and exposure are assessed simultaneously.

APPENDIX A-1
DESCRIPTIVE STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (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 study 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). 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. "Snuff" was not specifically defined in this paper.	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 (1.10 for snuff-takers, and 0.89 for non-snuff-takers: $p < 0.001$) after controlling for plaque index. Boy-smokers, consuming 1-9 cigarettes/day had a gingival index of 1.32 in the upper front jaw (0.85 for non-smokers). This difference was also statistically significant ($p < 0.05$).	**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. A limitation of this study is its cross-sectional study design. Causality cannot be determined since outcome and exposure are assessed simultaneously.

APPENDIX A-1
DESCRIPTIVE STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS
(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 study 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 odds ratios were adjusted for plaque, gingivitis, and tooth-brushing. The study involved a relatively small number of subjects (only 30 were current snus users). Another limitation of this study is its cross-sectional study design. Causality cannot be determined since outcome and exposure are assessed simultaneously.

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

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Rolandsson et al. 2005 Sweden This study investigated the effects of snuff use on dental effects and periodontal disease Results on oral mucosal lesions are presented in Appendix B.	Cross-sectional study Participants included 80 adolescent males between 16-25 years, selected among ice-hockey players in the Varmland region of Sweden. Tobacco habits, and oral health history was recorded via questionnaire. Clinical exams were also carried out. 40 of the 80 participants were snuff users. Snuff is defined as Swedish snuff.	There were no statistical differences between snuff users and nonusers regarding restored tooth surfaces, number of teeth, presence of plaque, prevalence of gingivitis, gingival index or probing pocket depth between snuff users and nonusers.	**The authors concluded that in spite of mucosal lesions caused by snuff, there were no statistical differences in prevalence in plaque and gingivitis between snuff users and non-users.** The study involved a relatively small number of subjects. A limitation of this study is its cross-sectional study design. Causality cannot be determined since outcome and exposure are assessed simultaneously.

APPENDIX A-1
DESCRIPTIVE STUDIES OF DENTAL EFFECTS AND PERIODONTAL DISEASE AMONG SWEDISH SNUS USERS (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 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). 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. "Snuff" was not specifically defined in this paper.	All groups of tobacco users had a significantly 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 (649 cases among current smokers) and periodontal disease (compared to never-smoking) (OR=2.41; 95% CI: 1.53-3.88), but there was no significant association between current snuff use (122 exposed cases) and periodontal disease (compared to never use) (OR=0.66; 95% CI: 0.30-1.32). There was an indication of association with former snuff use: the odds ratio associated with former snuff (31 exposed cases) 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. A higher prevalence of ever snuff users was observed for gingival index ≥2.0 and gingival recessions compared to never users of tobacco.	**The authors concluded that current use of snuff is not significantly associated with periodontal disease, while smoking is associated with periodontal disease in a dose-dependent fashion.** Odds ratios were adjusted for gender, age, education, plaque, and snuff or smoking. 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. The authors note that this investigation was based on a population sample rather than on a clinical one. This fact allowed the inclusion of conditions at the less severe end of the spectrum and prevented selection of subjects seeking care for clinically evident disease. A limitation of this study is its cross-sectional study design. Causality cannot be determined since outcome and exposure are assessed simultaneously.

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. The number of subjects using smokeless tobacco was not specified. "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.	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 B1

Descriptive Studies of Oral Mucosal Lesions

APPENDIX B-1
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (N=21)

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). 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.	<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-1
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)		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-1
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-1
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-1
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-1
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-1
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-1
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-1
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-1
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-1
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-1
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-1
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 were 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-1
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-1
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)		▪ 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-1
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-1
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-1
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)		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-1
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-1
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-1
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Rolandsson et al. 2005 Sweden This study investigated the effects of snuff use on oral mucosal lesions. Results on dental effects and periodontal disease are presented in Appendix A-1.	Cross-sectional study Participants included 80 adolescent males between 16-25 years, selected among ice-hockey players in the Varmland region of Sweden. Tobacco habits, and oral health history was recorded via questionnaire. Clinical exams were also carried out. 40 of the 80 participants were snuff users. Snuff is defined as Swedish snuff.	Out of 40 snuff users, 35 showed snuff induced lesions. No oral lesions were observed among non-users of snuff. A statistically significant relationship was found between snuff-induced oral mucosal lesions and the number of hours per day the snuff was used as well as with loose snuff vs. portion bag.	**The authors concluded that in spite of mucosal lesions caused by snuff, there were no statistical differences in prevalence in plaque and gingivitis between snuff users and non-users.** The study involved a relatively small number of subjects. A limitation of this study is its cross-sectional study design. Causality cannot be determined since outcome and exposure are assessed simultaneously.

APPENDIX B-1
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-1
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 B-1
DESCRIPTIVE STUDIES OF ORAL MUCOSAL LESIONS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Wallstrom et al. 2011 Sweden This clinical follow-up study assessed the possible reversibility of oral mucosal changes associated with the use of Swedish moist snuff.	Clinical follow-up study Subjects were 18 of 50 patients who agreed to have a second biopsy 6 months after cessation of snuff use to assess reversibility of snuff-induced lesions as part of a cessation program including nicotine replacement therapy (NRT - gum). The snuff induced lesions were photo-documented and graded, and biopsies examined. The thickness of the epithelium and the keratinized surface layer was measured and Student's *t*-test was used to compare the two biopsies. Lesions were graded according to Axell (1976). Participants did not use snuff or smoke during the 6 month period. Past and/or current habitual or occasional smokers were excluded. The type of snuff used by participants in this study was loose, and is assumed to be Swedish.	Participants used snuff for an average duration of 14.7± 2.7 hours/day and an average amount of 4.7 ± 3.0 cans/week. There was no significant correlation between the severity of the lesion and the total exposure to snuff in terms of the years with the habit, daily hours of consumption, and amount consumed on a daily basis. After 6 months 39% (n=7) showed remaining lesions (66% [n=2] with grade 4 lesions at baseline, 38% [n=3] with grade 3, and 40% [n=2] with grade 2). Five of these 7 subjects were still using NRT on a daily basis, 3 chewing the gum and 2 placing it under the lip, while two were nicotine-free. With respect to histomorphology, the epithelial thickness increased in 33% and decreased in 67% of participants, while the mean total thickness of the keratin surface layer decreased significantly from the baseline after cessation. Only one lesion had the same classification after 6 months of abstinence as baseline (grade 2) and the remaining lesions shifted from a higher to a lower grade.	**The authors concluded that after long and extensive snuff use, snuff induced lesions do not resolve completely. However, they also note that the overall clinical and histomorphological picture after 6 months of abstinence was improved. They noted 39% of the participants still exhibited clinical changes, although less severe, and the area of the affected mucosa had diminished in size.** The authors also noted that the fact that 71% of the subjects with remaining clinical lesions used NRT, suggests that these observations indicate that NRT may have a negative effect on the oral mucosa.

Appendix B2

Descriptive Studies of P53 Expression in Oral Mucosal Lesions

APPENDIX B-2
STUDIES OF P53 EXPRESSION IN ORAL MUCOSAL LESIONS (N=5)

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>Summary, Conclusions and Comments</th></tr>
<tr>
<td>Schildt et al. 2003</td>
<td>Objective: to investigate correlation between markers of cell proliferation and apoptosis, incl. mutation analysis of TP53[1] and exposure data from an extended group of tumors, with special focus on infections (HSV)

Case control Study, Univariate analysis

p53 antibody: DO7 (detects wild-type & mutant p53)</td>
<td>Oral snuff use, smoking, alcohol use in Sweden

Samples of 114 verified primary squamous cell oral cancer (SCC) (a subset of cases from a population-based case control study diagnosed in Sweden in 1980-1989) and matched (age, sex, county) controls; of the cases: 12 active and 8 ex-snuff users, of the matched controls: 20 active and 6 ex-snuff users</td>
<td><u>Immunohistochemistry & Gene Mutation PCR</u>
72/114 (63%) p53 positive
41/114 (36%) mutations in TP53 exons 5-9

<table>
<tr><th rowspan="2">Tumors</th><th colspan="6">Cases/matched Controls</th></tr>
<tr><th colspan="2">Snuff</th><th colspan="2">Smoking</th><th>Alcohol</th><th>Total Infections</th></tr>
<tr><th></th><th>ex</th><th>active</th><th>ex</th><th>active</th><th></th><th></th></tr>
<tr><td>All</td><td>8/6</td><td>12/20</td><td>26/32</td><td>33/27</td><td>66/55*</td><td>23/6**</td></tr>
<tr><td>p53 positive</td><td>5/5</td><td>9/12</td><td>16/20</td><td>17/15</td><td>42/37</td><td>17/3**</td></tr>
<tr><td>p53 negative</td><td>3/1*</td><td>3/8</td><td>10/12</td><td>16/12*</td><td>24/18*</td><td>6/3*</td></tr>
<tr><td>TP53 mutation</td><td>2/2</td><td>2/7</td><td>6/8</td><td>9/8</td><td>22/16*</td><td>8/3*</td></tr>
<tr><td>No TP53 mutation</td><td>6/6</td><td>10/13</td><td>20/24</td><td>24/19*</td><td>44/39*</td><td>15/3**</td></tr>
<tr><td>PCNA (14-93%)</td><td>7/5</td><td>11/7</td><td>24/29</td><td>32/26</td><td>61/50*</td><td>22/6**</td></tr>
<tr><td>Ki-67 (11-60%)</td><td>8/6</td><td>12/20</td><td>25/32</td><td>33/27</td><td>64/55*</td><td>23/6**</td></tr>
</table>
*Odds Ratio ≥1.5; ** Odds Ratio ≥5.0</td>
<td>• No clear relationship between snuff use and any of the biological markers studied, but the number of snus users involved in the study was small
• No correlation between smoking and p53 aberrations were detected
• Liquor consumption was a risk factor for increased biomarker levels associated with oral cancer, but odd ratios were not significant.
• The only exposure factor studied that was significantly associated with increased risk for all tumors as well as for p53 protein positive tumors only were oral infection (especially herpes simplex virus (HSV) infection)
• The authors concluded that almost all cases in this study had an aberration in p53 status (detected either as ↑ p53 protein expression or as mutation detected in exons 5-9 of the TP53 gene
• Study limitations: univariate analysis, no information how alcohol use and smoking overlapped with snuff use</td>
</tr>
</table>

[1] *TP53:* tumor suppressor gene encoding the p53 protein; a marker of cell cycle regulation;

APPENDIX B-2
STUDIES OF P53 EXPRESSION IN ORAL MUCOSAL LESIONS (continued)

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>Summary, Conclusions and Comments</th></tr>
<tr>
<td>Merne et al. 2002</td>
<td>Objective: To elucidate cellular mechanisms involved in snuff-induced epithelial changes

Oral mucosa biopsy specimen

Immunohisto-chemistry staining of Ki67, PCNA[2], p53, p21[3], HSP70[4], cytokeratins, collagen type IV; p53 antibody: DO7 (detects wild-type & mutant p53)

Histology by light microscopy.</td>
<td>Scandinavian-type moist snuff

Regular snuff use (average 5.9 years (range, 2-15 years), mean frequency 6.8 x/day (range, 2-10);

Biopsy specimens of snuff dipper’s lesions (oral mucosa in upper labial sulcus of the anterior region) of 14 male snuff users in Finland; all used loose snuff, 3 also portion-packed, 5 occasional smokers

Controls from normal oral buccal mucosa of 12 never-tobacco users</td>
<td><u>Histology of biopsy specimens</u>:
Snuff-induced lesions: characterized by a thick hyper-keratinized surface layer; epithelium had thickened and elongated rete ridges, mild vacuolation; mild chronic inflammation, but no eosinophils; no changes compatible with dysplasia.
Control samples: normal epithelial structure with thick, stratified epithelia and non-keratinized surface

<u>Immunohistochemistry</u>:
<table><tr><th colspan="3">Protein Levels
(mean percentage of positively stained cells)</th></tr><tr><th></th><th>Healthy Tissue from Controls</th><th>Lesions from Snuff Dipper</th></tr><tr><td>p53</td><td>2.0 ±1.7</td><td>3.1 ±4.6</td></tr><tr><td>p21</td><td>36.0 ±7.6</td><td>28.6 ±31.3</td></tr><tr><td>PCNA</td><td>100 ±0</td><td>34.4 ±16.15*</td></tr><tr><td>Ki-67</td><td>71.5 ±6.6</td><td>24.6 ±9.7*</td></tr></table>* significant

• Expression of cellular proliferation proteins (PCNA and Ki-67) was lower (by number of cells) in snuff lesions than in controls ($p < 0.001$), but staining intensity in snuff lesions was higher.
• Expression of cell cycle proteins (p53, p21) was not statistically different in snuff lesions compared to controls. p53 levels ↑ in 2/14 (14%), p21 levels ↑ in 7/14 (4 high, 3 low expression) snuff users’ lesions. These two samples (non-smokers) with ↑ p53 also had strong ↑ p21 and lesions were clinically graded category 2.</td>
<td>• Study results indicate that epithelial thickening may be due to increased life-span and differentiation of cells, rather than increased cellular proliferation. Authors hypothesized high pH and calcium salts may cause excessive keratinization; vacuolization may be degenerative response to high pH.
• Keratinization and epithelial thickening have been regarded as protective response.
• The authors concluded that lesions seen among snuff users are associated with suppressed cellular proliferation and infrequent p53 dysfunction and this may in part explain why dysplastic changes are seldom observed in mucosal lesions induced by Swedish snuff.
• Study limitations: small sample size, no data on alcohol use available</td>
</tr>
</table>

[2] PCNA: Proliferating nuclear antigen; marker of cell proliferation
[3] p21: downstream target of p53; marker of cell cycle regulation
[4] HSP: Heat-shock protein; marker of cell stress

APPENDIX B-2
STUDIES OF P53 EXPRESSION IN ORAL MUCOSAL LESIONS (continued)

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>Summary, Conclusions and Comments</th></tr>
<tr>
<td>Ibrahim et al. 1996</td>
<td>Objective: To determine the relative frequency of p53 expression in suspected oral premalignant lesions and in oral SCCs in relation to use of snuff, as a marker of p53 mutation

Oral mucosa biopsy specimen

Immunohisto-chemistry staining of p53;
p53 antibodies: DO7 & DO1 (detect wild-type & mutant p53)</td>
<td>Swedish snuff, Sudanese snuff

Specimens from oral lesions in patients diagnosed with premalignant or malignant oral lesions (lip and intra-oral):

15 Swedish snuff dippers, 22 Sudanese snuff dippers, 17 Sudanese controls (no tobacco use), 60 Swedish controls (8 smokers), 60 Norwegian controls (11 smokers);

Sudanese: average snuff use 32 years, 42 g/day, 18 hrs/day M; 21 years, 19 g/day, 7 hrs/day F; Swedish average snuff use 11 years M, 36.1 g/day, 13.1 hrs/day

Negative controls: 5 samples from patients with no history of tobacco or alcohol use</td>
<td>Immunohistochemistry:
<table>
<tr><th rowspan="3">Oral Lesion</th><th colspan="4">P53 Expression in Oral Lesions</th></tr>
<tr><th colspan="2">Controls</th><th colspan="2">Snuff Users</th></tr>
<tr><th>Swedish/ Norwegian</th><th>Sudanese</th><th>Swedish</th><th>Sudanese</th></tr>
<tr><td>Fibro-epithelial hyperplasia</td><td>0/12</td><td>0</td><td>2/15 (13%)</td><td>0</td></tr>
<tr><td>Epithelial dysplasia</td><td>1/2</td><td>0/3</td><td>0</td><td>0/7</td></tr>
<tr><td>Carcinoma in situ</td><td>4/5</td><td>0</td><td>0</td><td>0/1</td></tr>
<tr><td>Squamous cell carcinoma</td><td>67/101 (66%)</td><td>9/14 (64%)</td><td>0</td><td>3/14 (21%)</td></tr>
</table>
• 0/15 lesions of Swedish snuff dippers were SCCs compared to 14/22 (64%) lesions of Sudanese snuff dippers.
• All 60 lesions of Swedish, 41/60 lesions of Norwegian (84% total), and 14/17 (82%) Sudanese non-snuff dipping controls were SCC.
• 2/15 oral fibro-epithelial hyperplasias of Swedish snuff-dippers expressed p53 compared with 0/12 of Norwegian controls. 5/7 other suspected premalignant lesions of Norwegian controls expressed p53, compared to 0/3 of Sudanese controls or 0/8 of Sudanese snuff dippers.
• 5/8 (63%) Swedish and 7/11 (63%) smokers among the non-snuff dipping controls expressed p53.
• Expression of p53 in SCCs from Sudanese snuff dippers was statistically significant ↓ compared to those from non-snuff-dippers from all 3 countries.</td>
<td>• 2/15 (13%) oral lesions of Swedish and 3/22 (14%) of Sudanese snuff dippers expressed p53 compared to 72/120 (60%) oral lesions of Swedish and Norwegian non-snuff dippers (incl. 19 smokers) and 9/17 (53%) oral lesions of Sudanese non-snuff dippers, who were all non-smokers.
• The authors concluded that there was a lower relative frequency of p53 expression in oral lesions from Sudanese and Swedish snuff dippers compared to oral lesions from non-snuff dippers.
• Study limitations: small sample size; no data on alcohol use available; comparison with healthy tissue from controls was not given</td>
</tr>
</table>

APPENDIX B-2
STUDIES OF P53 EXPRESSION IN ORAL MUCOSAL LESIONS (continued)

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>Summary, Conclusions and Comments</th></tr>
<tr>
<td>Wedenberg et al. 1996</td>
<td>Objective:
Immunohisto-chemical expression of p53 in snuff-dippers' lesions

Oral mucosa biopsy specimen

Immunohisto-chemistry staining of p53 & Ki-67;
p53 antibody: DO7 (detects wild-type & mutant p53)</td>
<td>Swedish snuff

Upper lip biopsy specimens from oral lesions of 15 Swedish non-smoking snuff-dippers, control samples of normal oral mucosa from 4 never-tobacco users</td>
<td>Histology of biopsy specimens:
Snuff-induced lesions were identified as whitish-yellowish to brown wrinkled lesions with intervening normal or reddened furrows. Evenly distributed, slight-moderate hyperkeratinization, ↑ epithelial thickness, rare atrophic lesions, frequent vacuolated epithelia cells in superficial cell layers, stromal chronic inflammation (lymphocytes, plasma cells); no ↑ number of mitotic figures, no sign of epithelial dysplasia or evidence of invasion of squamous carcinoma.

Immunohistochemistry:
p53 staining always confided to nuclei, positive cells found generally close to basal cell layers; no clear relationship between number of positive cells and histomorphological changes in lesion
Ki-67 staining was nuclear in epithelial cells located in basal and suprabasal cells layers in normal mucosa and lesions

<table>
<tr><th colspan="3">Protein Levels (number of positively stained nuclear profiles/mm^2 epithelium)</th></tr>
<tr><th></th><th>Lesions from Snuff Dipper</th><th>Lesions from Controls</th></tr>
<tr><td>p53</td><td>45.9 (11.5-172.9)</td><td>0.18 (0-0.73)</td></tr>
<tr><td>Ki-67</td><td>566.1 (106-1152)</td><td>20.2 (14.6-32.0)</td></tr>
</table>
</td>
<td>
• p53 and Ki-67 staining ↑ among biopsy samples from oral lesions of Swedish snuff users compared to biopsy samples of normal mucosa from controls
• No clear correlation was seen between positive cells and histomorphological changes in lesion
• Authors noted that not one of the snuff-induced lesions showed any clinical or histopathological signs of epithelial dysplasia or SCC, confirming earlier findings that overexpression of p53 is an early event in tumorigenesis
• Authors concluded that results may indicate that overexpression of p53 gene contributes to subsequent malignant cell transformation related to snuff-dipping
• Study limitations: small sample size; no data on alcohol use available; only limited statistical analysis
</td>
</tr>
</table>

APPENDIX B-2
STUDIES OF P53 EXPRESSION IN ORAL MUCOSAL LESIONS (continued)

<table>
<tr><th>CITATION</th><th>STUDY OBJECTIVE & DESIGN</th><th>TEST PRODUCTS, DOSAGE, DURATION,& GROUPS</th><th>RESULTS</th><th>Summary, Conclusions and Comments</th></tr>
<tr>
<td>Wood et al. 1994</td>
<td>Objective: to determine whether accumulation of tumor-suppressor gene p53 protein occurs in oral leukoplakia of snuff users; to determine whether correlation exists between accumulation of p53 and degree of epithelial dysplasia present in oral leukoplakia

Oral mucosa biopsy specimen

Immunohisto-chemistry staining of p53
(4 primary anti-bodies: PAb 421, 1801, CM-1 recognize both wild-type and mutant p53, and PAb 240, which recognizes mutant form only)

Retrospective analysis of archival tissue specimens</td>
<td>Oral snuff (not specified)
Same individuals as in Wedenberg study according to Merne et al. 2002

Part 1: Oral mucosa biopsy specimens of leukoplakia lesions from 12 snuff users and normal tissue from 12 healthy non-tobacco users

Part 2: 43 archived leukoplakia specimens collected 1985-1992</td>
<td><u>Histology of biopsy specimens</u>:
All 12 snuff-user lesions: mild epithelial dysplasia;
Controls and normal appearing mucosa of snuff users: histologically normal.

<u>Immunohistochemistry</u>:
p53 accumulation in 5/12 (41.7%) leukoplakia specimens
Number of P53 positive cells was significantly ↑ in leukoplakia specimens compared to healthy tissue of same person; while ↑ number of positive cells compared to normal tissue from controls, this was not statistically significant (p=0.06). No difference in number of positive cells between normal tissue from controls and snuff users.

<table>
<tr><th colspan="3">p53 Levels (average number of positively stained cells)</th></tr>
<tr><th colspan="2">Normal mucosa</th><th rowspan="2">Leukoplakia from Snuff Users</th></tr>
<tr><th>Controls</th><th>Snuff Users</th></tr>
<tr><td>7.00 ±5.04</td><td>4.00 ±1.0</td><td>21.89 ±4.33</td></tr>
</table>

Part 2: 50% of leukoplakia specimens contained at least one positive stained cell. Number of positive cells correlated with degree of dysplasia (r=0.853) and significant difference between moderate/severe dysplasia lesions compared to those graded as mild.

<table>
<tr><th>Histology</th><th>N</th><th>p53 Expression in Archived Leukoplakia Specimens</th></tr>
<tr><td>Mild dysplasia</td><td>24</td><td>14.53 ±3.33</td></tr>
<tr><td>Moderate dysplasia</td><td>11</td><td>140.36 ±30.03</td></tr>
<tr><td>Severe dysplasia</td><td>8</td><td>232.86 ±26.85</td></tr>
</table>
</td>
<td>• p53 accumulation was significantly ↑ in leukoplakia lesions compared to healthy tissue in the same snuff users.
• p53 accumulation in oral leukoplakia was correlated with degree of epithelial dysplasia.
• Leukoplakia from snuff users was graded at the lower end with mild epithelial dysplasia and number of P53 positive cells was similar to that seen in archived leukoplakia graded as mild epithelial dysplasia.
• Authors noted that it is not possible to ascertain that p53 detected in leukoplakia specimens is actually result of mutation; none of the specimen stained positive for PAb 240.
• The authors concluded that accumulation of p53 may be a biomarker/ intermediate endpoint useful for monitoring the effect of prevention intervention in persons with oral leukoplakia.
• Study limitations: small sample size; no data on alcohol use available</td>
</tr>
</table>

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 are 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. Among esophageal adenocarcinoma cases, 35 were ever-users of snuff while 33 of the squamous-cell carcinoma cases were ever-users of snuff. Controls were 820 individuals randomly selected from age and sex strata to resemble the age and sex distribution among the esophageal adenocarcinoma subjects. Snuff users were those taking a quid of snuff at least once per week for 6 months or more. The Swedish snuff used in this study is produced through a heat processing system instead of fermentation.	<u>Oral Snuff Usage</u> **Esophageal adenocarcinoma** Never used Ever used **Esophageal squamous-cell carcinoma** Never used Ever used <u>Duration of Snuff Usage</u> **Esophageal adenocarcinoma** 1-10 years 11-25 years > 25 years **Esophageal squamous-cell carcinoma** 1-10 years 11-25 years > 25 years	<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) *p for trend=0.31* 1.2 (0.5-2.5) 0.9 (0.4-2.1) 2.0 (0.9-4.1) *p for trend=0.18*	**The authors concluded that there was no statistically significant association between snuff dipping and the risk of either type of esophageal tumor.** Odds ratios were adjusted for age, gender, tobacco smoking or snuff use, 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. Smoking was strongly associated with squamous-cell carcinoma. 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 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 Snuff 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 Smoking Status **Esophageal adenocarcinoma** Never Previous Current **Esophageal squamous-cell carcinoma** Never Previous Current	Odds Ratios (95% CI) 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* 1.0 (reference) 1.9 (1.2-2.9)* 1.6 (0.9-2.7) 1.0 (reference) 2.5 (1.4-4.7)* 9.3 (5.1-17.0)*	

* 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)		Duration of Smoking **Esophageal adenocarcinoma** 1-20 years 21-35 years > 35 years **Esophageal squamous-cell carcinoma** 1-20 years 21-35 years > 35 years Intensity of Smoking **Esophageal adenocarcinoma** 1-9 cigs/day 10-19 cigs/day > 19 cigs/day **Esophageal squamous-cell carcinoma** 1-9 cigs/day 10-19 cigs/day > 19 cigs/day	Odds Ratios (95% CI) 1.8 (1.1-3.1)* 1.5 (0.9-2.6) 2.0 (1.2-2.3)* 2.3 (1.1-4.6)* 2.9 (1.5-5.8)* 8.8 (4.9-16.1)* 1.2 (0.7-2.2) 1.7 (1.0-2.9) 1.1 (0.6-2.0) 2.8 (1.5-5.2)* 3.9 (2.2-6.9)* 4.9 (2.7-9.0)*	

* 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. 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. "Snuff" was defined as moist oral snuff in this paper.	<u>Head and Neck Cancer</u> **Oral snuff usage** Never used Ever used Current users Ex-users **Smoking** Never smoked Ever smoked Current smokers Ex-smokers **Age at start (snuff)** Never used < 25 years ≥ 25 years **Age at start (smoking)** Never smoked < 15 years 15-19 years 20-24 years ≥ 25 years **Duration of snuff usage** Never used < 30 years ≥ 30 years	<u>Relative Risk Estimates (95% CI)</u> 1.0 (reference) 1.1 (0.7-1.5) 1.0 (0.6-1.6) 1.2 (0.7-1.9) 1.0 (reference) 4.0 (2.8-5.7)* 6.5 (4.4-9.5)* 1.9 (1.3-2.8)* 1.0 (reference) 1.0 (0.6-1.6) 1.1 (0.7-1.8) 1.0 (reference) 5.0 (3.2-7.9)* 4.0 (2.7-5.9)* 3.8 (2.4-5.9)* 2.6 (1.5-4.6)* 1.0 (reference) 1.0 (0.7-1.6) 1.1 (0.6-2.0)	**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 number 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 (continued)		Head and Neck Cancer	Relative Risk Estimates (95% CI)	
		Duration of smoking		
		Never smoked	1.0 (reference)	
		< 30 years	1.2 (0.7-1.9)	
		30-44 years	3.9 (2.6-5.9)*	
		≥ 45 years	7.2 (4.8-10.8)*	
		Total snuff consumption		
		Never used	1.0 (reference)	
		< 125 kg	1.0 (0.7-1.6)	
		≥ 125 kg	1.1 (0.6-2.0)	
		Total smoking consumption		
		Never smoked	1.0 (reference)	
		< 125 kg	1.5 (1.0-2.4)	
		125-250 kg	4.3 (2.9-6.5)*	
		≥ 250 kg	5.9 (4.0-8.8)*	
		Intensity of snuff usage		
		Never used	1.0 (reference)	
		≤ 50 g/week	0.8 (0.5-1.3)	
		> 50 g/week	1.6 (0.9-2.6)	
		Intensity of smoking		
		Never smoked	1.0 (reference)	
		< 15 g/day	3.4 (2.3-5.1)*	
		15-24 g/day	4.4 (2.9-6.5)*	
		≥ 25 g/day	4.8 (2.9-8.1)*	

* 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 (continued)		Usage By Site	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)	
		Current smokers	4.9 (2.6-9.2)*	
		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)	
		Current smokers	7.5 (3.9-14.2)*	
		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)	
		Current smokers	5.2 (2.6-10.3)	
		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)	
		Current smokers	8.5 (4.0-18.2)*	

* 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. Among current snuff users, mucosal changes at the site of snuff placement were classified according to clinical severity using a 4-point scale. "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.	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
Rosenquist et al. 2005 (continued)		Smoking **Consumption** Never smoked 1-10 cigs/day 11-20 cigs/day > 20 cigs/day **Total Consumption** Never smoked < 125 kg 125-250 kg > 250 kg	Odds Ratios (95% CI) 1.0 (reference) 1.1 (0.6-2.1) 2.4 (1.3-4.1)* 2.8 (1.3-6.1) 1.0 (reference) 1.1 (0.6-2.0) 1.8 (1.0-3.5) 4.7 (2.4-9.1)	

* 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. 67 cases and 72 controls were active or ex-users of snuff. "Snuff" was defined as moist snuff in this paper.	<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 among never smokers** ≤ 156.0 kg > 156.0 kg <u>Smoking</u> **Smoking** Never smoker Active smoker Ex-smoker Ever-smoker **Smoking among never snuff-users** Never smoker Ex-smoker Active smoker **Lifetime use among never snuff users** ≤ 124.8 kg > 124.8 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 (0.4-1.6) 1.3 (0.6-2.6) 1.0 (reference) 1.8 (1.1-2.7)* 1.0 (0.6-1.6) 1.3 (0.9-1.9) 1.0 (reference) 0.9 (0.6-1.4) 1.7 (1.1-2.6)* 1.2 (0.7-1.9) 1.8 (1.1-2.9)*	**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. Snus is not defined in this study but assumed to be Swedish snus.	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.	<u>Tobacco Usage</u> **Risk of Oral Cancer Among all Cohort Members** Never-users of tobacco Ever-users of snus Ever-smokers Ex-smokers Current smokers **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)** 2.0 (1.4-2.7)* 1.1 (0.8-1.7) 2.5 (1.7-3.5)* 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, and that the oral use of snus does not seem to be linked to the risk for cancer of the oral cavity.** 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." The results can only be confidently generalized to Swedish male construction workers. The relative risks were not adjusted for alcohol consumption.

* 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 (continued)	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).			

* 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, all-cause mortality and respiratory death in Appendix Q-1 and cardiovascular diseases in Appendix J-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. Snus is defined as Scandinavian moist snuff in this study.	**Oral Snuff and Smoking Usage** **Oral/Pharyngeal Cancer** Snus use Never daily use Ever daily use (n=11) 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 (n=5)	**Hazard Ratio (95% CI)** 1.0 (reference) 3.1 (1.5-6.6)* 1.0 (reference) 0.5 (0.1-1.4) 1.0 (reference) 5.6 (1.6-19.6)* 1.0 (reference) 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. Snus is defined as Scandinavian moist snuff in this study.	**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 (n=1) Squamous cell carcinoma (n=10) [See Zendehdel et al. 2008 for additional analyses]	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)*	**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. Snus is not defined in this study but assumed to be Swedish snus.	Tobacco Usage **Risk of Pancreatic Cancer Among All Snus Users Regardless of Smoking Status** Never user of snus Ever snus use (n=45) Former snus use (n=18) Current snus use (n=27) **Risk of Pancreatic Cancer Among Ever-Users of Snus According to Smoking Status** Never users of snus Never smokers (n=3) Former smokers (n=14) Current smokers (n=28)	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) 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>Tobacco Usage</u> **Risk of Pancreatic Cancer Among all Cohort Members** Never-users of tobacco Ever-users of snus Ever-smokers Ex-smokers Current smokers **Risk of Pancreatic Cancer Among 125,576 Never-Smokers** Never-users of tobacco Ever-users of snus (n=20) Ex-users of snus (n=2) Current users of snus (n=18) Amount of snus consumed 1-9 g/day (n=6) ≥ 10 g/day (n=13)	<u>Relative Risk (95% CI)</u> 1.0 (reference) 0.9 (0.7-1.2) 2.8 (2.1-3.7)* 1.8 (1.3-2.4)* 3.5 (2.6-4.6)* 1.0 (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 sample 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. Snuff is not defined in this study. Individuals who use snuff seem to be combined with those who also chew tobacco.	<u>Chewing tobacco or snuff usage (regular use vs. never used)</u> **All cases of pancreatic cancer** Among all individuals with chewing data (n=12) **Histologically-verified cases only** Among all individuals with chewing data (n=9) Among men with alcohol, cigarette and chewing data (n=6) Among men with alcohol, cigarette and chewing data, with adjustment for alcohol use and cigarette smoking (n=4) <u>Cigarette smoking (≥10 cigs/day vs. never smoked)</u> **All cases of pancreatic cancer** Among men with cigarette data (n=6) **Histologically-verified cases only** Among all individuals with chewing data (n=5)	<u>Relative Risk (p-value)</u> 1.34 (0.21) 2.20 (0.045)* 2.31 (0.067) 2.85 (0.060) 1.13 (0.35) 2.04 (0.087)	**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.** 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. 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. Strengths of this study include its large sample size and prospective study design, however the number of cases available in general and to study the joint effects of the various risk factors was small; limiting the reliability of adjusted relative risks.

* 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 (continued)			Relative Risk (p-value)	
		Among men with alcohol, cigarette and chewing data (n=4)	1.88 (0.13)	
		Among men with alcohol, cigarette and chewing data, with adjustment for alcohol use and cigarette smoking (n=4)	2.13 (0.12)	

* 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.	<u>Oral Snuff Usage</u> Snuff Dipping <u>Cigarette smoking</u> Non-users of tobacco Ex-smokers Current smokers	<u>Odds Ratio (95% CI)</u> 0.70 (0.47-1.06) *(Reference group was not specified.)* 1.0 (reference) 1.09 (0.75-1.59) 1.72 (1.16-2.54)*	**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. The odds ratios for gastric cancer associated with cigarette use were adjusted for age, gender, SES 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. Among the cases, 50 were ever-users of snuff. Controls were 820 individuals randomly selected from age and sex strata to resemble the age and sex distribution among the esophageal adenocarcinoma subjects. Snuff users were those taking a quid of snuff at least once per week for 6 months or more. The Swedish snuff used in this study is produced through a heat processing system instead of fermentation.	<u>Oral Snuff Usage</u> **Snuff Status** Never used Ever used (n=53) **Duration of Usage** 1-10 years (n=18) 11-25 years (n=19) > 25 years (n=15) **Intensity of Usage** 1-14 quids/week (n=19) 15-35 quids/week(n=15) > 35 quids/week (n=18) <u>Cigarette Usage</u> **Smoking Status** Never Previous (n=124) Current (n=95) **Duration of Usage** 1-20 years (n=38) 21-35 years (n=77) > 35 years (n=104) **Intensity of Usage** 1-9 cigs/day (n=46) 10-19 cigs/day (n=73) > 19 cigs/day (n=86)	<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* 1.0 (reference) 3.4 (2.2-5.2)* 4.5 (2.9-7.1)* 2.1 (1.2-3.4)* 3.9 (2.4-6.2)* 5.7 (3.6-9.1)* 2.3 (1.4-3.7)* 3.1 (2.0-4.9)* 3.6 (2.3-5.7)*	**The authors concluded that there was no statistically significant association between snuff dipping and the risk of gastric cardia adenocarcinoma.** Odds ratios were adjusted for age, gender, tobacco smoking or snuff use, 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 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). 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 (n=6) Current users (n=9) Ever-users (n=15) **Distal stomach cancer-intestinal** Never-users Ex-users (n=18) Current users (n=26) Ever-users (n=44) **Distal stomach cancer-diffuse** Never-users Ex-users (n=8) Current users (n=11) Ever-users (n=19) **Total gastric and cardia cancer** Never tobacco Ever-user (never smoke) (n=11) <u>Smoking</u> **Cardia cancer** Never-smokers Ex-smokers (n=25) Current smokers (n=31)	<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) 1.0 (reference) 0.5 (0.2-1.2) 1.0 (reference) 0.9 (0.5-1.6) 1.7 (1.0-3.1)	**The authors found no evidence that snuff dipping increased the risk of gastric cancer (of any sub-site or histologic type).** 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. Odds ratios among smokers were adjusted for age, gender, residence area, BMI, SES, use of smokeless tobacco, and use of beer, wine and liquor. 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. 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 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
	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.	**Distal stomach cancer-intestinal** Never-smokers Ex-smokers (n=101) Current smokers (n=67) **Distal stomach cancer-diffuse** Never-smokers Ex-smokers (n=46) Current smokers (n=57) **Total gastric and cardia cancer** Never tobacco Current smoker (never snuff) (n=101) [See Ye et al. 1999 for additional analyses]	Odds Ratios (95% CI) 1.0 (reference) 1.4 (1.0-2.0) 1.8 (1.2-2.7)* 1.0 (reference) 1.3 (0.8-2.0) 2.2 (1.4-3.5)* 1.0 (reference) 2.0 (1.3-2.9)*	

* 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. There were 74 cases among ever users of snus and 42 cases among current users. 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. Snus is not defined but assumed to be Swedish.	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. Snus is defined as Scandinavian moist snuff in this study.	<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 (n=58) Noncardia (n=253) Snus users, additionally adjusted for smoking intensity Cardia (n=58) Noncardia (n=253) Among never-smokers: Users of snus only Cardia (n=8) Noncardia (n=68) [See Zendehdel et al. 2008 for additional analyses]	<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)*	**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 analysis of cases with cancer of the gastric cardia was based on a small number of cases (n=8).

* 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.	Oral Snuff Usage **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	Relative Risk (95% CI) 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. There were 22 and 69 kidney and bladder cancer cases among ever users of snus, and 9 and 40 kidney and bladder cancer cases, respectively, among current users of snus. 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. Snus is not defined in this study but assumed to be Swedish snus.	<u>Tobacco Usage</u> **Risk of All Types of Lung Cancer Regardless of Smoking Status** Never user of snus Ever users of snus (n=72) Former users of snus (n=28) Current users of snus (n=44) **Risk of All Types of Lung Cancer Among Ever-Users of Snus According to Smoking Status** Never users of snus Never smokers (n=3) Former smokers (n=7) Current smokers (n=62) **Risk of Lung Adenocarcinoma Regardless of Smoking Status** Never user of snus Ever users of snus (n=11) Former users of snus (n=4) Current users of snus (n=7)	<u>Relative Risk (95% CI)</u> 1.00 (reference) 0.80 (0.61-1.05) 0.80 (0.54-1.19) 0.80 (0.58-1.11) 1.00 (reference) 0.96 (0.26-3.56) 0.64 (0.24-1.68) 0.68 (0.51-0.90)** 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.	Death due to Lung Cancer by Snus Use or Non-Use of Tobacco **Among ages 35-54 at study entry** Nonusers Smokeless tobacco users (n=1) **Among ages 55-65 at study entry** Nonusers Smokeless tobacco users (n=2) Death due to Lung Cancer by Smoking or Non-Use of Tobacco **Among ages 35-54 at study entry** Nonusers < 15 cig/day (n=16) > 15 cig/day (n=43) Ex-smokers, 1-5 years (n=7) Ex-smokers, > 5 years (n=3) **Among ages 55-65 at study entry** Nonusers < 15 cig/day (n=36) > 15 cig/day (n=57) Ex-smokers, 1-5 years (n=14) Ex-smokers, > 5 years (n=12)	Relative Risk of Death (95% CI) 1.0 (reference) 1.2 (0.2-9.1) 1.0 (reference) 0.8 (0.1-3.9) 1.0 (reference) 8.1 (3.2-20.4)* 21.4 (8.5-54.1)* 6.7 (2.3-19.7)* 1.2 (0.3-4.5) 1.0 (reference) 11.9 (5.5-25.6)* 30.6 (14.6-64.1)* 9.4 (3.9-22.3)* 2.3 (1.0-5.7)*	**The authors stated that no excess risk of death due to cancer was observed in smokeless tobacco users when compared to nonusers.** 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. There were only 3 lung cancer deaths among smokeless tobacco users.

* 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 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).	<u>Tobacco Usage</u> **Risk of Lung Cancer Among all Cohort Members** Never-users of tobacco Ever-users of snus Ever-smokers (n=2062) Ex-smokers (n=329) Current smokers (n=1733) **Risk of Lung Cancer Among 125,576 Never-Smokers** Never-users of tobacco Ever-users of snus (n=18) Ex-users of snus (n=3) Current users of snus (n=15) Amount of snus consumed 1-9 g/day (n=7) > 10 g/day (n=10)	Relative Risk (95% CI) 1.0 (reference) 0.7 (0.6-0.7)** 7.2 (6.0-8.5)* 2.6 (2.2-3.2)* 10.2 (8.6-12.2)* 1.0 (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

Cohort Studies of Other Cancers

APPENDIX H
COHORT STUDIES OF OTHER CANCERS AMONG SWEDISH SNUS USERS (N=8)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS 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. 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, all-cause mortality, cardiovascular disease, and stroke are presented in Appendices G, Q-1, 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 Tobacco Usage</u> **Among all subjects** Nonusers of tobacco Smokeless tobacco (n=96) Smokers < 15 cig/day (n=216) Smokers ≥ 15 cig/day (n=276) **Among ages 35-54 at study entry** Nonusers of tobacco Smokeless tobacco (n=22) Smokers < 15 cig/day (n=62) Smokers ≥ 15 cig/day (n=116) **Among ages 55-65 at study entry** Nonusers of tobacco Smokeless tobacco (n=69) Smokers < 15 cig/day (n=145) Smokers ≥ 15 cig/day (n=148)	<u>Relative Risk (95% CI) Of Death</u> 1.0 (reference) 1.1 (0.9-1.4) 1.5 (1.3-1.8)* 2.5 (2.2-3.0)* 1.0 (reference) 1.2 (0.8-1.9) 1.2 (0.9-1.7) 2.2 (1.8-2.9)* 1.0 (reference) 1.0 (0.8-1.3) 1.7 (1.4-2.1)* 2.9 (2.3-3.0)*	**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
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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 and assumed to be Swedish).	<u>Tobacco Usage -- Men</u> **Acute Lymphocytic Leukemia** Never tobacco user Pure snuff dipper (n=4) Current pure smoker (n=19) **Acute Myelogenous Leukemia** Never tobacco user Pure snuff dipper (n=10) Current pure smoker (n=92) **Chronic Myelogenous Leukemia** Never tobacco user Pure snuff dipper (n=12) Current pure smoker (n=28) **Multiple Myeloma** Never tobacco user Pure snuff dipper (n=26) Current pure smoker (n=168)	<u>Incidence Rate Ratios (95% CI)</u> 1.00 (reference) 1.24 (0.39-4.01) 1.80 (0.83-3.90) 1.00 (reference) 0.81 (0.41-1.60) 1.50 (1.06-2.11)* 1.00 (reference) 1.17 (0.60-2.28) 0.69 (0.42-1.14) 1.00 (reference) 0.92 (0.61-1.40) 0.96 (0.77-1.20)	**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 and smoking were restricted to pure users of snuff and smokers respectively. 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.

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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 Hodgkin's 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. There was only 1 female who used snuff, and no cases of NHL or HD. 28% of the male subjects had ever used snuff (defined as moist snuff and assumed to be Swedish).	<u>Tobacco Usage -- Men</u> **Non-Hodgkin's Lymphoma** Never tobacco user Ever snuff dipper (n=66) 1-30 years snuff dip. (n=49) > 30 years snuff dip. (n=16) Ever Cigarette (n=357) Current smoking (n=455) **Hodgkin's Disease** Never tobacco user Ever snuff dipper (n=15) 1-30 years snuff dip. (n=11) > 30 years snuff dip. (n=4) Ever Cigarette (n=66) Current smoking (n=73) <u>Tobacco Usage -- Women</u> **Non-Hodgkin's Lymphoma** Never tobacco user Ever snuff dipper (n=0) Ever Cigarette (n=14) Current smoking (n=13) **Hodgkin's Disease** Never tobacco users Ever snuff dipper (n=0) Ever Cigarette (n=3) Current smoking (n=2)	<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) 1.00 (0.86-1.16) 1.00 (0.87-1.15) 1.0 (reference) 0.88 (0.49-1.58) 0.70 (0.36-1.37) 3.78 (1.23-11.60)* 1.32 (0.91-1.91) 1.32 (0.91-1.90) 1.0 (reference) 1.36×10^{-15} (~0) 0.68 (0.35-1.31) 0.75 (0.38-1.47) 1.0 (reference) 8.72×10^{-16} (~0) 0.50 (0.12-2.11) 0.38 (0.72-2.00)	**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.

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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
Nordenvall et al. 2012 Sweden This study aimed to investigate the relationship between tobacco use and cancer-specific death. [Subjects were selected from the same overall study population as Bolinder et al. 1992 and Bolinder et al. 1994] Results on all-cause mortality and death from causes other than cancer (among those diagnosed with cancer) are presented in Appendix Q-1.	Cohort study Subjects were 336,381 male Swedish construction workers 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, 2007, whichever occurred first. Cancer-specific death had to be the same as the first cancer diagnosis (i.e. the cause of death was cancer at the same site as the primary cancer). 40,230 new first cancers were diagnosed during follow up (who did not die the same day as diagnosis). Of these cases, 14,533 died from the primary cancer and 9,716 died from other causes. Cause of death was ascertained through the year 2007 by record linkage with nationwide cancer and death registries. Survival was investigated among never-smoking snus users, never-snus-using smokers, and combined users (currently or in sequence). Snus is defined as Scandinavian moist snuff (snus) in this study.	<u>Among Those Diagnosed with Cancer</u> **Cancer-Specific Death** Never-users of any tobacco Ever tobacco users Pure snus users Pure smokers Combined users <u>Among Those Diagnosed with Smoking-Related Cancer</u> **Cancer-Specific Death** Never-users of any tobacco Ever tobacco users Pure snus users Pure smokers Combined users <u>Cancer-Specific Death by Comorbidity</u> **No Comorbidity** Never-users of any tobacco Ever tobacco users Pure snus users Pure smokers Combined users	<u>Hazard ratio (95% CI)</u> 1.00 (reference) 1.14 (1.09-1.19)* 1.15 (1.05-1.26)* 1.15 (1.10-1.21)* 1.08 (1.02-1.15)* 1.00 (reference) 1.15 (1.09-1.20)* 1.17 (1.06-1.30)* 1.16 (1.11-1.15)* 1.08 (1.01-1.15)* 1.00 (reference) 1.22 (1.10-1.36)* 1.10 (0.86-1.40) 1.24 (1.11-1.38)* 1.20 (1.04-1.40)*	**The authors concluded that use of tobacco, and also the less harmful moist snuff (snus), is associated with moderately increased cancer-specific mortality.** The authors further stated that their data provide little guidance in regard to the mechanisms, which warrant further investigations, but suggest that their results concerning snus users seem to narrow in on nicotine as a conceivable culprit. Hazard ratios were adjusted for age at cancer diagnosis, calendar period of diagnosis, cancer site, and BMI at entry. This study has several strengths. It was a large, prospective study with almost complete follow-up and accurate identification of outcomes using national registers. The authors also state that confounding due to occupation or socioeconomic status was unlikely given the homogeneous population of construction workers.

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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
Nordenvall et al. 2012 (continued)			Hazard ratio (95% CI)	A limitation of this study is that 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 also state that the results may not be generalizable to women, and a healthy worker effect cannot be disregarded. In an earlier study of the same population (Nordenvall et al. 2010), the authors noted that there were no data on other possible confounders such as diet, alcohol intake and physical activity.
		Chronic Pulmonary / Cerebrovascular Disease / Myocardial Infarction		
		Never-users of any tobacco	1.00 (reference)	
		Ever tobacco users	1.13 (1.04-1.23)*	
		Pure snus users	1.08 (0.89-1.29)	
		Pure smokers	1.16 (1.06-1.26)*	
		Combined users	1.07 (0.96-1.20)	
		Other Comorbidity		
		Never-users of any tobacco	1.00 (reference)	
		Ever tobacco users	1.17 (1.12-1.23)*	
		Pure snus users	1.15 (1.04-1.27)*	
		Pure smokers	1.19 (1.13-1.25)*	
		Combined users	1.12 (1.05-1.19)*	

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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
Nordenvall et al. 2010 Sweden This study evaluated the potential association between tobacco use and anal and colorectal cancer.	Cohort study Subjects were 336,381 male Swedish construction workers 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, 2007, whichever occurred first. Incidence of anal and colon cancer was ascertained through the year 2007 by record linkage with nationwide cancer, migration, and death registries. 42% of the workers were ever smokers at time of entry, 12% were only snus users, and 16% were combined smokers and snus users. Snus is defined as Scandinavian moist snuff (snus) in this study.	<u>Tobacco Usage</u> **Colon** Non-users of any tobacco Pure snus users (n=153) Duration at inclusion: 1-9 years (n=39) 10-24 years (n=43) ≥ 25 years (n=71) Estimated total duration: 1-24 years (n=27) 25-34 years (n=27) 35-44 years (n=33) ≥ 45 years (n=66) Pure smokers (n=1,282) Pure cigarette (n=690) Duration at inclusion: 1-14 years (n=166) 15-24 years (n=210) ≥ 25 years (n=308) Estimated total duration: 1-29 years (n=114) 30-39 years (n=162) 40-49 years (n=217) ≥ 50 years (n=191) Mixed users (n=440) **Right-sided colon** Non-users of any tobacco Pure snus users (n=59) Duration at inclusion: 1-9 years (n=16) 10-24 years (n=17) ≥ 25 years (n=26)	<u>Hazard ratio (95% CI)</u> 1.00 (reference) 1.08 (0.91-1.29) 1.33 (0.94-1.88) 1.02 (0.74-1.38) 1.06 (0.83-1.36) 1.15 (0.75-1.76) 0.97 (0.65-1.43) 1.01 (0.71-1.44) 1.16 (0.89-1.50) 1.08 (0.99-1.19) 1.10 (0.99-1.22) 1.06 (0.89-1.26) 1.12 (0.96-1.32) 1.12 (0.98-1.28) 0.98 (0.79-1.22) 1.02 (0.85-1.22) 1.14 (0.97-1.33) 1.22 (1.03-1.45)* 1.17 (1.04-1.32)* 1.00 (reference) 0.86 (0.65-1.13) 1.09 (0.64-1.86) 0.84 (0.51-1.37) 0.80 (0.53-1.19)	**The authors concluded that the results do not convincingly support an important role of tobacco use in the etiology of colorectal cancer. A link between smoking and anal cancer was confirmed.** The authors stated that overall, their results are mostly nonsignificant and the excess so small that they could be explained by confounding from unmeasured factors. Hazard ratios were adjusted for age and BMI. This study has several strengths. It was a large, prospective study with almost complete follow-up and accurate identification of outcomes using national registers. The authors also state that confounding due to occupation or socioeconomic status was unlikely given the homogeneous population of construction workers. No obvious "healthy worker effect" was observed. A limitation of this study is that 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.

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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
Nordenvall et al. 2010 (continued)		Estimated total duration:		The authors also noted that some of the results were imprecise, suggesting that multiple significance testing may have generated borderline significant results by chance. Additionally, anal cancer specifically is a low-incidence type of cancer. The authors further noted that there were no data on other possible confounders such as diet, alcohol intake, physical activity, and NSAID use. For example, physical activity is inversely associated with risk of colon cancer but not rectal cancer. Smokers are known to perform less physical activity than non-smokers.
		1-24 years (n=13)	1.07 (0.58-1.98)	
		25-34 years (n=12)	0.92 (0.51-1.66)	
		35-44 years (n=9)	0.63 (0.32-1.22)	
		≥ 45 years (n=25)	0.87 (0.57-1.31)	
		Pure smokers (n=602)	1.07 (0.93-1.22)	
		Pure cigarette (n=331)	1.12 (0.96-1.31)	
		Duration at inclusion:		
		1-14 years (n=75)	1.01 (0.78-1.31)	
		15-24 years (n=103)	1.19 (0.95-1.49)	
		≥ 25 years (n=153)	1.16 (0.96-1.41)	
		Estimated total duration:		
		1-29 years (n=49)	0.85 (0.62-1.18)	
		30-39 years (n=80)	1.13 (0.87-1.46)	
		40-49 years (n=101)	1.19 (0.94-1.50)	
		≥ 50 years (n=101)	1.16 (0.96-1.41)	
		Mixed users (n=194)	1.09 (0.91-1.30)	
		Left-sided colon		
		Non-users of any tobacco	1.00 (reference)	
		Pure snus users (n=60)	1.28 (0.97-1.71)	
		Duration at inclusion:		
		1-9 years (n=11)	1.55 (0.83-2.90)	
		10-24 years (n=19)	1.35 (0.85-2.17)	
		≥ 25 years (n=30)	1.21 (0.82-1.78)	
		Estimated total duration:		
		1-24 years (n=5)	1.09 (0.43-2.76)	
		25-34 years (n=11)	1.19 (0.64-2.21)	
		35-44 years (n=20)	1.66 (1.05-2.63)*	
		≥ 45 years (n=24)	1.19 (0.77-1.82)	
		Pure smokers (n=468)	1.12 (0.95-1.30)	

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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
Nordenvall et al. 2010 (continued)		Pure cigarette (n=238)	1.08 (0.90-1.29)	
		Duration at inclusion:		
		1-14 years (n=55)	1.10 (0.81-1.48)	
		15-24 years (n=68)	1.03 (0.78-1.35)	
		≥ 25 years (n=109)	1.07 (0.85-1.35)	
		Estimated total duration:		
		1-29 years (n=39)	1.17 (0.82-1.67)	
		30-39 years (n=53)	0.94 (0.69-1.28)	
		40-49 years (n=73)	1.01 (0.77-1.33)	
		≥ 50 years (n=67)	1.18 (0.89-1.56)	
		Mixed users (n=171)	1.30 (1.06-1.58)*	
		Rectum		
		Non-users of any tobacco	1.00 (reference)	
		Pure snus users (n=99)	1.05 (0.85-1.31)	
		Duration at inclusion:		
		1-9 years (n=15)	0.71 (0.42-1.20)	
		10-24 years (n=33)	1.07 (0.75-1.53)	
		≥ 25 years (n=49)	1.18 (0.88-1.60)	
		Estimated total duration:		
		1-24 years (n=12)	0.81 (0.44-1.50)	
		25-34 years (n=17)	0.78 (0.47-1.27)	
		35-44 years (n=27)	1.10 (0.74-1.63)	
		≥ 45 years (n=41)	1.27 (0.92-1.77)	
		Pure smokers (n=978)	1.16 (1.04-1.30)*	
		Pure cigarette (n=539)	1.18 (1.04-1.34)*	
		Duration at inclusion:		
		1-14 years (n=230)	1.18 (1.01-1.39)*	
		15-24 years (n=7)	----	
		≥ 25 years (n=532)	----	
		Estimated total duration:		
		1-29 years (n=181)	1.23 (1.03-1.47)*	
		30-39 years (n=115)	1.14 (0.92-1.41)	
		40-49 years (n=7)	----	
		≥ 50 years (n=--)	----	

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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
Nordenvall et al. 2010 (continued)		Mixed users (n=319)	1.21 (1.05-1.39)*	
		Anus		
		Non-users of any tobacco	1.00 (reference)	
		Pure snus users (n=1)	0.61 (0.07-5.07)	
		Duration at inclusion:		
		1-9 years (n=0)	----	
		10-24 years (n=0)	----	
		≥ 25 years (n=1)	2.05 (0.23-18.1)	
		Estimated total duration:		
		1-24 years (n=0)	----	
		25-34 years (n=0)	----	
		35-44 years (n=0)	----	
		≥ 45 years (n=1)	2.88 (0.31-26.9)	
		Pure smokers (n=31)	2.41 (1.06-5.48)*	
		Pure cigarette (n=18)	2.57 (1.07-6.17)*	
		Duration at inclusion:		
		1-14 years (n=8)	2.84 (1.02-7.94)*	
		15-24 years (n=0)	----	
		≥ 25 years (n=18)	----	
		Estimated total duration:		
		1-29 years (n=9)	4.58 (1.60-13.1)*	
		30-39 years (n=2)	1.26 (0.25-6.42)	
		40-49 years (n=0)	----	
		≥ 50 years (n=--)	----	
		Mixed users (n=14)	3.48 (1.40-8.64)*	

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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>Tobacco Usage</u> **All Melanoma** Tobacco nonuser Pure cigarette smoker Pure snuff user 1-29 years ≥ 30 years **CMM** Tobacco nonuser Pure cigarette smoker Pure snuff user 1-29 years ≥ 30 years **MIS** Tobacco nonuser Pure cigarette smoker Pure snuff user 1-29 years ≥ 30 years **IMM** Tobacco nonuser Pure cigarette smoker Pure snuff user 1-29 years ≥ 30 years	<u>Incidence Rate Ratio (95% CI)</u> 1.00 (reference) 0.69 (0.61-0.79)** 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 (reference) 0.69 (0.59-0.80)** 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 (reference) 0.67 (0.49-0.94)** 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 (reference) 0.86 (0.45-1.62) 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 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.

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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, but is assumed to be Swedish.) Snuff dippers were categorized by length of use (< 30 years or ≥ 30 years). 28% of the subjects had ever used snuff. 13% had only ever used snuff. There were 29 cases of CSCC among snuff dippers.	<u>Tobacco Usage</u> **Tobacco Usage** Nontobacco User Snuff Dipper Cigarette smoker **Years of Snuff Dipping** < 30 ≥ 30	<u>Incidence Rate Ratio (95% CI)</u> 1.00 (reference) 0.64 (0.44-0.95)** 1.04 (0.85-1.26) 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.** The incidence rate ratios were adjusted for age and for all other categories of tobacco use. 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. 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, and all-cause mortality and respiratory death are presented in Appendix C-3 and Q-1 respectively.	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. Snus is defined as Scandinavian moist snuff (snus) in this study.	Tobacco Usage **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	Hazard Ratio (95% CI) 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 concluded 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."

[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 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 (continued)				Additionally, there was no information on the 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 I

Appendix to Table 5-3: Effect Estimates for Cardiovascular Disease Risk Factors and Events

Appendix I: Appendix to Table 5-3: Effect Estimates for Cardiovascular Disease Risk Factors and Events

Cardiovascular Disease Risk Factors and Events among Swedish Snus Users		
Reference	**Outcome**	**Effect Estimate (95% CI)**
Acute Effects on Heart Rate		
Associations examined but effect estimates were not reported by: Bolinder et al. 1997b; Hirsch et al. 1992; Lunell and Curvall 2011; Rohani and Agewall 2004		
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		
Acute Effects on Blood Pressure		
Associations examined but effect estimates were not reported by: Bolinder et al. 1997b; Hirsch et al. 1992; Rohani and Agewall 2004		
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)

Cardiovascular Disease Risk Factors and Events among Swedish Snus Users		
Reference	**Outcome**	**Effect Estimate (95% CI)**
	>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		
Cholesterol		
Hergens et al. 2005	**Hyperlipidemia** Snuff use Former Current	 1.1 (0.63-2.0) 0.99 (0.66-1.5)
Norberg et al. 2006	**Low HDL** ≤4 cans/week >4 cans/week	 1.0 (0.86-1.18) 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 >4 cans/week	 1.2 (1.05-1.35)* 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		
Eliasson et al. 2004	**Impaired glucose tolerance** Consistent exclusive snus users Ex-snus users Smokers who switched to snus **Pathological glucose tolerance** Consistent exclusive snus users Ex-snus users Smokers who switched to snus	 0.23 (0.03-1.80) 0.75 (0.16-3.57) 1.18 (0.51-2.74) 0.45 (0.10-2.04) 1.85 (0.60-5.70) 1.05 (0.46-2.44)
Norberg et al. 2006	**f- P-glucose ≥5.6 or diabetes** ≤4 cans/week >4 cans/week	 1.0 (0.86-1.08) 0.8 (0.69-1.02)
Persson et al. 2000	**Impaired glucose tolerance** Moist snuff Former Current No. of boxes of snuff week^{-1} in current snuffers ≤2 3+	 0.7 (0.4-1.2) 0.8 (0.4-1.4) 0.7 (0.4-1.4) 0.8 (0.4-1.4)
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 Current No. of boxes of snuff week^{-1} in current snuffers ≤2	 0.4 (0.1-1.3) 0.9 (0.4-2.0) 0.5 (0.2-1.6)

Cardiovascular Disease Risk Factors and Events among Swedish Snus Users		
Reference	**Outcome**	**Effect Estimate (95% CI)**
	3+	0.7 (0.3-1.7)
	2 h insulin response, lowest third	
	Moist snuff	
	Former	2.2 (1.1-4.4)*
	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; Hirsch et al. 1992; Wennmalm et al. 1991		
Impaired Endothelial Function		
An association was examined but an effect estimate was not reported by: Rohani and Agewall 2004		
MetSy		
Gustafsson et al. 2011b	**Metabolic Syndrome**	
	Snuff use at age 43	
	Men	0.79 (0.33-1.86)
	Women	0.96 (0.58-1.56)
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		
Eliasson et al. 2004	Prevalence Results	
	Known Type 2 diabetes	

Cardiovascular Disease Risk Factors and Events among Swedish Snus Users		
Reference	**Outcome**	**Effect Estimate (95% CI)**
	Ever snus use (exclusive) Current snus user Ex-snus user <u>Incidence Results</u> **Known Type 2 diabetes** Consistent exclusive snus Ex-snus users Smokers who switched to snus <u>Among 513 men with normal OGT at baseline</u> **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
Hergens et al. 2005	**Diabetes** Snuff use Former Current	 1.1 (0.40-3.3) 1.5 (0.76-2.9)
Ostenson et al. 2012	**Type 2 diabetes** Consistent snus use (n=16) Former snus use (n=6) 1-5 boxes/week (n=7) >5 boxes/week (n=9) Never-smoking high consump. (n=3)	 1.1 (0.6-2.0) 0.5 (0.2-1.2) 0.6 (0.2-1.4) 3.3 (1.4-8.1)* 2.3 (0.5-9.8)
Persson et al. 2000	**Type 2 diabetes** Moist snuff Former Current No. of boxes of snuff week^{-1} in current snuffers ≤2 3+	 0.8 (0.3-2.0) 1.5 (0.8-3.0) 0.2 (0.0-2.0) 2.7 (1.3-5.5)*
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)
BMI, Waist-Hip Ratio (WHR)		
Bolinder et al. 1992	**BMI<22** Age (years) ≤35 36-45 46-55	 1.0 (0.9-1.1) 1.0 (0.7-1.2) 1.0 (0.8-1.3)

Cardiovascular Disease Risk Factors and Events among Swedish Snus Users		
Reference	**Outcome**	**Effect Estimate (95% CI)**
	≥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)*
Engstrom et al. 2010	Underweight	
	Males	
	Exclusive snus use	0.46 (0.22-0.97)
	Dual use	1.27 (0.45-3.59)
	Females	
	Exclusive snus use	0.91 (0.55-1.51)
	Dual use	1.76 (0.88-3.52)
	Overweight	
	Males	
	Exclusive snus use	1.04 (0.93-1.15)
	Dual use	1.30 (1.01-1.66)*
	Females	
	Exclusive snus use	1.00 (0.79-1.26)
	Dual use	1.63 (0.96-2.76)
	Obese	
	Males	
	Exclusive snus use	1.01 (0.85-1.20)
	Dual use	1.59 (1.12-2.26)*
	Females	
	Exclusive snus use	0.99 (0.70-1.39)
	Dual use	1.76 (0.88-3.52)
Hansson et al. 2011	**Weight Gain**	
	Exclusive current use	1.31 (1.04-1.65)*
	Exclusive former use	1.36 (0.89-2.10)
	Quit during follow up	1.25 (0.88-1.77)
	Began during follow up	0.97 (0.50-1.86)
	Obese	
	Exclusive current use	1.93 (1.13-3.30)*
	Exclusive former use	0.85 (0.25-2.88)
	Quit during follow up	1.13 (0.51-2.50)
	Began during follow up	----
Hergens et al. 2005	**Overweight**	
	Snuff use	
	Former	1.5 (0.79-2.8)
	Current	1.9 (1.2-2.9)*
Nafziger et al. 2007	**Weight non-gain**	
	Snuff use	0.83 (0.74-0.92)*
Rodu et al. 2004	Prevalence of Overweight at Study Entry	
	Tobacco Use	
	Current exclusive smoking	0.87 (0.73-1.03)

Cardiovascular Disease Risk Factors and Events among Swedish Snus Users		
Reference	**Outcome**	**Effect Estimate (95% CI)**
	Current exclusive snus use	1.20 (1.01-1.42)*
	Current combined use	1.25 (1.03-1.63)*
	<u>Development of Overweight During Follow-up Among Men Not Overweight at Entry</u>	
	Tobacco Use At Entry/At Follow-Up	
	Smoking/snus	80 (22-205)
	Snus/snus	120 (84-167)
	Snus/no tobacco	142 (78-264)
Saarni et al. 2004	**Recurrent intentional weight loss**	
	Lifetime frequency of snuff use	
	Men	
	2-50 times	1.51 (1.08-2.13)*
	>50 times	1.41 (0.91-2.19)
	Women	
	2-50 times	1.63 (0.98-2.70)
	>50 times	----
Sundbeck et al. 2009	**BMI ≥30 kg/m^2**	
	All snuff users	
	≤4 cans/week	1.27 (0.73-2.20)
	>4 cans/week	1.18 (0.50-2.79)
	All	1.24 (0.75-2.06)
	Current exclusive snuff users	
	≤4 cans/week	0.67 (0.24-1.82)
	>4 cans/week	1.36 (0.36-5.10)
	All	0.83 (0.36-1.90)
	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)
Vaezghasemi et al. 2012	**Overweight & Obesity**	
	Simple logistic regression	
	Girls	1.3 (0.7-2.4)
	Boys	2.3 (1.7-3.2)
	Multiple logistic regression	
	Boys	1.6 (1.1-2.4)
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; Aro et al. 2010		
Incidence of Myocardial Infarction (fatal or nonfatal)		

Cardiovascular Disease Risk Factors and Events among Swedish Snus Users		
Reference	**Outcome**	**Effect Estimate (95% CI)**
Hergens et al. 2005	**All Cases** Snuff use Former Current **Nonfatal Cases** Snuff use Former Current **Fatal Cases** Snuff use Former Current	 1.1 (0.78-1.5) 0.98 (0.77-1.3) 1.1 (0.79-1.6) 0.98 (0.76-1.3) 1.1 (0.54-2.1) 1.9 (0.65-1.6)
Hergens et al. 2007	MI risk among never smokers **Total MI** Current snuff users Former snuff users **MI - Nonfatal** Current snuff users Former snuff users **Total MI – by snuff use** ≤ 12.5 g/day 12.5-24.9 g/day 25-49.9 g/day ≥ 50 g/day **MI – Nonfatal – by snuff use** ≤ 12.5 g/day 12.5-24.9 g/day 25-49.9 g/day ≥ 50 g/day	 1.02 (0.92-1.14) 0.76 (0.55-1.05) 0.94 (0.83 -1.06) 0.70 (0.48-1.02) 1.12 (0.95-1.30) 0.93 (0.79-1.09) 0.95 (0.73-1.24) 1.24 (0.89-1.73) 1.02 (0.84-1.22) 0.85 (0.70-1.03) 0.95 (0.71-1.29) 1.06 (0.71-1.58)
Huhtasaari et al. 1992	**Snuff Use --Cans/Week** <2 cans weekly ≥2 cans weekly **Snuff Dippers Vs. No Tobacco (by Age Group of Snuff Dippers)** 35-54 years 55-64 years All subjects	 0.63 (0.41-0.98)** 0.93 (0.61-1.41) 0.96 (0.56-1.67) 1.24 (0.67-2.30) 0.89 (0.62-1.29)
Huhtasaari et al. 1999	**Fatal and nonfatal acute MI** Regular use of snuff Regular smoking	 0.58 (0.35-0.94)** 3.53 (2.48-5.03)*
Janzon and Hedblad 2009	First ever MI **Males – risk factor adjusted** Snuff user, never smoker **Females** Snuff user	 0.75 (0.3-1.8) 0 cases
Wennberg et al. 2007	**MI** Never smoked, current snuff Former smoker, current snuff user	 0.82 (0.46-1.43) 1.25 (0.80-1.96)

Cardiovascular Disease Risk Factors and Events among Swedish Snus Users		
Reference	**Outcome**	**Effect Estimate (95% CI)**
	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)
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 J1

Descriptive Studies of Cardiovascular Effects

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (N=10)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Angman and Eliasson 2008 Sweden This study examined the effects of Swedish snus on blood pressure in Swedish men. [This study was published in Swedish, and the information presented here is based on an English abstract, and information from tables and rough translation.]	Cross-sectional study The study population included 4,305 Swedish men between 25 and 74 years of age, randomly selected from the MONICA cohort (Monitoring of Trends and Determinants in Cardiovascular Disease). The MONICA database included information from five population surveys from 1986, 1990, 1994, 1999, and 2004. Blood pressure was measured twice in a sitting, after five minutes rest. Participants were classified into nine groups of tobacco users: Never users of tobacco Snus users, non-smokers Snus users, ex-smokers Snus users who are also smokers Smokers, non-snus users Smokers, ex-snus users Ex-snus users, non-smokers Ex-smokers, non-snus users Ex-smokers who are ex-snus users "Snuff" is defined as Swedish moist oral snuff, or snus, in this study.	No differences in blood pressure in never-smoking snus users were observed compared to non-tobacco users. Adjusted Blood Pressure (mmHg) **Systolic** Never tobacco: 133.3 Exclusive snus: 132.8 Exclusive smoker: 131.5 **Diastolic** Never tobacco: 82.5 Exclusive snus: 82.4 Exclusive smoker: 81.9	**The authors concluded that the found no differences in blood pressure between those who never used tobacco and those who were snus users without having previously smoked.** The authors noted that the results were unchanged when possible confounding factors, such as age, gender, waist circumference, blood pressure medication, and physical inactivity. Due to the nature of the cross-sectional study design, a firm conclusion cannot be drawn from this data.

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Bolinder 1997a Sweden 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.	Cross-sectional 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	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.]	Cross-sectional 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 ($\geq$ 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	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.]	Cross-sectional 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 (serum cholesterol, serum triglycerides, fibrinogen) 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	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.]	Cross-sectional 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	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, body weight and other health effects observed in this study are summarized in Appendices L-1, O-1, and Q-2 respectively.	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>Measured Effect of Cardiovascular Disease Risk Factors for Ages 46-55</u> **Cardiovascular diagnosis** Non-user Smokeless tobacco (n=8) Cigarettes (≥ 15/day) (n=22) **Hypertension** Non-user Smokeless tobacco (n=28) Cigarettes (≥ 15/day) (n=9) **Diastolic BP>90** Non-user Smokeless tobacco (n=298) Cigarettes (≥ 15/day)(n=333) **Systolic BP>160** Non-user Smokeless tobacco (n=111) Cigarettes (≥ 15/day)(n=139) <u>Measured Effect of Cardiovascular Disease Risk Factors for Ages 56-65</u> **Cardiovascular diagnosis** Non-user Smokeless tobacco (n=69) Cigarettes (≥ 15/day) (n=33) **Diastolic BP>90** Non-user Smokeless tobacco (n=625) Cigarettes (≥ 15/day)(n=225) **Systolic BP>160** Non-user Smokeless tobacco (n=389) Cigarettes (≥ 15/day)(n=148)	<u>Odds Ratios (95% CI)</u> 1.0 (reference) 1.6 (0.7-3.5) 2.2 (1.3-3.9)* 1.0 (reference) 3.0 (1.9-4.9)* 0.9 (0.4-1.9) 1.0 (reference) 1.8 (1.5-2.1)* 0.8 (0.7-0.9)** 1.0 (reference) 1.7 (1.3-2.1)* 0.9 (0.7-1.1) 1.0 (reference) 1.5 (1.1-1.9)* 1.3 (0.9-1.9) 1.0 (reference) 1.3 (1.1-1.4)* 0.7 (0.5-0.8)** 1.0 (reference) 1.2 (1.1-1.4)* 0.7 (0.6-0.8)**	**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	RESULTS		AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Bolinder et al. 1992 (continued)		Measured Effect of Cardiovascular Disease Symptoms	Odds Ratios (95% CI)	
		Breathlessness on slight effort		
		Non-user	1.0 (reference)	
		Smokeless tobacco	1.4 (1.3-1.6)*	
		Cigarettes (≥ 15/day)	2.5 (2.2-2.7)*	
		Chest pain Walking up hill		
		Non-user	1.0 (reference)	
		Smokeless tobacco	1.2 (1.1-1.4)*	
		Cigarettes (≥ 15/day)	1.8 (1.7-2.1)*	
		Pain in the leg while walking		
		Non-user	1.0 (reference)	
		Smokeless tobacco	1.3 (1.1-1.5)*	
		Cigarettes (≥ 15/day)	2.1 (1.8-2.4)*	
		White finger symptoms		
		Non-user	1.0 (reference)	
		Smokeless tobacco	1.4 (1.3-1.6)*	
		Cigarettes (≥ 15/day)	1.6 (1.5-1.8)*	
		*Denotes statistically significant increase in risk.		
		**Denotes statistically significant decrease in risk.		

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	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.	Cross-sectional study Subjects included 1,583 participants of the MONICA study (Monitoring of 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 female 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 was not significantly associated with 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 mediated through the atherosclerotic pathway.

APPENDIX J-1
DESCRIPTIVE STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	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.	Cross-sectional 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. Serum insulin levels were significantly higher in smokers than in non-tobacco users. No differences in pulse rate or systolic blood pressure were found between smokers and non-tobacco users, however a significantly higher diastolic blood pressure was observed among smokers compared to non-tobacco users. Serum lipids and fibrinogen were significantly elevated in smokers compared to non-tobacco users.	**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 lifestyle 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	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.	Cross-sectional study 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. Tobacco smoking was associated with an increase of the IMT and the occurrence of atherosclerotic plaques in the carotid and femoral arteries. Smoking was also accompanied by dyslipidemia, hyperinsulinemia and inflammation (factors that are associated with development of atherosclerotic disease).	**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	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.	Cross-sectional study 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. Smoking was found to facilitate the formation of thromboxane A_2.	**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

Experimental Studies of Cardiovascular Effects

APPENDIX J-2
EXPERIMENTAL STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (N=5)

CITATION, LOCATION	STUDY TYPE, POPULATION	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, and also acute effects after short term exposure. [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.]	Experimental human 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-2
EXPERIMENTAL STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	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.	Experimental human 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 taking 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 during exercise.** There was no adjustment for possible confounding factors.

APPENDIX J-2
EXPERIMENTAL STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Lunell and Curvall 2011 Sweden This study examined the acute effects of snus and nicotine chewing gum on heart rate.	Experimental human study Participants included 15 regular cigarette smokers (9 males/6 females), age 19-49. Ever users of snus or gum were excluded. After fasting and abstinent overnight (12 hours) from cigarette smoking, participants were given two strengths of portion snus (Snus 1: General Onyx Portion Snus White Large: 9.9 mg nicotine; Snus 2: General Portion Snus White Large, 8.7 mg nicotine) and nicotine gum. Suns was kept in the upper lip and the gum for 30 minutes. Heart rate was measured after 10-min rest before administration of trial products (timepoint 0), and at the timepoints 10, 20, and 30 minutes.	Heart rate increased rapidly to reach a maximum at 20 min. The mean 20-min increase of heart rate was 9.3±9.6, 8.9±6.4, and 9.9±5.1 beats/min for Snus 1, Snus 2, and chewing gum, respectively. After 30 min, heart rate had leveled out at 7.5±7.4, 9.3±5.5, and 9.3±6.3 beats/min, respectively, for Snus 1, Snus 2, and chewing gum.	Though the authors do not comment on the significance of the changes in heart rate, it appears that the products influenced heart rate.

APPENDIX J-2
EXPERIMENTAL STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	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. "Snuff" is defined as portion-bag-packed moist snuff of the same brand, and is assumed to be Swedish snus.	35 min. after beginning snuff use, there was a statistically significant 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 J-2
EXPERIMENTAL STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	RESULTS	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Sundstrom et al. 2012 Sweden This study examined the acute effects of snuff use on blood pressure, heart rate, and ventricular heart function.	Experimental human study Subjects were 27 men and four women who were habitual snus users (smoking habits not described). Only healthy persons with no history of CVD and with a normal ultrasound of the heart with left ventricular ejection fraction (LVEF) > 50% were included. All volunteers had been instructed not to smoke or use snuff for at least 5 h before the examination. Measures were recorded at four different times: before snuff intake, 5 and 30 minutes after placing snuff in their mouth, and 30 minutes after snuff withdrawal from their mouth. The type of snus used in this study was "Ettan Original Portion", 8 mg/g nicotine.	Heart rate and blood pressure were not significantly increased at any of the three time points following snuff use compared to pre-snuff measurements. With respect to ventricular function, the authors reported a transient decrease in diastolic heart function attributed primarily to a statistically significant decrease in E/A ratio (the ratio between early (E) and late (atrial - A) ventricular filling velocity) at 5 and 30 minutes following snuff consumption and the delay in left- and right ventricular relaxation.	**The authors noted that these results, along with the finding that systolic function was unaffected following snuff use, are consistent with findings observed among cigarette smokers.** The authors cited numerous references for this comparison to smokers, though this study did not examine and compare snuff users with either smokers or unexposed controls (nontobacco users). The authors also point out that even though the diastolic heart function parameters tended towards a pattern of impairment following the use of snuff compared to each participants own baseline, these parameters were still within the normal range of function (e.g. the E/A ratio following snuff use was not considered clinically abnormal).

Appendix J3

Case-Control Studies of Cardiovascular Effects

APPENDIX J-3
CASE-CONTROL STUDIES OF CARDIOVASCULAR EFFECTS 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 hyperlipidemia and hypertension. See Appendix O-3 for results on body weight, Appendix M-4 for results on diabetes and Appendix J-2 for results on MI.	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 hypertension (6.5 mmol/L at exam or treatment with lipid-lowering meds) and hyperlipidemia (170/95 mmHg at exam or hypertension in questionnaire). "Snuff" was defined as Swedish moist snuff.	Snuff Use **Hyperlipidemia** Never Former Current **Hypertension** Never Former Current	Odds Ratio (95% CI) 1.00 (reference) 1.10 (0.63-2.00) 0.99 (0.66-1.50) 1.00 (reference) 0.98 (0.58-1.60) 1.80 (1.30-2.50)*	**The authors state that the consumption of smokeless tobacco was strongly associated with hypertension.** A significant association between snuff use and hyperlipidemia was not observed. Odds ratios of being overweight were adjusted for age, hospital catchment area, and smoking. Additionally, the prevalence of hypertension and hyperlipidemia was analyzed only among controls, so technically this was a cross-sectional analysis.

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

Appendix J4

Cohort Studies of Cardiovascular Effects

APPENDIX J-4
COHORT STUDIES OF CARDIOVASCULAR EFFECTS AMONG SWEDISH SNUS USERS (N=2)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hergens et al. 2008b Sweden This study examined the relationship between the use of snus and risk of hypertension	Cohort study Subjects were 120,930, never-smoking, male Swedish construction workers who underwent regular preventive health check-ups and had at least one visit from 1978-1993, 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 the National Inpatient Register. Adjusted relative risks were derived from Cox proportional hazards regression models. Hypertension was defined by ICD-9 codes: 401-405 and ICD-10 codes: I10-I15. High blood pressure was defined as > 160 mmHg (systolic) or diastolic blood pressure > 100 mmHg. Snuff is defined as "Swedish moist snuff."	<u>Healthy baseline (N=77,469)</u>: **Hypertension (n)** Never snuff use (581) Ever snuff use (158) Former snuff use (12) Current snuff use (146) < 12.5 g/day (37) 12.5-24.9 g/day (66) 25-49.9 g/day (30) > 50 g/day (13) <u>Healthy baseline with repeat exams (N=42,005)</u>: **High blood pressure (n)** Never snuff use (337) Ever snuff use (124) Former snuff use (10) Current snuff use (114) < 12.5 g/day (34) 12.5-24.9 g/day (51) 25-49.9 g/day (18) > 50 g/day (11) **Hypertension (n)** Never snuff use (397) Ever snuff use (91) Former snuff use (7) Current snuff use (84) < 12.5 g/day (22) 12.5-24.9 g/day (36) 25-49.9 g/day (17) > 50 g/day (9)	<u>Relative Risk (95% CI)</u> 1.00 (reference) 1.08 (0.89-1.29) 0.78 (0.43-1.41) 1.10 (0.91-1.33) 1.03 (0.74-1.43) 1.15 (0.88-1.49) 1.15 (0.79-1.69) 1.03 (0.59-1.79) 1.00 (reference) 1.39 (1.08-1.79)* 1.49 (0.76-2.90) 1.34 (1.03-1.74)* 1.49 (0.97-2.27) 1.24 (0.86-1.80) 1.19 (0.69-2.05) 1.67 (0.86-3.28) 1.00 (reference) 1.36 (1.07-1.72)* 0.85 (0.40-1.79) 1.43 (1.12-1.83)* 1.18 (0.77-1.82) 1.43 (1.01-2.02)* 1.77 (1.08-2.90)* 1.76 (0.90-3.42)	**The authors concluded that the use of Swedish moist snuff appears to be associated with a moderately increased risk of hypertension.** Relative risks were adjusted for age, BMI, and region of residence. Strengths of this study include the prospective design, large sample size and a homogeneous population. Additionally, only those who never smoked were included in the analysis. A limitation of this study is that misclassification of exposure may have occurred because of subsequent changes in tobacco use during the prolonged follow-up. The authors state that though the results of this study may partly be due to a short-term effect from snuff use, subjects were not allowed to use tobacco during the health check-up and the blood pressure was measured after 5 minutes of rest in a supine position. The authors suggested the use of a high cut-off for the definition of high blood pressure for this reason.

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

APPENDIX J-4
COHORT 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
Norberg et al. 2006 Sweden This study was done to investigate associations between metabolic syndrome (MetSy) and components of MetSy, with a focus on the role of snus. Results on obesity and metabolic syndrome are presented in Appendices O-2 and N1 respectively.	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 hypertension, fasting plasma glucose ≥ 5.6 mmol/L, triglycerides ≥ 1.7 mmol/L, and low HDL cholesterol. 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. "Snuff" was defined as Swedish moist snuff (snus) in this study.	Snus Use **Glucose ≥ 5.6 mmol/L or Diabetes** No use ≤ 4 cans/week > 4 cans/week **Triglycerides ≥1.7 mmol/L** 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	Odds Ratio (95% CI) 1.0 (reference) 1.0 (0.86-1.08) 0.8 (0.69-1.02) 1.0 (reference) 1.2 (1.05-1.35)* 1.6 (1.30-1.95)* 1.0 (reference) 1.0 (0.86-1.18) 1.1 (0.82-1.42) 1.0 (reference) 0.9 (0.84-1.05) 1.2 (0.99-1.46)	**The authors concluded that snus has the greatest effect on hypertriglyceridemia and obesity. Snus was not associated with hypertension, dysglycemia and low HDL cholesterol.** Odds ratios for these components of MetSy 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 major strengths of this were that 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, it appears 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. Furthermore, odds ratios are not adjusted for smoking or energy intake.

APPENDIX J-4
COHORT 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
Norberg et al. 2006 (continued)		Smoking	Odds Ratio (95% CI)	
		Glucose ≥ 5.6 mmol/L or Diabetes		
		Non-smoking	1.0 (reference)	
		Ex-smoker	1.2 (1.15-1.35)*	
		Daily smoker	1.3 (1.23-1.45)*	
		Triglycerides ≥ 1.7 mmol/L		
		Non-smoking	1.0 (reference)	
		Ex-smoker	1.3 (1.16-1.41)*	
		Daily smoker	1.6 (1.43-1.73)*	
		Low HDL Cholesterol		
		Non-smoking	1.0 (reference)	
		Ex-smoker	1.1 (1.00-1.27)	
		Daily smoker	1.2 (1.07-1.35)*	
		Hypertension		
		Non-smoking	1.0 (reference)	
		Ex-smoker	1.2 (1.07-1.27)*	
		Daily smoker	0.8 (0.75-0.89)**	

Appendix J5

Descriptive Studies of Cardiovascular Diseases

APPENDIX J-5
DESCRIPTIVE STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (N=1)

CITATION, LOCATION	STUDY TYPE, POPULATION	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.	Cross-sectional 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 did not find any association between prevalence of smoking and coronary mortality.	**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 J6

Case-Control Studies of Cardiovascular Diseases

APPENDIX J-6
CASE-CONTROL STUDIES OF CARDIOVASCULAR DISEASES 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. Among all non-fatal and fatal cases, there were 122 and 25 current snuff users respectively. Among never-smokers, there were 7 and 3 nonfatal and fatal cases respectively. "Snuff" was defined as Swedish moist snuff.	Tobacco Use Among All Individuals **All Cases** Never-tobacco user Former snuff user Current snuff user **Nonfatal Cases** Never-tobacco user Former snuff user Current snuff user **Fatal Cases** Never-tobacco user Former snuff user Current snuff user Among Never Smokers **All Cases** Never-tobacco user Former snuff user Current snuff user **Nonfatal Cases** Never-tobacco user Former snuff user Current snuff user **Fatal Cases** Never-tobacco user Former snuff user Current snuff user	Odds Ratio (95% CI) 1.0 (reference) 1.1 (0.78-1.5) 0.98 (0.77-1.3) 1.0 (reference) 1.1 (0.79-1.6) 0.98 (0.76-1.3) 1.0 (reference) 1.1 (0.54-2.1) 1.9 (0.65-1.6) 1.0 (reference) 1.2 (0.46-3.1) 0.73 (0.35-1.5) 1.0 (reference) 1.2 (0.43-3.2) 0.59 (0.25-1.4) 1.0 (reference) 1.7 (0.21-13.6) 1.7 (0.48-5.5)	**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-6
CASE-CONTROL STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Hergens et al. 2005 (continued)		Among Current Smokers	Odds Ratio (95% CI)	
		All Cases		
		Never-tobacco user	1.0 (reference)	
		Never snuff user	2.8 (2.7-10.6)*	
		Former snuff user	5.3 (2.7-10.6)*	
		Current snuff user	2.3 (1.6-3.4)*	
		All cases	2.8 (2.4-3.4)*	
		Nonfatal Cases		
		Never-tobacco user	1.0 (reference)	
		Never snuff user	2.7 (2.2-3.3)*	
		Former snuff user	5.3 (2.6-10.7)*	
		Current snuff user	2.1 (1.4-3.1)*	
		All cases	2.7 (2.2-3.3)*	
		Fatal Cases		
		Never-tobacco user	1.0 (reference)	
		Never snuff user	3.6 (2.4-5.2)*	
		Former snuff user	6.0 (1.8-20.3)*	
		Current snuff user	3.8 (1.9-7.5)*	
		All cases	3.5 (2.4-5.0)*	

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

APPENDIX J-6
CASE-CONTROL STUDIES OF CARDIOVASCULAR DISEASES 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. No Tobacco (by Age Group of Tobacco Users)</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> Non-users of tobacco 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) 1.00 (reference) 3.22 (1.82-5.70)* 1.09 (0.55-2.16) 2.09 (1.39-3.15)* 1.00 (reference) 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 <u>≤</u>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-6
CASE-CONTROL STUDIES OF CARDIOVASCULAR DISEASES 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-6
CASE-CONTROL STUDIES OF CARDIOVASCULAR DISEASES 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 (n=21) Former smoker, current snuff user (n=37) Current smoker, no current snuff (n=136) Current smoker, current snuff user (n=30) Never smoked, former snuff user (n=11) Former smoker, never snuff user (n=58) Former smoker, former snuff user (n=33) **Fatal MI within 28 Days** Never used tobacco Never smoked, current snuff (n=7) Former smoker, current snuff user (n=7) Current smoker, no current snuff (n=37) Current smoker, current snuff user (n=5) Never smoked, former snuff user (n=2) Former smoker, never snuff user (n=11) Former smoker, former snuff user (n=4)	Odds Ratios (95% CI) 1.00 (reference) 0.82 (0.46-1.43) 1.25 (0.80-1.96) 2.60 (1.91-3.54)* 2.14 (1.28-3.60)* 0.66 (0.32-1.34) 1.18 (0.82-1.70) 1.34 (0.84-2.12) 1.00 (reference) 1.12 (0.38-3.29) 1.24 (0.44-3.53) 3.53 (1.83-6.84)* 1.11 (0.34-3.69) 0.64 (0.13-3.18) 1.02 (0.45-2.31) 0.60 (0.18-2.02)	**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, and 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.

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

APPENDIX J-6
CASE-CONTROL STUDIES OF CARDIOVASCULAR DISEASES 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 (continued)			Odds Ratios (95% CI)	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 associated with increased risk (Bolinder et al. 1994) involved a defined socioeconomic group (i.e., construction workers), while other studies were population-based. The authors note that the study was limited by sample size, especially analyses of SCD.
		SCD with Survival < 24 Hr		
		Never used tobacco	1.00 (reference)	
		Never smoked, current snuff (n=7)	1.18 (0.38-3.70)	
		Former smoker, current snuff user (n=6)	1.39 (0.44-4.42)	
		Current smoker, no current snuff (n=31)	3.12 (1.53-6.33)*	
		Current smoker, current snuff user (n=3)	0.75 (0.17-3.28)	
		Never smoked, former snuff user (n=2)	0.70 (0.14-3.64)	
		Former smoker, never snuff user (n=7)	0.74 (0.28-1.97)	
		Former smoker, former snuff user (n=3)	0.50 (0.12-2.03)	
		SCD with Survival < 1 Hr		
		Never used tobacco	1.00 (reference)	
		Never smoked, current snuff (n=4)	0.38 (0.08-1.89)	
		Former smoker, current snuff user (n=5)	2.67 (0.52-13.80)	
		Current smoker, no current snuff (n=21)	4.54 (1.55-13.25)*	
		Current smoker, current snuff user (n=1)	0.13 (0.01-2.10)	
		Never smoked, former snuff user (n=1)	0.35 (0.03-4.56)	
		Former smoker, never snuff user (n=4)	0.35 (0.07-1.78)	
		Former smoker, former snuff user	0 cases	

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

Appendix J7

Cohort Studies of Cardiovascular Diseases

APPENDIX J-7
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (N=8)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Arefalk et al. 2012 Sweden This study examined the relationship between the use of snus and risk of heart failure.	Cohort study Subjects were drawn from two independent Swedish Prospective cohorts. They included a community based sample of 1,076 elderly men from the Uppsala Longitudinal Study of Adult Men (ULSAM) and a sample of 118,425 never-smoking male construction workers from the Swedish Construction Worker Cohort (CWC). ULSAM: In 1970-73, all 50-year-old men residing in Uppsala County in Central Sweden were invited to a health survey and reinvestigated in 1991-95. Smokeless tobacco use was collected using a self-administered questionnaire. First hospitalization for heart failure, was validated through chart review. Median follow-up was 8.9 years. CWC: Subjects included those who underwent regular 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 hospitalization for heart failure, death, emigration, or December 31, 2003, whichever occurred first. Follow-up was carried out through linkage with nationwide death, emigration, and cancer incidence	<u>Snuff Usage</u> **ULSAM** Snus non-use Snus use (n=14) Model A Model B Model C **CWC** Never tobacco use Former snus use (n=6) Model A Model B Model C Current snus use (n=75) Model A Model B Model C < 12.5 g/day (n=28) Model A Model B Model C 12.5-24.9 g/day (n=35) Model A Model B Model C 25-49.9 g/day (n=8) Model A Model B Model C ≥ 50 g/day (n=4) Model A Model B Model C p trend	<u>Hazard ratio (95% CI)</u> 1.00 (reference) 2.42 (1.37-4.27)* 2.08 (1.03-4.22)* 2.09 (1.00-4.39) 1.00 (reference) 1.02 (0.46-2.29) 1.00 (0.45-2.23) 0.99 (0.44-2.22) 1.35 (1.05-1.72)* 1.28 (1.00-1.64) 1.24 (0.97-1.59) 1.19 (0.81-1.74) 1.18 (0.80-1.73) 1.15 (0.78-1.68) 1.57 (1.11-2.21)* 1.46 (1.03-2.06)* 1.40 (0.99-1.98) 1.13 (0.56-2.27) 1.03 (0.51-2.08) 1.02 (0.50-2.06) 1.48 (0.55-3.98) 1.25 (0.47-3.84) 1.24 (0.46-3.34) 0.9	**The authors concluded that the data from two independent cohorts suggest that use of snus may be associated with a higher risk of heart failure.** The authors noted that they observed an increased risk for subsequent heart failure among elderly male users of Swedish snus and a similar, but less pronounced association in a younger and larger cohort of never-smoking men. The authors further note that their findings need confirmation in future studies and that underlying mechanisms remain to be elucidated. ULSAM: The authors presented data from three different proportional hazard models. Model A was adjusted only for age. Model B was adjusted also for current smoking dose, pack-years of smoking, diabetes, BMI, occupational classification, alcohol use, and MI before baseline. Model C was adjusted also for office systolic blood pressure, antihypertensive medication use, electrocardiogram-left ventricular hypertrophy and replacing MI before baseline with MI during follow-up (as a time-dependent covariate). CWC: The authors presented data from three different proportional hazard models. Model A was adjusted only for age. Model B was adjusted also for BMI, region of residence and MI before

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

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Arefalk et al. 2012 (continued)	registries. Median follow up was 18 years. Snus is defined as oral moist snuff (snus) and is assumed to be Swedish snus.			baseline. Model C was adjusted also systolic and diastolic blood pressures and replacing MI before baseline with MI during follow-up (as a time-dependent covariate). The ULSAM study is based on a well characterized cohort, with validated heart failure diagnosis. The authors suggest that the association in the general population compared to the ULSAM cohort may be lower, because of residual confounding of smoking and imprecision due to limited sample size. Non-smoking snus users were too few to study separately. The CWC is a large cohort with a high prevalence of exposure, which allowed the authors to restrict this sample to never-smoking men. Tobacco habits were assessed only at study entry; changes in tobacco habits over time could influence the results.

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

APPENDIX J-7
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (continued)

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, all-cause mortality and stroke are presented in Appendices G, H, Q-1, and K-2, respectively. [Updated and extended by Hergens et al. 2007]	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 of Death By Tobacco Usage **All Cardiovascular Disease** Nonusers Smokeless tobacco (n=220) Smokers (< 15 cig/day) (n=450) Smokers (≥ 15 cig/day) (n=381) Cause-Specific Mortality Ages 35-54 **All Cardiovascular Disease** Nonusers Smokeless tobacco (n=44) Smokers (< 15 cig/day) (n=164) Smokers (≥ 15 cig/day) (n=199) **Ischemic Heart Disease** Nonusers Smokeless tobacco (n=35) Smokers (< 15 cig/day) (n=128) Smokers (≥ 15 cig/day) (n=162) Cause-Specific Mortality Ages 55-65 **All Cardiovascular Disease** Nonusers Smokeless tobacco (n=174) Smokers (< 15 cig/day) (n=272) Smokers (≥ 15 cig/day) (n=167) **Ischemic Heart Disease** Nonusers Smokeless tobacco (n=137) Smokers (< 15 cig/day) (n=225) Smokers (≥ 15 cig/day) (n=122)	Relative Risk (95% CI) of death 1.0 (reference) 1.4 (1.2-1.6)* 1.8 (1.6-2.0)* 1.9 (1.7-2.2)* 1.0 (reference) 2.1 (1.5-2.9)* 2.7 (2.2-3.4)* 3.2 (2.6-3.9)* 1.0 (reference) 2.0 (1.4-2.9)* 2.6 (2.1-3.4)* 3.3 (2.6-4.2)* 1.0 (reference) 1.1 (1.0-1.4) 1.5 (1.3-1.7)* 1.5 (1.3-1.7)* 1.0 (reference) 1.2 (1.0-1.5) 1.7 (1.4-1.9)* 1.4 (1.2-1.8)*	**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-7
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (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 Johansson 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 study 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 (n=28) Smoke and snuff (n=15) Smoke (n=153) No tobacco Snuff (n=8) Smoke and snuff (n=3) Smoke (n=52)	<u>Incidence Rate Ratios</u> 1.00 (reference) 0.77 (0.51-1.15) 1.64 (0.96-2.79) 1.74 (1.41-2.14)* <u>Mortality Risk Ratio</u> 1.00 (reference) 1.15 (0.54-2.41) 1.69 (0.52-5.46) 1.98 (1.35-2.91)*	**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.** 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). 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. Tobacco use was only recorded at baseline.

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

APPENDIX J-7
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (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. "Snus" is defined as a moist smokeless tobacco product, commonly used in Sweden.	<u>IHD risk by tobacco exposure</u> Never tobacco users Current pure snus use (n=18) Current pure smoking (n=155) Former pure snus use (n=11) Former pure smoking (n=229) Never snus users Snus use ≤ 4 cans/week (n=55) Snus use > 4 cans/week (n=14) Never snus users Snus use < 20 years (n=22) Snus use ≥ 20 years (n=47) <u>All CVD risk by tobacco exposure</u> Never tobacco users Current pure snus use (n=32) Current pure smoking (n=230) Former pure snus use (n=19) Former pure smoking (n=318) Never snus users Snus use ≤ 4 cans/week (n=79) Snus use > 4 cans/week (n=24) Never snus users Snus use < 20 years (n=34) Snus use ≥ 20 years (n=68) [see Hansson et al. 2009 for additional analyses]	<u>Hazard ratio (95% CI) for IHD</u> 1.00 (reference) 0.85 (0.51-1.41) 1.99 (1.59-2.50)* 1.07 (0.56-2.03) 1.34 (1.10-1.64)* 1.00 (reference) 0.84 (0.62-1.13) 0.92 (0.52-1.63) 1.00 (reference) 0.87 (0.55-1.38) 0.85 (0.62-1.18) 1.00 (reference) 1.00 (0.69-1.46) 1.86 (1.56-2.22)* 1.21 (0.75-1.97) 1.17 (1.00-1.38) 1.00 (reference) 0.85 (0.67-1.09) 1.15 (0.75-1.77) 1.00 (reference) 0.97 (0.67-1.40) 0.87 (0.67-1.13)	**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. The strengths of this study include the large population size, and up-to-date information on tobacco use and potential confounding and mediating factors. Additionally, the authors were able to reproduce established associations between smoking and the risk of CVD, indicating the validity of the data. A major limitation was the low number of CVD deaths, so the authors could not address the relationship between snus use and fatal CVD.

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

APPENDIX J-7
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (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 (n=416) Former snuff users (n=37) <u>MI - Nonfatal</u> Never tobacco users Current snuff users (n=298) Former snuff users (n=27) <u>MI - Fatal</u> Never tobacco users Current snuff users (n=118) Former snuff users (n=10) <u>Total MI – by snuff use</u> Never tobacco users ≤ 12.5 g/day (n=167) 12.5-24.9 g/day (n=158) 25-49.9 g/day (n=56) ≥ 50 g/day (n=35) <u>MI – Nonfatal – by snuff use</u> Never tobacco users ≤ 12.5 g/day (n=117) 12.5-24.9 g/day (n=113) 25-49.9 g/day (n=44) ≥ 50 g/day (n=24) <u>MI – Fatal – by snuff use</u> Never tobacco users ≤ 12.5 g/day (n=50) 12.5-24.9 g/day (n=45) 25-49.9 g/day (n=12) ≥ 50 g/day (n=11)	<u>Hazard Ratio for MI</u> 1.00 (reference) 1.02 (0.92-1.14) 0.76 (0.55-1.05) 1.00 (reference) 0.94 (0.83 -1.06) 0.70 (0.48-1.02) 1.00 (reference) 1.32 (1.08-1.61)* 1.00 (0.54-1.88) 1.00 (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) 1.00 (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) 1.00 (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. The authors noted that when fatal MI was further adjusted for high blood pressure, 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.

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** denotes statistically significant decrease in risk

APPENDIX J-7
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (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. 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. "Smokeless tobacco" is defined as snuff in this study and is assumed to be Swedish snus.	First ever MI **Males – risk factor adjusted:** Nontobacco users Snuff user, never smoker Smokers, snuff users **Females** Nontobacco users Snuff user	Relative Risk (95% CI) 1.00 (reference) 0.75 (0.3-1.8) 1.31 (0.8-2.0) 1.00 (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.** 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). There were too few cardiovascular events (MI or stroke) among female snuff users to examine this outcome in this cohort. 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-7
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (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-7
COHORT STUDIES OF CARDIOVASCULAR DISEASES AMONG SWEDISH SNUS USERS (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, head and neck cancers in Appendix C and all-cause mortality and respiratory death in Appendix Q-1.	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. Snus is defined as Scandinavian moist snuff in this study.	<u>Tobacco Usage</u> **Cardiovascular death** Snus use Never daily use Ever daily use Restricted to never smokers Snus use Never daily use Ever daily use Smoking Never daily use Age < 75 Age 75+	<u>Hazard Ratio (95% CI)</u> 1.00 (reference) 1.11 (0.98-1.25) 1.00 (reference) 1.15 (0.97-1.37) 1.00 (reference) 1.63 (1.37-1.93)* 1.23 (1.09-1.38)*	**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 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 ex-smokers) (n=30) Exclusive snuff users All cigarette smokers (including snuffers) (n=67) 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 (0.37-2.94) 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.	<u>Tobacco Use</u> **Among Men** Reference not defined Smokers Snuffers **Among Women** Reference not defined Smokers Snuffers	<u>Relative Risk (95% CI)</u> 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, all-cause mortality and cardiovascular disease are presented in Appendices G, H, Q-1, 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.	Tobacco Use **Stroke Mortality Ages 35-54** Nonusers of tobacco Smokeless tobacco (n=4) < 15 cig/day (n=17) > 15 cig/day (n=19) Ex-smokers, 1-5 years (n=4) Ex-smokers, >5 years (n=5) **Stroke Mortality Ages 55-65** Nonusers of tobacco Smokeless tobacco (n=26) < 15 cig/day (n=19) > 15 cig/day (n=25) Ex-smokers, 1-5 years (n=20) Ex-smokers, >5 years (n=35)	Relative Risk (95% CI) of death 1.0 (reference) 1.9 (0.6-5.7) 2.7 (1.4-5.4)* 3.0 (1.5-5.7)* 1.2 (0.4-3.7) 0.7 (0.2-1.9) 1.0 (reference) 1.2 (0.7-1.8) 0.7 (0.4-1.2) 1.6 (1.0-2.5) 1.5 (0.9-2.5) 0.8 (0.5-1.2)	**The authors concluded that there was an apparent excess risk of death from cardiovascular and cerebrovascular diseases of about 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 and sex (men only). 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. It was not possible for the authors to consider potential confounding due to alcohol consumption. 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 (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 Johansson 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-3.	Cohort study 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 were assessed. Information on prior tobacco use not assessed. "Snuff" is defined as Swedish moist snuff (snus) in this study.	<u>Stroke risk by tobacco habits</u> No tobacco Snuff (n=19) Smoke and snuff (n=9) Smoke (n=66) No tobacco Snuff (n=4) Smoke and snuff (n=3) Smoke (n=12)	<u>Incidence Rate Ratios</u> 1.0 (reference) 1.07 (0.65-1.77) 1.98 (1.00-3.95) 1.40 (1.03-1.91)* <u>Mortality Risk Ratio</u> 1.0 (reference) 1.01 (0.35-2.92) 4.30 (1.22-15.1)* 1.02 (0.50-2.05)	**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 (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. "Snus" is not defined in this study but is assumed to be Swedish.	<u>Stroke risk among snus users, never smokers</u> Never tobacco users (n=155) Current snus use (n=14) Former snus use (n=8) Never snus users (n=351) Snus use ≤ 4 cans/week (n=24) Snus use > 4 cans/week (n=12) Never snus users (n=351) Snus use < 20 years (n=13) Snus use ≥ 20 years (n=22) <u>Stroke risk among smokers, never snus users</u> Never tobacco users (n=155) Current smoking (n=81) Former smoking (n=115)	<u>Hazard ratio (95% CI) for stroke</u> 1.00 (reference) 1.18 (0.67-2.08) 1.35 (0.65-2.82) 1.00 (reference) 0.75 (0.49-1.15) 1.75 (0.95-3.21) 1.00 (reference) 1.13 (0.63-2.01) 0.80 (0.51-1.25) 1.00 (reference) 1.61 (1.22-2.13)* 1.01 (0.78-1.30)	**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. The authors were unable to address the relationship between snus use and fatal CVD (including stroke), because of the low number of cardiovascular deaths.

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

APPENDIX K-2
COHORT STUDIES OF STROKE AMONG SWEDISH SNUS USERS (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%	<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 (n=2569) Current snuff users (n=368) Former snuff users (n=30) <u>All stroke types - Fatal</u> Never tobacco users (n=236) Current snuff users (n=44) Former snuff users (n=1) <u>Ischemic stroke - All</u> Never tobacco users (n=1979) Current snuff (n=284) Former snuff users (n=20) <u>Ischemic stroke - Nonfatal</u> Never tobacco users (n=1887) Current snuff users (n=263) Former snuff users (n=19) <u>Ischemic stroke - Fatal</u> Never tobacco users (n=92) Current snuff users (n=21) Former snuff users (n=1)	<u>Hazard Ratio for stroke</u> 1.00 (reference) 1.05 (0.95-1.17) 0.72 (0.50-1.02) 1.00 (reference) 1.02 (0.91-1.14) 0.75 (0.53-1.08) 1.00 (reference) 1.38 (0.99-1.91) 0.30 (0.04-2.11) 1.00 (reference) 1.07 (0.94-1.22) 0.68(0.44-1.06) 1.00 (reference) 1.04 (0.91-1.18) 0.67(0.43-1.06) 1.00 (reference) 1.72 (1.06-2.78)* 0.82(0.12-5.93)	**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 is 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 (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
	were current snuff users. "Snuff" is defined as Swedish moist snuff in this study.	<u>Hemorrhagic stroke - All</u> Never tobacco users (n=474) Current snuff users (n=68) Former snuff users (n=8) <u>Hemorrhagic stroke - Nonfatal</u> Never tobacco users (n=378) Current snuff users (n=52) Former snuff users (n=8) <u>Hemorrhagic stroke - Fatal</u> Never tobacco users (n=96) Current snuff users (n=16) Former snuff users (n=0) <u>Unspecified stroke - All</u> Never tobacco users (n=352) Current snuff users (n=60) Former snuff users (n=3) <u>Unspecified stroke - Nonfatal</u> Never tobacco users (n=304) Current snuff users (n=53) Former snuff users (n=3) <u>Unspecified stroke - Fatal</u> Never tobacco users (n=48) Current snuff users (n=7) Former snuff users (n=0) [See Hergens et al. 2008 for additional analyses]	 1.00(reference) 0.85 (0.65-1.10) 0.90(0.45-1.82) 1.00 (reference) 0.77 (0.57-1.04) 1.10(0.54-2.21) 1.00 (reference) 1.17(0.68-2.01) 0 1.00 (reference) 1.35 (1.02-1.80)* 0.66(0.21-2.06) 1.00 (reference) 1.31 (0.98-1.77) 0.680.22-2.14) 1.00 (reference) 1.14 (0.51-5.54) 0	

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

APPENDIX K-2
COHORT STUDIES OF STROKE AMONG SWEDISH SNUS USERS (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. 7% of males and 0.4% of females were snuff users; of these, 34% of males and 28% of females were dual users. "Smokeless tobacco" is defined as snuff in this study and is assumed to be Swedish snus.	First ever stroke **Males – risk factor adjusted:** Snuff nonusers Snuff user, never smoker (n=4) Smokers, snuff users (n=13) **Females** Snuff nonusers Snuff user	Relative Risk (95% CI) 1.00 (reference) 0.59 (0.2-1.5) 1.13 (0.6-2.0) 1.00 (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. 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=2)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Aro et al. 2010 Sweden The aim of this study was to investigate the relationship between different forms of tobacco (including Swedish snus) and upper gastrointestinal (GI) symptoms, histology, and frequency of *H. pylori* infection.	Cross-sectional (population-based) The population under study involved a random sample of adults from two northern Swedish municipalities (Kalix and Haparanda). Every seventh adult (n=3,000) from the target population (18-80 years of age, n=21,610 in September 1998) was drawn. Respondents filled out a validated questionnaire, the Abdominal Symptom Questionnaire (ASQ) (n=2,122). 1,001 individuals underwent an esophagogastroduodenoscopy (EGD), with biopsies evaluated by two experienced pathologists. *H. pylori* was detected by Warthin-Starry silver staining. Snus users and smokers were exclusive users without a past history of smoking or snus use. Dual users were defined as current snus users and smokers. "Snus" is not defined in this study, but is assumed to be Swedish snus. Snus users included one user of chewing tobacco.	<u>Tobacco Use</u> **Gastroesophageal reflux symptoms** Never-users of tobacco Current snus user Current smoker Using both Former snus user Former smoker Former user of both **Dyspepsia** Never-users of tobacco Current snus user Current smoker Using both Former snus user Former smoker Former user of both **Irritable bowel syndrome** Never-users of tobacco Current snus user Current smoker Using both Former snus user Former smoker Former user of both	<u>Odds ratio (95% CI)</u> 1.00 (reference) 1.22 (0.72-2.06) 0.90 (0.60-1.39) 2.40 (0.89-6.56) 1.89 (0.65-5.51) 1.12 (0.77-1.65) 0.83 (0.40-1.74) 1.00 (reference) 1.29 (0.76-2.19) 1.61 (1.09-2.19)* 2.78 (1.06-7.28)* 1.93 (0.64-5.86) 1.18 (0.81-1.73) 0.84 (0.40-1.77) 1.00 (reference) 0.87 (0.49-1.55) 1.12 (0.74-1.70) 3.25 (1.28-8.22)* 2.60 (0.91-7.41) 1.62 (1.12-2.35)* 0.59 (0.26-1.35)	**The authors concluded that snus use was associated with histological markers of chronic chemical irritation consistent with gastroesophageal reflux disease at the esophago-gastric junction. They also concluded that smoking cigarettes was associated with overall peptic ulcer disease while snus users had less peptic ulcer disease than expected.** Snus use was not significantly associated with any of the gastrointestinal symptom groups investigated in this study. There was also no significant association between current *H.* pylori infection and current snus use. Dual snus and cigarette use was significantly associated with dyspepsia, irritable bowel syndrome, and epigastric pain. Odds ratios were adjusted for *H. pylori* infection, use of aspirin, use of non-steroidal anti-inflammatory drugs, high alcohol consumption, education level, categorized BMI, use of acid reducing drugs and GERS and adjusting for age and sex. Odds ratios for histological markers were adjusted for *H. pylori* infection, GERS, and categorized age and sex. The response rate to all parts of this study was high, suggesting that the results are likely to be reliable and representative.

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

APPENDIX L-1
DESCRIPTIVE STUDIES OF GASTROINTESTINAL EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Aro et al. 2010 (continued)			Odds ratio (95% CI)	Due to the nature of their design, causality cannot be determined from cross-sectional studies since disease and exposure are measured simultaneously. The small number of individuals in some sub-groups is also a limitation.
		Epigastric pain		
		Never-users of tobacco	1.00 (reference)	
		Current snus user	1.48 (0.77-2.85)	
		Current smoker	1.49 (0.94-2.35)	
		Using both	5.66 (2.18-14.69)*	
		Former snus user	3.15 (0.91-10.96)	
		Former smoker	1.17 (0.75-1.83)	
		Former user of both	1.28 (0.52-3.11)	
		Abdominal pain		
		Never-users of tobacco	1.00 (reference)	
		Current snus user	1.05 (0.64-1.73)	
		Current smoker	1.06 (0.72-1.57)	
		Using both	2.08 (0.77-5.66)	
		Former snus user	2.54 (0.87-7.47)	
		Former smoker	1.48 (1.03-2.12)*	
		Former user of both	0.96 (0.49-1.87)	
		No or minor GI symptoms		
		Never-users of tobacco	1.00 (reference)	
		Current snus user	0.81 (0.49-1.35)	
		Current smoker	0.97 (0.65-1.46)	
		Using both	0.63 (0.22-1.80)	
		Former snus user	0.27 (0.07-0.99)	
		Former smoker	0.63 (0.43-0.93)	
		Former user of both	0.79 (0.40-1.57)	

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

APPENDIX L-1
DESCRIPTIVE STUDIES OF GASTROINTESTINAL EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Aro et al. 2010 (continued)			Odds ratio (95% CI)	
		Esophagitis		
		Never-users of tobacco	1.00 (reference)	
		Current snus user	1.13 (0.62-2.08)	
		Current smoker	1.07 (0.61-1.88)	
		Using both	1.08 (0.33-3.60)	
		Former snus user	1.33 (0.40-4.43)	
		Former smoker	1.09 (0.66-1.78)	
		Former user of both	0.74 (0.32-1.69)	
		Gastric ulcer		
		Never-users of tobacco	1.00 (reference)	
		Current snus user	0.93 (0.11-8.08)	
		Current smoker	2.60 (0.84-8.08)	
		Using both	2.88 (0.32-26.23)	
		Former snus user	----	
		Former smoker	1.49 (0.46-4.87)	
		Former user of both	----	
		Duodenal ulcer		
		Never-users of tobacco	1.00 (reference)	
		Current snus user	----	
		Current smoker	2.20 (0.77-6.30)	
		Using both	2.12 (0.23-19.46)	
		Former snus user	----	
		Former smoker	0.64 (0.17-2.51)	
		Former user of both	0.93 (0.11-8.11)	

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

APPENDIX L-1
DESCRIPTIVE STUDIES OF GASTROINTESTINAL EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Aro et al. 2010 (continued)			Odds ratio (95% CI)	
		Overall peptic ulcer disease		
		Never-users of tobacco	1.00 (reference)	
		Current snus user	0.34 (0.04-2.69)	
		Current smoker	2.32 (1.04-5.19)*	
		Using both	2.57 (0.49-13.55)	
		Former snus user	----	
		Former smoker	1.00 (0.41-2.44)	
		Former user of both	0.64 (0.08-5.23)	
		Basal cell hyperplasia	1.74 (1.02-3.00)*	
		Elongation of papillae	1.79 (1.05-3.05)*	

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

APPENDIX L-1
DESCRIPTIVE STUDIES OF GASTROINTESTINAL EFFECTS AMONG SWEDISH SNUS USERS (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.] Data on cardiovascular outcomes, other health effects and body weight effects in this study are also summarized in Appendices J-1, Q-2, 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 ≥ 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 Smokers (≥ 15 cig/day) **Peptic Ulcer** Non-users of tobacco Smokeless tobacco users Smokers (≥ 15 cig/day)	Odds Ratio (95% CI) 1.0 (reference) 0.9 (0.8-0.9)** 1.3 (1.3-1.4)* 1.0 (reference) 1.1 (0.9-1.2) 2.8 (2.5-3.1)*	**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 (except for tobacco use). Smokers of ≥ 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. 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 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 tobacco Ever **Ulcerative colitis** Never tobacco Ever <u>Moist Snuff Use Among All Subjects (Never, Former, Current Smokers):</u> **Crohn's disease** Never tobacco Ever **Ulcerative colitis** Never tobacco Ever <u>Cigarette Use among Never-users of Snuff</u> **Crohn's disease** Never tobacco Former Current **Ulcerative colitis** Never tobacco Former Current	<u>Relative Risks (95% CI)</u> 1.0 (reference) 0.9 (0.3-3.1) 1.0 (reference) 1.1 (0.4-3.1) 1.0 (reference) 2.1 (1.0-4.6) 1.0 (reference) 2.2 (1.1-4.4)* 1.0 (reference) 1.1 (0.4-3.3) 1.1 (0.5-2.3) 1.0 (reference) 1.3 (0.5-3.4) 0.7 (0.3-1.5)	**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. The exposed number of cases in this study was small.

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

APPENDIX L-2
STUDIES OF GASTROINTESTINAL EFFECTS AMONG SWEDISH SNUS USERS (Continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUS USE AND COMMENTS
Persson et al. 1993		Cigarette use Among All Subjects (Never, Former, Current Snuff Users) **Crohn's disease** Never tobacco Former Current **Ulcerative colitis** Never tobacco Former Current	Relative Risks (95% CI) 1.0 (reference) 1.2 (0.5-3.1) 1.3 (0.7-2.7) 1.0 (reference) 1.5 (0.7-3.4) 0.9 (0.5-1.8)	

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

Appendix L3

Cohort Studies of Gastrointestinal Effects

APPENDIX L-3
COHORT STUDIES OF GASTROINTESTINAL EFFECTS AMONG SWEDISH SNUS USERS (N=1)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Carlens et al. 2010 Sweden This study investigated the potential relationship between chronic inflammatory diseases (including ulcerative colitis (UC) and Crohn's disease (CD)) and the use of "Swedish moist snuff" (snus). See Appendix Q-1 for results on rheumatoid arthritis, sarcoidosis and multiple sclerosis.	Cohort study Subjects were 277,777 male Swedish construction workers who underwent regular health check-ups and had at least one visit from 1978-1993, when information on smoking and snus was obtained through personal interviews with nurses. Subjects were followed until date of diagnosis, death, emigration or December 31, 2004, whichever occurred first. Follow-up was carried out through linkage with nationwide death, emigration, and Swedish Hospital Discharge registries. Adjusted relative risks were derived from Cox proportional hazards regression models. Categories of use included various smoked tobacco as well as snuff use status (never, former, current). Amount of snuff use was also reported (< 22g or ≥ 22g). 13% of the subjects were current or former snus users (among never-smokers). "Snuff" is defined as Swedish moist snuff in this study.	<u>Tobacco Usage</u> **Risk of Ulcerative Colitis Among all Cohort Members** Never-users of tobacco Ever-smoker (n=616) Ever-user of snuff (n=305) **Risk of Ulcerative Colitis Among Pure or Dual Tobacco Users** Never-users of tobacco Ever-smoker (n=425) Ever-user of snuff (n=114) Ever-smoker/ever-snuff (n=191) **Risk of Crohn's Disease Among all Cohort Members** Never-users of tobacco Ever-smoker (n=405) Ever-user of snuff (n=174) **Risk of Crohn's Disease Among Pure or Dual Tobacco Users** Never-users of tobacco Ever-smoker (n=297) Ever-user of snuff (n=66) Ever-smoker/ever-snuff (n=108)	<u>Relative Risk (95% CI)</u> 1.0 (reference) 1.3 (1.1-1.5)* 1.1 (0.9-1.2) 1.0 (reference) 1.2 (1.1-1.4)* 1.0 (0.8-1.2) 1.4 (1.1-1.6)* 1.0 (reference) 1.5 (1.2-1.8)* 0.9 (0.8-1.1) 1.0 (reference) 1.5 (1.3-1.9)* 1.0 (0.8-1.4) 1.4 (1.1-1.8)*	**The authors concluded that smokeless tobacco does not increase the risk of chronic inflammatory diseases (including UC and CD), suggesting that inhaled nonnicotinic components of cigarette smoke are more important than nicotine itself in the etiology of these diseases.** The authors also note that none of the diseases exhibited a dose-response relationship with the amount of snus used (data not shown). Relative risks were adjusted for age, smoking or snus use (among all cohort members), and region of residence. The study population of this cohort was large with prospectively collected data, had a high prevalence of exposure and had high power. However, there were some limitations: Tobacco habits were assessed only at study entry; changes in tobacco habits over time could influence the results. Other exposures among construction workers and selection into the cohort ("healthy worker effect") may limit the generalizability of the results.

* 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/GLUCOSE IMPAIRMENT 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 study (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. "Snuff" is defined as oral moist snuff and is assumed to be Swedish.	<u>Tobacco Use</u> **Impaired glucose tolerance** Never users of tobacco Moist snuff only (n=6) ≤ 2 boxes/week (n=10) > 3 boxes/week (n=15) Cigarettes only (n=31) 1-24 cigs/day (n=81) 25+ cigs/day (n=22) **Type 2 diabetes** Never users of tobacco Moist snuff only (n=4) ≤ 2 boxes/week (n=1) > 3 boxes/week (n=12) Cigarettes only (n=15) 1-24 cigs/day (n=25) 25+ cigs/day (n=13)	<u>Prevalence Odds Ratios (95% CI)</u> 1.0 (reference) 0.9 (0.4-2.1) 0.7 (0.4-1.4) 0.8 (0.4-1.4) 1.0 (0.6-1.6) 0.9 (0.6-1.3) 1.3 (0.7-2.2) 1.0 (reference) 3.9 (1.1-14.3)* 0.2 (0.0-2.0) 2.7 (1.3-5.5)* 1.8 (0.7-4.5) 1.1 (0.5-2.1) 2.6 (1.1-5.8)*	**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 (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.	Cross-sectional study (population-based) 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). "Snuff" is not defined in this study but is assumed to be Swedish snus.	<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. Odds ratios were adjusted for age (all 60), sex (men only), BMI, waist circumference, employment, educational level, living in an apartment, physical activity, alcohol intake and smoking and snuff duration. 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

Case-Control Studies of Insulin Resistance and Diabetes

APPENDIX M-2
CASE-CONTROL STUDIES OF INSULIN/GLUCOSE IMPAIRMENT 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.	Snuff Use **Diabetes** Never Former Current	Odds Ratio (95% CI) 1.0 (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. Odds ratios for diabetes were adjusted for age, hospital catchment area, and smoking. A limitation of this study, however, is that odds ratios were not adjusted for energy intake, which could have led to some residual confounding. Additionally, the risk of diabetes was analyzed only among controls, so technically this was a cross-sectional analysis.

* 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/GLUCOSE IMPAIRMENT 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. "Snuff" is defined as portion-bag packed snuff and is assumed to be Swedish snus.	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

Cohort Studies of Insulin Resistance and Diabetes

APPENDIX M-4
COHORT 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 SNUFF 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). Snus is defined as Swedish snus in this study.	<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-4
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 SNUFF USE AND COMMENTS
Eliasson et al. 2004 (continued)		Among 513 men with normal glucose tolerance test 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 M-4
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 SNUFF USE AND COMMENTS
Ostenson et al. 2012 Sweden This study investigated the effect of smoking and snus use on the risk of type 2 diabetes.	Prospective study Subjects were 2,383 middle-aged (aged 35-56 years) Swedish men who participated in the Stockholm Diabetes Prevention Programme, performed during 1992-1994 in four municipalities within Stockholm County. An oral glucose tolerance test was administered at the 10 year follow-up point. Those diagnosed with diabetes before the end of the 10 year period were excluded (n=84). Tobacco use was assessed at baseline and follow-up 10 years later. Subjects were classified as consistent or former snus users or smokers and also categorized by use: 1-5 boxes of snus/week, >5 boxes/week, and 1-15 cigarettes/day or >15 cigarettes/day. Snus is defined as "oral moist snuff," and is assumed to be Swedish snus.	<u>Tobacco Use</u> **Snus Use** Consistent never snus use Consistent snus use (n=16) Former snus use (n=6) 1-5 boxes/week (n=7) >5 boxes/week (n=9) Never-smoking high consumption (n=3) **Cigarette Use** Consistent never smoking Consistent smoking (n=17) Former smoking (n=30) 1-15 cigarettes/day (n=7) >15 cigarettes/day (n=10)	<u>Odds Ratios (95% CI)</u> 1.0 (reference) 1.1 (0.6-2.0) 0.5 (0.2-1.2) 0.6 (0.2-1.4) 3.3 (1.4-8.1)* 2.3 (0.5-9.8) 1.0 (reference) 1.5 (0.8-3.0) 0.9 (0.5-1.7) 0.8 (0.3-2.1) 2.4 (1.0-5.8)	**The authors concluded that high consumption of snus, similar to cigarette smoking, predicts the risk of developing type 2 diabetes.** Odds ratios were adjusted for tobacco use, age, BMI, glucose tolerance at baseline, physical activity, alcohol consumption, socioeconomic position, and family history of diabetes. This study presents several limitations. Though the title implies that the study is prospective, in fact, participants who were free of type 2 diabetes at baseline but diagnosed prior to the follow-up exam were not considered in this study. Only 99 participants who had newly-discovered type II diabetes following an OGTT at the final exam were included in the analysis of the study, which only tested for an association with self-reported tobacco use categories for outcomes determined at a single time point rather than taking into account risk of developing the disease over time. It's possible that these 84 individuals may have been different compared to the 99 included at follow-up, including tobacco use characteristics. Though the authors controlled for many important confounders, there was no adjustment for any potential dietary confounders. The authors noted that a limitation of the study is the small number of cases developing diabetes, especially when attempting to evaluate the effects of snus in subjects who did not have a record of previous smoking. Never-smoking was significantly less prevalent among cases.

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

APPENDIX M-4
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 SNUFF USE AND COMMENTS
Ostenson et al. 2012 (continued)				It's also possible that the results may have been influenced by other characteristics noted among snus users. Consistent snus users had a higher BMI, higher alcohol consumption and a higher frequency of individuals in the lowest socioeconomic position. Former smoking was more prevalent among cases than controls with high consumption of snus. This could have influenced BMI, waist circumference or waist-to-hip ratio that could have influenced risk of type II diabetes. Body weight (BMI) and central adiposity (waist circumference or waist-to-hip ratio) are both associated with smoking (where BMI tends to be lower in smokers but central adiposity tends to be higher in smokers). Central adiposity in snus users who were never smokers tends to be comparable to nontobacco users, as noted in section 5.8.

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

Appendix N1

Descriptive Studies of Metabolic Syndrome

APPENDIX N-1
DESCRIPTIVE STUDIES OF METABOLIC SYNDROME AMONG SWEDISH SNUS USERS (N=2)

CITATION, LOCATION,	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Gustafsson et al. 2011b Sweden This study examined whether BMI, blood pressure, and socioeconomic status I adolescence and early adulthood are related to metabolic syndrome. Snuff use was investigated as a potential independent contributor to the risk of metabolic syndrome at age 43.	Cohort study, but snuff analyzed only at age 43 (cross-sectional) Subjects were all adolescents (drawn from the Northern Swedish Cohort) who entered or should have entered 9th grade in the town of Lulea in 1981 (n=506 girls and 577 boys). Follow-up surveys were conducted in 1983, 1986, 1995, and 2008, at participant age 18, 21, 30, and 43 years, respectively. Participants completed a questionnaire at each survey. When participants were 16, 21, and 43 years of age, health exams were performed. The definition of the International Diabetes Federation was used to operationalize metabolic syndrome. Daily snuff was assessed as "yes" (n=62 among women and 156 among men) or "no." "Snuff" is not defined in this study but is assumed to be Swedish snus.	<u>Oral Snuff and Smoking Usage</u> **Snuff** Women Men **Smoking** Women Men	<u>Odds ratio (95% CI)</u> 0.79 (0.33-1.86) 0.96 (0.58-1.56) 1.44 (0.76-2.76) 1.74 (0.97-3.14)	**Though the authors do not come to a conclusion about snuff use. Among both men and women, snuff use at age 43 was not identified as a significant independent contributor of metabolic syndrome.** Though the odds ratios among snuff users and smokers were not significant, odds ratios among smokers were elevated. Odds ratios were adjusted for socioeconomic status, BMI, blood pressure, daily smoking, daily snuff use, alcohol consumption, and physical inactivity. A reference group for tobacco users is not identified in this study. Even though this is a prospective cohort study, snuff use is only assessed at one point in time (at age 43). Therefore causality cannot be determined.

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

APPENDIX N-1
DESCRIPTIVE STUDIES OF METABOLIC SYNDROME AMONG SWEDISH SNUS USERS (continued)

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.	Cross-sectional study (population based) 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). "Snuff" is not defined in this study but is assumed to be Swedish snus.	<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.** Odds ratios were adjusted for age (all 60), sex (men only), BMI, waist circumference, employment, educational level, living in an apartment, physical activity, alcohol intake and smoking and snuff duration. 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 N2

Cohort Studies of Metabolic Syndrome

APPENDIX N-2
COHORT STUDIES OF METABOLIC SYNDROME AMONG SWEDISH SNUS USERS (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 obesity and cardiovascular effects are presented in Appendices O-2 and J-5 respectively.	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.	<u>Snus Use</u> **Metabolic Syndrome** No use ≤ 4 cans/week (n=174) > 4 cans/week (n=74) **Components of MetSy** <u>Glucose ≥ 5.6 mmol/L or Diabetes</u> No use ≤ 4 cans/week > 4 cans/week <u>Triglycerides ≥1.7 mmol/L</u> No use ≤ 4 cans/week > 4 cans/week <u>Low HDL Cholesterol</u> No use ≤ 4 cans/week > 4 cans/week <u>Hypertension</u> No use ≤ 4 cans/week > 4 cans/week <u>Body Mass Index ≥ 30</u> No use ≤ 4 cans/week > 4 cans/week	<u>Odds Ratio (95% CI)</u> 1.0 (reference) 1.0 (0.85-1.22) 1.6 (1.26-2.15)* 1.0 (reference) 1.0 (0.86-1.08) 0.8 (0.69-1.02) 1.0 (reference) 1.2 (1.05-1.35)* 1.6 (1.30-1.95)* 1.0 (reference) 1.0 (0.86-1.18) 1.1 (0.82-1.42) 1.0 (reference) 0.9 (0.84-1.05) 1.2 (0.99-1.46) 1.0 (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. The components of MetSy were adjusted for those factors, as well as education, exercise, and alcohol use. 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. Furthermore, odds ratios are not adjusted for smoking or energy intake. 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 N-2
COHORT STUDIES OF METABOLIC SYNDROME AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION,	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Norberg et al. 2006 (continued)		Smoking **Metabolic Syndrome** Non-smoking Ex-smoker (n=416) Daily smoker (n=402) **Components of MetSy** Glucose ≥ 5.6 or Diabetes Non-smoking Ex-smoker Daily smoker Triglycerides ≥ 1.7 Non-smoking Ex-smoker Daily smoker Low HDL Cholesterol Non-smoking Ex-smoker Daily smoker Hypertension Non-smoking Ex-smoker Daily smoker Body Mass Index ≥ 30 Non-smoking Ex-smoker Daily smoker	Odds Ratio (95% CI) 1.0 (reference) 1.2 (1.06-1.38)* 1.0 (0.89-1.16) 1.0 (reference) 1.2 (1.15-1.35)* 1.3 (1.23-1.45)* 1.0 (reference) 1.3 (1.16-1.41)* 1.6 (1.43-1.73)* 1.0 (reference) 1.1 (1.00-1.27) 1.2 (1.07-1.35)* 1.0 (reference) 1.2 (1.07-1.27)* 0.8 (0.75-0.89)** 1.0 (reference) 1.2 (1.04-1.30)* 1.1 (0.98-1.23)	

* 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=11)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Aro et al. 2010 Sweden The aim of this study was to investigate the relationship between different forms of tobacco (including Swedish snus) and upper gastrointestinal (GI) symptoms, histology, and frequency of *H. pylori* infection. The authors also present demographic data including mean BMI. Data on gastrointestinal effects are summarized in Appendix L-1.	Cross-sectional (population-based) The population under study involved a random sample of adults from two northern Swedish municipalities (Kalix and Haparanda). Every seventh adult (n=3,000) from the target population (18-80 years of age, n=21,610 in September 1998) was drawn. Respondents filled out a validated questionnaire, the Abdominal Symptom Questionnaire (ASQ) (n=2,122). Snus users and smokers were exclusive users without a past history of smoking or snus use. Dual users were defined as current snus users and smokers. "Snus" is not defined in this study, but is assumed to be Swedish snus. Snus users included one user of chewing tobacco.	Tobacco Use **Mean BMI** Never-users of tobacco Current snus user Current smoker Using both Former snus user Former smoker Former user of both	Difference compared to never-users reference Nonsignificant P < 0.05 (lower) Nonsignificant Nonsignificant Nonsignificant Nonsignificant	**The authors present results that indicate a significantly lower BMI among current smokers. The mean BMI among snus users was not significantly different compared to never-users of tobacco.** The response rate to all parts of this study was high, suggesting that the results are likely to be reliable and representative. 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 O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (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, cardiovascular and other health effects observed in this study are summarized in Appendices L-1, J-1, and Q-2 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 Use **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	Odds Ratios (95% CI): 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. 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 O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Bolinder et al. 1992 (continued)	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 (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.	Tobacco Use **BMI** Never-users of tobacco Smokeless tobacco users Smokers **Waist/hip-Ratio** Never-users of tobacco Smokeless tobacco users Smokers	Level of significance for differences between smokers or smokeless tobacco users and never-users of tobacco reference Nonsignificant Nonsignificant reference Nonsignificant $p < 0.001$* (increased in smokers)	**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.** 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 O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (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>Tobacco Use</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.** 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 O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (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 snuff users, 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>Tobacco Use</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. 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 O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (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 female 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>Tobacco Use</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. 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 O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Engstrom et al. 2010 Sweden This study examined the relationship between various socio-demographic, lifestyle, and health-related characteristics (including BMI).	Cross-sectional study (population-based) Subjects included a population-based sample of 34,707 men and women aged 18-84 years from the 2006 Stockholm Public Health Survey. Current tobacco use was categorized into four mutually exclusive groups - no daily use (including former use), exclusive daily use of snus, exclusive daily smoking or daily dual use (both smoking and snus use). Occasional tobacco use was not elicited in the survey and therefore not taken into account in this study. 17% and 3.1% of males and females respectively were current exclusive users of snus. "Snus" is defined as a moist, smokeless tobacco product and is assumed to be Swedish.	<u>Exclusive Tobacco Use</u> <u>Underweight</u> **Males** Non-use Snus use Smoking Dual use Smoking Snus use Dual use **Females** Non-use Snus use Smoking Dual use Smoking Snus use Dual use <u>Overweight</u> **Males** Non-use Snus use Smoking Dual use Smoking Snus use Dual use **Females** Non-use	<u>Odds ratio (95% CI)</u> 1.00 (reference) 0.46 (0.22-0.97)** 1.68 (1.01-2.78)* 1.27 (0.45-3.59) 1.00 (reference) 0.18 (0.08-0.42)** 0.59 (0.20-1.78) 1.00 (reference) 0.91 (0.55-1.51) 1.24 (0.98-1.58) 1.76 (0.88-3.52) 1.00 (reference) 0.51 (0.30-0.87)** 1.03 (0.36-2.95) 1.00 (reference) 1.04 (0.93-1.15) 0.75 (0.65-0.86)** 1.30 (1.01-1.66)* 1.00 (reference) 1.26 (1.08-1.47)* 1.55 (1.18-2.04)* 1.00 (reference)	**The authors of this study concluded that underweight was inversely associated with snus use while the opposite was true for smoking. Smoking was less common among overweight and obese individuals while snus use was not related to overweight.** Odds ratios were adjusted for age, occupational class, past tobacco use, and physical activity. Strengths included the large size of the sample allowed for the inclusion of women in the analysis, and the wide range of available information on social and health related characteristics. Limitations included high non-participation in the survey (39%) which may have led to selection bias, self-report of behavioral characteristics (including tobacco use), and the nature of study itself. No conclusions can be drawn regarding causality from this cross-sectional study.

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

APPENDIX O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Engstrom et al. 2010 (continued)		Snus use Smoking Dual use Smoking Snus use Dual use <u>Obese</u> **Males** Non-use Snus use Smoking Dual use Smoking Snus use Dual use **Females** Non-use Snus use Smoking Dual use Smoking Snus use Dual use	1.00 (0.79-1.26) 1.01 (0.89-1.14) 1.63 (0.96-2.76) 1.00 (reference) 0.97 (0.77-1.24) 1.53 (0.91-2.59) 1.00 (reference) 1.01 (0.85-1.20) 0.76 (0.61-0.94) 1.59 (1.12-2.26)* 1.00 (reference) 1.28 (1.01-1.63)* 1.89 (1.28-2.77)* 1.00 (reference) 0.99 (0.70-1.39) 0.92 (0.77-1.10) 1.76 (0.88-3.52) 1.00 (reference) 1.10 (0.77-1.56) 1.87 (0.93-3.74)	

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

APPENDIX O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (continued)

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>Smoking Status</u> **Men** Never Former Occasional Daily **Women** Never Former Occasional Daily	<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) ----- 1.00 (reference) 1.17 (0.69-1.96) 1.42 (0.90-2.24) 2.00 (1.37-2.90)* 1.00 (reference) 1.27 (0.90-1.78) 1.44 (1.02-2.02)* 1.87 (1.39-2.50)*	**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 (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 whose 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	<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 concluded 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 (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
		[See Sundbeck et al. 2009 for further analyses.]		

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

APPENDIX O-1
DESCRIPTIVE STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Vaezghasemi et al. 2012 Sweden This study investigated the relationship between overweight and obesity and various lifestyle characteristics (including snuff use).	Cross-sectional study Participants were 6,768 13-15 year olds from Västerbotten County, Sweden. Participants filled out an on-line survey in 2007, with an 82% participation rate, and responses were considered reliable for 74% (2,517 boys/2,470 girls). The survey addressed demography, self-rated health, self-reported weight, height, and lifestyle characteristics. 1,219 of the boys were snus users, while 1,174 of the girls were snus users. Participants responded either "yes" or "no" for snuff use. Yes indicated very seldom to everyday use. "Snuff" is assumed to be Swedish snus.	<u>Tobacco use</u> **Snuff use** Simple logistic regression Girls Boys Multiple logistic regression Boys **Smoking** Simple logistic regression Girls Boys Multiple logistic regression Boys	 1.3 (0.7-2.4) 2.3 (1.7-3.2)* 1.6 (1.1-2.4)* 0.9 (0.6-1.3) 1.9 (1.5-2.5)* 1.2 (0.9-1.7)	**The authors concluded that overweight/obesity was associated with using snuff for boys.** Snuff use was not associated with overweight and obesity in girls. The authors also noted that overweight/obese boys and girls were more often physically inactive, and that for the boys, overweight/obesity was also associated with skipping breakfast, and insufficient tooth brushing. For the girls, overweight/obesity was also associated with living with one parent and more television watching. Girls reported better dietary and tooth brushing habits, while boys reported healthier habits concerning sleep duration, physical activity, eating breakfast, and smoking compared to the girls. Among boys, multiple logistic regression odds ratios were adjusted for age, self-rated health, breakfast, tooth brushing, TV watching, physical activity, smoking, alcohol use, and drug use. A limitation of this study is its cross-sectional study design. Additionally, it is unclear that the authors adjusted for former smoking among snuff 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 (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, waist circumference (WC) 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.	Tobacco Use **Snuff-years** BMI WHR WC **Cigarette-years** BMI WHR WC **BMI** Ex-snuff user Current snuff user Ex-smoker Current smoker **WHR** Ex-snuff user Current snuff user Ex-smoker Current smoker **WC** Ex-snuff user Current snuff user Ex-smoker Current smoker	Spearman's r-value 0.09 0.11* ($p < 0.01$) 0.07 0.14* ($p < 0.01$) 0.29* ($p < 0.001$) 0.21* ($p < 0.001$) Level of significance compared to never-users or never-smokers Nonsignificant Nonsignificant Increased* ($p < 0.05$) Nonsignificant Nonsignificant Nonsignificant Increased* ($p < 0.05$) Increased* ($p < 0.05$) Nonsignificant Nonsignificant Increased* ($p < 0.05$) 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, WHR, or WC were observed among 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 O2

Case-Control Studies of Body Weight

APPENDIX O-2
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 Appendices J-2, M-4, and J-6, for results on MI, diabetes, and cardiovascular effects respectively.	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: BMI ≥ 30 kg/m^2). "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. Odds ratios of being overweight 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 for MI. A limitation of this study, however, is that odds ratios were not adjusted for energy intake, which could have led to some residual confounding. Additionally, the risk of being overweight was analyzed only among controls, so technically this was a cross-sectional analysis.

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

Appendix O3

Cohort Studies of Body Weight

APPENDIX O-3
COHORT STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (N=4)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Hansson et al. 2011 Sweden This study investigated the relationship between the use of snus or smoking, weight gain, and the incidence of obesity.	Cohort study Subjects were 9,954 males aged 18-84 living in Stockholm County, Sweden, recruited in 2002 and reassessed in 2007. Tobacco use was categorized according to information obtained in both baseline and follow-up surveys. Outcomes were assessed by comparing self-reported weight and BMI between baseline and follow-up surveys. Weight gain was defined as ≥ 5% increase in body weight and obesity was defined as BMI ≥ 30 kg/m^2. 18% of participants reported current snus use at baseline but among these only 839 were never-smokers. "Snus" is defined as a "moist smokeless tobacco product" and is assumed to be Swedish snus.	<u>Exclusive Daily Snuff Usage</u> **Weight gain** Never tobacco Stable current use (n=139) Stable former use (n=31) Quit during follow up (n=51) Began during follow up (n=14) **Obese** Never tobacco Stable current use (n=21) Stable former use (n=3) Quit during follow up (n=8) Began during follow up (n=0) <u>Exclusive Daily Cigarette Usage</u> **Weight gain** Never tobacco Stable current use (n=174) Stable former use (n=235) Quit during follow up (n=120) Began during follow up (n=9) **Obese** Never tobacco Stable current use (n=26) Stable former use (n=47) Quit during follow up (n=16) Began during follow up (n=6)	<u>Odds ratio (95% CI)</u> 1.00 (reference) 1.31 (1.04-1.65)* 1.36 (0.89-2.10) 1.25 (0.88-1.77) 0.97 (0.50-1.86) 1.00 (reference) 1.93 (1.13-3.30)* 0.85 (0.25-2.88) 1.13 (0.51-2.50) ---- 1.00 (reference) 1.24 (1.00-1.54) 1.04 (0.87-1.25) 3.15 (2.39-4.15)* 0.70 (0.29-1.67) 1.00 (reference) 1.31 (0.78-2.22) 1.09 (0.72-1.66) 1.87 (0.99-3.53) 3.53 (1.24-10.09)*	**The authors concluded that snus use is moderately associated with weight gain and incident obesity. They suggest that this indicates that there may be metabolic disturbances among snus users.** A significant association with weight gain was also observed among smokers who quit during follow up. Odds ratios were adjusted for age, alcohol consumption, physical activity, education, consumption of fruit and berries, frequency of having breakfast. A major strength of this study was its prospective design. The outcome definitions are clinically relevant and enable comparisons with other studies. The association between smoking cessation and weight gain, in line with other studies, suggests no major bias in the results. A limitation of this study is that the information was self-reported, which is a potential source of bias. Furthermore, the authors had no information on energy intake, and cannot exclude the possibility of residual confounding.

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

APPENDIX O-3
COHORT STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (continued)

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." "Snuff" was defined as Swedish moist snuff (snus) in this study.	<u>Weight Non-Gain in the Longitudinal Study</u> **Snuff Use** No Yes **Snuff Use** No Yes **Cigarette use** No Yes	<u>Odds Ratios (95% CI)</u> <u>Multivariate:</u> 1.00 (reference) 0.83 (0.74-0.92)** <u>Univariate:</u> 1.00 (reference) 0.82 (0.74-0.91)** 1.00 (reference) 0.99 (0.92-1.05)	**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-3
COHORT STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (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 obesity 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.	Tobacco Usage **Body Mass Index ≥ 30** Smoking Non-smoking Ex-smoker (n=416) Daily smoking (n=402) Use of snus No use ≤ 4 cans/week (n=174) > 4 cans/week (n=74)	Odds Ratio (95% CI) 1.0 (reference) 1.2 (1.04-1.30)* 1.1 (0.98-1.23) 1.0 (reference) 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 major strengths of this were that 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 O-3
COHORT STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Norberg et al. 2006 (continued)	"Snuff" was defined as Swedish moist snuff (snus) in this study.			

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

APPENDIX O-3
COHORT STUDIES OF BODY WEIGHT AMONG SWEDISH SNUS USERS (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 three mutually exclusive categories of snus users: ex, current, and never. "Snus" was defined as Swedish moist snuff (snus) in this study.	<u>Prevalence of Overweight at Study Entry</u> **Tobacco Use** Never use Current pure smoking (n=137) Current pure snus use (n=96) Current combined use (n=47) Ex-snus users (n=42) All ex-smokers (n=382) No current tobacco (n=249) Current snus (n=133) <u>Development of Overweight During Follow-up Among Men Not Overweight at Entry</u> **Tobacco Use At Entry/At Follow-Up** Never/no tobacco Smoking/smoking (n=93) Smoking/snus (n=23) Smoking/no tobacco (n=53) Snus/snus (n=152) Snus/no tobacco (n=39)	<u>Prevalence Ratios (95% CI)</u> 1.00 (reference) 0.87 (0.73-1.03) 1.20 (1.01-1.42)* 1.25 (1.03-1.63)* 0.93 (0.71-1.21) 1.24 (1.10-1.40)* 1.23 (1.07-1.40)* 1.33 (1.14-1.55)* <u>Standardized Incidence Ratios (95% CI)</u> ----- 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 P1

Descriptive Studies of Pregnancy Outcomes and Reproductive Effects

APPENDIX P-1
DESCRIPTIVE STUDIES OF REPRODUCTIVE EFFECTS AMONG SWEDISH SNUS USERS (N=1)

CITATION, LOCATION,	STUDY TYPE, POPULATION	RESULTS	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.	The authors did not find any effect of snuffing on any of the reproductive parameters evaluated (semen parameters, seminal biochemical biomarkers, hormone levels). Among smokers, the authors reported significantly reduced total sperm counts when compared with non-smokers. Smoking was also significantly associated with a dose-dependent decrease in hormone levels (serum FSH). Additionally, non-smokers had a significantly decreased zing levels per ejaculate compared to non-smokers.	**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.** p-Values were adjusted for tobacco, alcohol intake, and the length of abstinence period. Due to the nature of their design, causality cannot be determined from cross-sectional studies since disease and exposure are measured simultaneously.

Appendix P2

Cohort Studies of Pregnancy Outcomes and Reproductive Effects

APPENDIX P-2
COHORT STUDIES OF PREGNANCY OUTCOMES AMONG SWEDISH SNUS USERS (N=6)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Baba et al. 2012a Sweden This study examined the effects of snus use during pregnancy small-for-gestational-age (SGA) births.	Cohort study Subjects were 846,411 women who were delivered of singleton, live-born infants in Sweden from 1999 through 20010. Information on birth outcomes and tobacco use was obtained from the Swedish Medical Birth Register. The definition of SGA is a birthweight more than two standard deviations below the mean weight for gestational age according to the sex-specific Swedish fetal growth curves. There were 9,129 exclusive snuff users, 14,093 snuff users who stopped, 74,359 current, exclusive smokers, and 85,181 smokers who stopped. Tobacco use was self-reported. Snuff is defined as Swedish oral moist snuff (snus) in this study.	<u>Tobacco Usage</u> **SGA by change of habit in early pregnancy** Nonuser of tobacco Snuff user/nonuser Snuff user/snuff user Smoker/nonuser Smoker/smoker **Preterm (≤36 weeks) SGA by change of habit in early pregnancy** Nonuser of tobacco Snuff user/nonuser Snuff user/snuff user Smoker/nonuser Smoker/smoker **Term (>37 weeks) SGA by change of habit in early pregnancy** Nonuser of tobacco Snuff user/nonuser Snuff user/snuff user Smoker/nonuser Smoker/smoker **Term SGA by change of habit from early to late pregnancy** Nonuser of tobacco Snuff user/nonuser Snuff user/snuff user Smoker/nonuser Smoker/smoker	<u>Odds ratio (95% CI)</u> 1.00 (reference) 0.86 (0.75-0.98)** 1.26 (1.09-1.46)* 1.03 (0.98-1.09) 2.55 (2.43-2.67)* 1.00 (reference) 0.80 (0.60-1.08) 1.50 (1.13-1.98)* 0.86 (0.76-0.98)** 1.85 (1.67-2.06)* 1.00 (reference) 0.87 (0.75-1.02) 1.21 (1.02-1.43)* 1.07 (1.01-1.14)* 2.76 (2.62-2.91)* 1.00 (reference) 1.08 (0.86-1.35) 1.38 (1.01-1.88)* 1.82 (1.65-2.01)* 3.21 (3.02-3.40)*	**The authors concluded that both smoking, and to a lesser extent use of oral moist snuff, during pregnancy increase the risk of an SGA birth.** The authors noted that both nicotine and tobacco combustion products are involved in the mechanisms by which maternal tobacco use during pregnancy increases the risk of SGA birth, and that products containing nicotine should be avoided during pregnancy. Snuff use had, if anything, a stronger association with preterm SGA than term SGA, whereas the opposite was true for smoking. Women who stopped using snuff before their first visit to antenatal care had no increased risks of preterm or term SGA, and women who stopped using snuff later during pregnancy had no increased risk of term SGA. Odds ratios were adjusted for maternal age, parity, early pregnancy body mass index, maternal height, cohabitation, education, pregestational diabetes and essential hypertension Major strengths of this study include the large, population-based study sample and standardized records. Information on tobacco use was collected by interview in early pregnancy and at a second interview (gestational weeks 30-32). A weakness of the study is the self-

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

APPENDIX P-2
COHORT STUDIES OF PREGNANCY OUTCOMES AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Baba et al. 2012a (continued)				reported information on tobacco use during pregnancy. Underreporting is a possibility. The authors lacked information on other potential confounders including alcohol and other drugs, domestic violence, differences in diet, and exposure to infections. The authors also noted that tobacco-related risk may have been influenced by unmeasured health-related factors. For example, they note that compared with non-tobacco users, snuff users and especially smokers are more likely to have low education level and be overweight or obese during pregnancy.

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

APPENDIX P-2
COHORT STUDIES OF PREGNANCY OUTCOMES AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Baba et al. 2012b Sweden This study examined the effects of snus use during pregnancy on preterm birth.	Cohort study Subjects were 776,836 women who were delivered of singleton, live-born infants in Sweden from 1999 through 2009. Information on birth outcomes (including preterm birth) and tobacco use was obtained from the Swedish Medical Birth Register. There were 8,321 exclusive snuff users and 695 exclusive smokers during early pregnancy, and 11,983 exclusive snuff users and 78,817 exclusive smokers 3 months before pregnancy. Tobacco use was self-reported. Snuff is defined as Swedish oral moist snuff (snus) in this study.	<u>Tobacco Usage</u> **Preterm (<37 weeks)** Nonuser of tobacco Stopped using snuff Current snuff user Stopped smoking Current smoker **Very preterm birth (<32 weeks)** Nonuser of tobacco Stopped using snuff Current snuff user Stopped smoking Current smoker **Moderate preterm birth (32-36 weeks)** Nonuser of tobacco Stopped using snuff Current snuff user Stopped smoking Current smoker **Spontaneous onset** Nonuser of tobacco Stopped using snuff Current snuff user Stopped smoking Current smoker **Induced onset** Nonuser of tobacco Stopped using snuff Current snuff user Stopped smoking Current smoker	<u>Odds ratio (95% CI)</u> 1.00 (reference) 0.92 (0.84-1.01) 1.29 (1.17-1.43)* 0.90 (0.87-0.94)** 1.30 (1.25-1.36)* 1.00 (reference) 0.88 (0.68-1.14) 1.44 (1.12-1.86)* 0.91 (0.82-1.01) 1.68 (1.52-1.84)* 1.00 (reference) 0.93 (0.84-1.02) 1.27 (1.15-1.41)* 0.90 (0.86-0.94)** 1.25 (1.20-1.30)* 1.00 (reference) 0.92 (0.83-1.02) 1.30 (1.15-1.45)* 0.92 (0.88-0.96)** 1.32 (1.26-1.38)* 1.00 (reference) 0.93 (0.78-1.10) 1.27 (1.07-1.52)* 0.86 (0.79-0.92)** 1.20 (1.12-1.29)*	**The authors concluded that women who stop using snuff or stop smoking in early pregnancy reduce their risk of preterm birth. The similarities in risks, between persistent snuff users and smokers, and between women who quit using snuff or quit smoking, suggest that antenatal exposure to nicotine is involved in the mechanisms by which maternal use of tobacco increases the risk of preterm birth.** Odds ratios were adjusted for early pregnancy BMI, maternal age, parity, education and cohabitation. Major strengths of this study include the large, population-based study sample and standardized records. Information on tobacco use was collected by interview in early pregnancy. A weakness of the study is the self-reported information on tobacco use during pregnancy. Underreporting is a possibility. The authors lacked information on other potential confounders including alcohol and other drugs, domestic violence, differences in diet, and exposure to infections.

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

APPENDIX P-2
COHORT STUDIES OF PREGNANCY OUTCOMES AMONG SWEDISH SNUS USERS (continued)

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.	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. Tobacco use was self-reported. 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.	<u>Pregnancy Outcome</u> **Small-For-Gestational-Age Birth (> 2 SD below mean weight)** Nonusers of tobacco Snus users (n=17) Cigarette smokers (n=475) **Preterm Delivery (< 37 weeks gestation)** Nonusers of tobacco Snus users (n=59) Cigarette smokers (n=666) **Preeclampsia** Nonusers of tobacco Snus users (n=37) Cigarette smokers (n=234)	<u>Odds Ratio (95% CI)</u> 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 g (95% CI: 6-72 g), and in cigarette smokers by 190 g (95% CI: 178-202 g), 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).

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** denotes statistically significant decrease in risk

APPENDIX P-2
COHORT STUDIES OF PREGNANCY OUTCOMES AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Gunnerbeck et al. 2011 Sweden This study investigated whether the disturbances in cardiorespiratory control (and risk of apnea) associated with maternal smoking is also seen in infants prenatally exposed to snuff.	Cohort study Subjects were 609,551 women who were delivered of singleton, live-born infants in Sweden from 1999 through 2006. Information on birth outcomes (including neonatal apnea) and tobacco use was obtained from the Swedish Medical Birth Register. There were 7,599 exclusive snuff users, 41,391 exclusive light smokers, and 16,928 exclusive heavy smokers. Tobacco use was self-reported. Snuff is defined as Swedish snuff (snus) in this study.	<u>Tobacco Usage</u> **Apnea** Nonuser of tobacco Snuff user (n=26) Model 1 Model 2 Cigarette smoker 1-9 cigs/day (n=94) Model 1 Model 2 > 9 cigs/day (n=40) Model 1 Model 2	<u>Odds ratio (95% CI)</u> 1.00 (reference) 2.15 (1.44-3.20)* 1.96 (1.30-2.96)* 1.31 (1.04-1.65)* 1.08 (0.85-1.37) 1.49 (1.07-2.08)* 1.08 (0.76-1.52)	**The authors concluded that snuff use during pregnancy is associated with a higher risk of neonatal apnea than smoking.** The authors noted that this finding is consistent with animal studies of prenatal nicotine exposure. Odds ratios were adjusted using two models. Model 1 was adjusted for maternal age, height, parity, education, and tobacco use. Model 2 was further adjusted for cesarean delivery, gender, gestation age, and small for gestational age. Major strengths of this study include the large, population-based, study sample and standardized records. Information on tobacco use was collected in early pregnancy. The relatively homogeneous population of women born in the Nordic countries should further minimize the potential for confounding by unmeasured sociodemographic factors or differences in management. A weakness of the study is the self-reported information on tobacco use during pregnancy. Underreporting is a possibility. Some women may have stopped using tobacco later in pregnancy. The authors also lacked information on other potential confounders including alcohol and other drugs, domestic violence, differences in

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APPENDIX P-2
COHORT STUDIES OF PREGNANCY OUTCOMES AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Gunnerbeck et al. 2011 (continued)				diet, and exposure to infections.

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** denotes statistically significant decrease in risk

APPENDIX P-2
COHORT STUDIES OF PREGNANCY OUTCOMES AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Wikström et al. 2010a Sweden This study examined the effects of snus use during pregnancy on preterm birth.	Cohort study Subjects were 610,757 women who were delivered of singleton, live-born infants in Sweden from 1999 through 2006. Information on birth outcomes (including preterm birth) and tobacco use was obtained from the Swedish Medical Birth Register. There were 7,607 exclusive snuff users, 41,436 exclusive light smokers, and 16,951 exclusive heavy smokers. Tobacco use was self-reported. Snuff is defined as Swedish oral moist snuff (snus) in this study.	<u>Tobacco Usage</u> **Very preterm birth** Nonuser of tobacco Snuff user (n=56) Cigarette smoker 1-9 cigs/day (n=394) > 9 cigs/day (n=186) **Moderate preterm birth** Nonuser of tobacco Snuff user (n=378) Cigarette smoker 1-9 cigs/day (n=2,061) > 9 cigs/day (n=1,025) **Spontaneous onset** Nonuser of tobacco Snuff user (n=298) Cigarette smoker 1-9 cigs/day (n=1,713) > 9 cigs/day (n=839) **Induced onset** Nonuser of tobacco Snuff user (n=125) Cigarette smoker 1-9 cigs/day (n=654) > 9 cigs/day (n=311)	<u>Odds ratio (95% CI)</u> 1.00 (reference) 1.38 (1.04-1.83)* 1.60 (1.42-1.81)* 1.90 (1.61-2.25)* 1.00 (reference) 1.25 (1.12-1.40)* 1.18 (1.12-1.24)* 1.45 (1.35-1.56)* 1.00 (reference) 1.25 (1.10-1.41)* 1.24 (1.17-1.32)* 1.56 (1.44-1.69)* 1.00 (reference) 1.33 (1.10-1.61)* 1.17 (1.06-1.28)* 1.30 (1.14-1.48)*	**The authors concluded that the use of Swedish snuff was associated with increased risks of very and moderately preterm birth with both spontaneous and induced onsets.** The authors also confirmed earlier findings of a dose-response association between smoking and preterm birth. Odds ratios were adjusted for maternal age, early pregnancy, early-pregnancy BMI, parity and years of education. Major strengths of this study include the large, population-based, study sample and standardized records. Information on tobacco use was collected by interview in early pregnancy. The relatively homogeneous population of women born in the Nordic countries should further minimize the potential for confounding by unmeasured sociodemographic factors or differences in management. A weakness of the study is the self-reported information on tobacco use during pregnancy. Underreporting is a possibility. Some women may have stopped using tobacco later in pregnancy. The authors also lacked information on other potential confounders including alcohol and other drugs, domestic violence, differences in diet, and exposure to infections.

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APPENDIX P-2
COHORT STUDIES OF PREGNANCY OUTCOMES AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Wikström et al. 2010b Sweden This study examined the effects of snus use during pregnancy on risk of stillbirth and other pregnancy complications including preeclampsia, antenatal bleeding, or giving birth to a small for gestational age (SGA) infant.	Cohort study Subjects were 610,879 women who were delivered of singleton, live-born infants in Sweden from 1999 through 2006. Information on birth outcomes (including stillbirth) and tobacco use was obtained from the Swedish Medical Birth Register. There were 7,629 exclusive snuff users, 41,488 exclusive light smokers, and 17,014 exclusive heavy smokers. Tobacco use was self-reported. Snuff is defined as Swedish oral moist snuff (snus) in this study.	<u>Tobacco Usage</u> **Stillbirth** Nonuser of tobacco Snuff user (n=40) Cigarette smoker 1-9 cigs/day (n=172) > 9 cigs/day (n=120) **Restricted Model of Stillbirth** Nonuser of tobacco Snuff user (n=29) Cigarette smoker 1-9 cigs/day (n=99) > 9 cigs/day (n=58) **Preeclampsia** Nonuser of tobacco Snuff user (n=272) Cigarette smoker 1-9 cigs/day (n=994) > 9 cigs/day (n=310) **Antenatal Bleeding** Nonuser of tobacco Snuff user (n=90) Cigarette smoker 1-9 cigs/day (n=583) > 9 cigs/day (n=338) **Small for Gestational Age** Nonuser of tobacco Snuff user (n=150) Cigarette smoker 1-9 cigs/day (n=1653) > 9 cigs/day (n=833)	<u>Odds ratio (95% CI)</u> 1.00 (reference) 1.60 (1.13-2.29)* 1.40 (1.17-1.67)* 2.42 (1.96-2.99)* 1.00 (reference) 1.57 (1.03-2.41)* 1.15 (0.91-1.45) 1.85 (1.39-2.46)* 1.00 (reference) 1.12 (0.98-1.28) 0.66 (0.61-0.71)** 0.52 (0.46-0.60)** 1.00 (reference) 1.15 (0.92-1.44) 1.51 (1.37-1.66)* 1.88 (1.65-2.13)* 1.00 reference) 1.17 (0.98-1.39) 2.34 (2.21-2.49)* 3.20 (2.94-3.48)*	**The authors concluded that use of Swedish snuff during pregnancy was associated with a higher risk of stillbirth.** The authors also confirmed earlier findings of a dose-dependent increased stillbirth risk in cigarette smokers. Odds ratios were adjusted for maternal age, BMI, parity, years of education chronic hypertension, and pre-gestational diabetes. The restricted model of still birth excluded women with preeclampsia, antenatal bleeding, and infants born SGA. The authors note that the greatly reduced risk among smokers but not for snuff users in this model suggests different mechanisms for the increased stillbirth risk in snuff users and smokers. Major strengths of this study include the large, population-based, study sample and standardized records. Information on tobacco use was collected by interview in early pregnancy. The relatively homogeneous population of women born in the Nordic countries should further minimize the potential for confounding by unmeasured sociodemographic factors or differences in management. A weakness of the study is the self-reported information on tobacco use

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APPENDIX P-2
COHORT STUDIES OF PREGNANCY OUTCOMES AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Wikström et al. 2010b (continued)				during pregnancy. Underreporting is a possibility. Some women may have stopped using tobacco later in pregnancy. The authors also lacked information on other potential confounders including alcohol and other drugs, domestic violence, differences in diet, and exposure to infections.

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APPENDIX P-2
COHORT STUDIES OF PREGNANCY OUTCOMES AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Wikström et al. 2010c Sweden This study examined the effects of snus use during pregnancy on preeclampsia risk.	Cohort study Subjects were 612,712 women who were delivered of singleton, live-born infants in Sweden from 1999 through 2006. Information on birth outcomes (including preeclampsia) and tobacco use was obtained from the Swedish Medical Birth Register. There were 7,555 exclusive snuff users, 41,121 exclusive light smokers, and 16,806 exclusive heavy smokers. Tobacco use was self-reported. Snuff is defined as Swedish snuff (snus) in this study.	<u>Tobacco Usage</u> **Preeclampsia** Nonuser of tobacco Snuff user Cigarette smoker 1-9 cigs/day > 9 cigs/day **Gestational Hypertension** Nonuser of tobacco Snuff user Cigarette smoker 1-9 cigs/day > 9 cigs/day [See Wikström et al. 2010a for additional analyses]	<u>Odds ratio (95% CI)</u> 1.00 (reference) 1.11 (0.97-1.28) 0.66 (0.61-0.71)** 0.51 (0.44-0.58)** 1.00 (reference) 0.89 (0.68-1.15) 0.64 (0.55-0.73)** 0.49 (0.39-0.62)**	**The authors concluded that cigarette smoking but not snuff use during pregnancy decreased the risk for the development of preeclampsia and gestational hypertension.** The authors note that nicotine does not seem to be the protective ingredient against preeclampsia in cigarette smoke. Odds ratios were adjusted for early pregnancy BMI, maternal age, parity, and year of education. Major strengths of this study include the large, population-based, study sample and standardized records. Information on tobacco use was collected at both the first antenatal visit and from gestational weeks 30-32. The relatively homogeneous population of women born in the Nordic countries should further minimize the potential for confounding by unmeasured sociodemographic factors or differences in management. A major weakness of the study is the self-reported information on tobacco use during pregnancy. Underreporting is a possibility. The authors also lacked information on other potential confounders including alcohol and other drugs, domestic violence, differences in diet, and exposure to infections.

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Appendix Q1

Descriptive Studies of Other Health Effects

APPENDIX Q-1
DESCRIPTIVE STUDIES OF OTHER HEALTH EFFECTS 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. 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, cardiovascular, and body weight effects observed in this study are summarized in Appendices L-1, J-1, and O-1 respectively.	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.	Tobacco Use **Musculoskeletal diagnosis (ages 45-55)** Non-user Smokeless tobacco Cigarettes (≥ 15/day) **Musculoskeletal diagnosis (ages 56-65)** Non-user Smokeless tobacco Cigarettes (≥ 15/day) **Low back pain in the past year** Non-user Smokeless tobacco Cigarettes (≥ 15/day) **Pain in leg while walking** Non-user Smokeless tobacco Cigarettes (≥ 15/day) **Sleeping disturbances** Non-user Smokeless tobacco Cigarettes (≥ 15/day) **Nervous Problems** Non-user Smokeless tobacco Cigarettes (≥ 15/day)	Odds Ratios (95% CI) 1.0 (reference) 2.8 (1.6-4.8)* 2.4 (1.5-3.8)* 1.0 (reference) 1.5 (1.2-1.8)* 1.7 (1.3-2.2)* 1.0 (reference) 1.1 (1.0-1.2) 1.2 (1.2-1.3)* 1.0 (reference) 1.3 (1.1-1.5)* 2.1 (1.8-2.4)* 1.0 (reference) 1.2 (1.1-1.4)* 1.8 (1.7-2.0)* 1.0 (reference) 1.2 (1.1-1.4)* 1.8 (1.6-2.0)	**The authors stated that the results of this study confirm that there is a higher rate of back disorder symptoms among smokers, but although smokeless tobacco users did not show a similar excess risk in low back pain, symptoms, they were granted disability pensions due to musculoskeletal disorders twice as often as the non-users.** Due to the cross-sectional design of this study, causality cannot be determined.

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APPENDIX Q-1
DESCRIPTIVE STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION,	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Bolinder et al. 1992 (continued)			Odds Ratios (95% CI)	
		Frequent sick leave		
		Non-user	1.0 (reference)	
		Smokeless tobacco	1.1 (1.0-1.2)	
		Cigarettes (≥ 15/day)	1.7 (1.6-1.8)*	
		Long sick leave		
		Non-user	1.0 (reference)	
		Smokeless tobacco	1.2 (1.1-1.2)*	
		Cigarettes (≥ 15/day)	1.7 (1.6-1.8)*	

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

APPENDIX Q-1
DESCRIPTIVE STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION,	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Edwards et al. 2011 Sweden The aim of this study was to investigate the relationship between tobacco consumption habits and major depression.	Cross-sectional study Subjects in this population were same-sex twins (12,744 males and 15,249 females) from the Swedish Screening Across the Lifespan Twin (SALT) study, which is based on the Swedish Twin Registry. Measure of major depression was accomplished using the computerized Composite International Diagnostic Interview Short-Form, adapted to assess lifetime prevalence of depression. A small number (n=205) volunteered that they were taking anti-depressants, and were considered positive for history of major depression. The authors considered an individual as a 'regular smoker' or a ' regular snus user' if at least one of the following was true: (i) they reported ever regularly smoking/snusing; (ii) they reported at least 1 year of smoking/snusing; (iii) they reported weekly use of cigarettes/snus. Nicotine dependence was assed using items from the Fagerstrom Test for Nicotine Dependence, and scores were categorized into five levels of dependence. The type of snuff in this study is assumed to be Swedish snus.	<u>Tobacco Use</u> **Males** Non-tobacco Regular cigarette use Regular snus use Cigarettes: nicotine dependence Very low Low Medium High Very high Snus: nicotine dependence Very low Low Medium High Very high **Females** Non-tobacco Regular cigarette use Regular snus use Cigarettes: nicotine dependence Very low Low Medium High Very high Snus: nicotine dependence Very low Low Medium High Very high	<u>Odds Ratios (95% CI)</u> 1.00 (reference) 1.21 (1.07-1.36)* 1.28 (1.14-1.45)* 1.00 (0.86-1.15) 1.02 (0.87-1.20) 1.82 (1.48-2.24)* 2.04 (1.65-2.52)* 2.80 (1.89-4.16)* 1.24 (0.98-1.57) 1.09 (1.90-1.31)* 1.39 (1.17-1.66)* 1.71 (1.29-2.27)* ---- 1.00 (reference) 1.52 (1.38-1.67)* 2.01 (1.52-2.66)* 1.21 (1.09-1.36)* 1.51 (1.32-1.72)* 2.32 (1.95-2.77)* 2.95 (2.46-3.55)* 4.34 (2.69-7.02)* 2.45 (1.53-3.95)* 1.00 (0.99-1.00) 1.82 (0.90-3.69) 0.93 (0.31-2.77) ----	**The authors concluded that their results support the hypothesis that major depression, regular tobacco use, and nicotine dependence share a common liability. The genetic correlation between major depression and regular tobacco use or nicotine dependence are modest. The relationship between regular tobacco use and MD is positive and statistically significant for both sexes, and for both types of tobacco use in males.** The authors state that these analyses were not corrected for potentially confounding factors, such as other types of psychopathology, neuroticism, or social-economic status. Nicotine dependence measures did not take into account the fact that many individuals use both cigarettes and snus. Also, the data is cross-sectional; therefore, causality cannot be determined from this study. The authors note that evidence suggests that nicotine can have detrimental effects on neurotransmitter systems and neural integrity, which could in turn have an impact on depressive symptoms. Alternatively, individuals experiencing depressed mood might use nicotine as a form of self-medication, in which case depressive symptoms could lead to tobacco use rather than vice versa.

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** denotes statistically significant decrease in risk

APPENDIX Q-1
DESCRIPTIVE STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION,	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Engstrom et al. 2010 Sweden The aim of this study was to investigate socio-demographic, lifestyle and health characteristics (including self-rated health and psychosocial distress) among snus users.	Cross-sectional study Subjects in this study included a population-based sample of 34,707 men and women aged 18-84 years from the Stockholm Public Health Survey from 2006. Information on a variety of lifestyle factors was collected. Perceived health was self-reported on a 5-points Likert scale, collapsed into three categories: very poor/poor; fair; very good/good. Psychological distress was assessed via the twelve-item version of the General Health Questionnaire (GHQ-12). Current tobacco use was categorized into four mutually exclusive groups - no daily use (including former use), exclusive daily use of snus, exclusive daily smoking or daily dual use (both smoking and snus use). The type of snus used in this study is Swedish snus.	Tobacco Use **Males** **Self-Rated Health** Very Good/Good, No tobacco Fair Snus use Smoking Dual use Very Poor/Poor Snus use Smoking Dual use **Psychosocial Distress** No Distress, No tobacco Snus use Smoking Dual use **Females** **Self-Rated Health** Very Good/Good, No tobacco Fair Snus use Smoking Dual use Very Poor/Poor Snus use Smoking Dual use **Psychosocial Distress** No Distress, No tobacco Snus use Smoking Dual use	Odds Ratios (95% CI) 1.00 (reference) 1.11 (0.98-1.26) 1.69 (1.46-1.95)* 2.16 (1.69-2.77)* 1.11 (0.86-1.42) 2.25 (1.76-2.87)* 2.67 (1.75-4.08)* 1.00 (reference) 0.96 (0.83-1.10) 1.33 (1.12-1.58)* 1.68 (1.28-2.20)* 1.00 (reference) 1.08 (0.86-1.37) 1.74 (1.55-1.95)* 1.44 (0.84-2.48) 1.02 (0.64-1.64) 2.65 (2.22-3.16)* 3.34 (1.66-6.72)* 1.00 (reference) 1.14 (0.91-1.42) 1.54 (1.37-1.73)* 1.63 (1.00-2.66)	**The authors concluded that the social, lifestyle and health profiles of exclusive snus users in Stockholm County are more unfavorable compared to non-users of tobacco, but less so than those of exclusive smokers. However, the authors note that perceived poor general health and psychological distress were not associated with snus use, in contrast with both smoking and dual use.** Odds ratios were adjusted for age, occupational class, disposable income, education, and past smoking and snuff use. Strengths of this study include the large sample size, which allowed the authors to include women in the analysis. However, the non-participation rate was 39%, which may have led to selection bias. Due to the nature of their design, causality cannot be determined from cross-sectional studies since outcome and exposure are measured simultaneously.

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** denotes statistically significant decrease in risk

APPENDIX Q-1
DESCRIPTIVE STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (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>Tobacco Use</u> Snuff, Have quit Snuff, Occasionally Snuff, Daily Smoking, Have quit Smoking, Occasionally Smoking, 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 0.365 (0.016-0.714) *p*=0.040* -0.500 (-1.234-0.235) *p*=0.182) 0.657 (0.136-1.178) *p*=0.014*	**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 outcome and exposure are measured simultaneously. Because tobacco is often used for coping with stress, the authors suggest that it is possible that occasional smokers resorted to using tobacco more to cope with chronic pain and end up grouped daily smokers.

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** denotes statistically significant decrease in risk

APPENDIX Q-1
DESCRIPTIVE STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION,	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Mattila et al. 2008 Finland This study examined the relationship between low back pain and smokeless tobacco use.	Cross-sectional study Subjects were 7,040 male military conscripts who filled out a nationwide questionnaire as part of the Finish Conscript Health Survey. Conscripts were selected randomly from ten garrisons for the years 2002, 2003, 2005 and 2006. The median age of the respondents was 19 (range 18-29) years. The main outcome was lifetime low back pain prompting a visit to a physician by the time the person entered the military service. 908 of the men reported using smokeless tobacco. Smokeless tobacco is not defined in this study.	<u>Tobacco Use</u> **Use of Smokeless Tobacco** No Yes **Daily Smoking** No Yes	<u>Odds Ratios (95% CI)</u> 1.0 (reference) 1.4 (1.2-1.7)* 1.0 (reference) 1.2 (1.1-1.4)*	**The authors concluded that the strongest risk indicators of low back pain were number of diseases (other than back-related) diagnosed by physician during past year, below average self-perceived health and use of smokeless tobacco.** Odds ratios were adjusted for age, tobacco use, perceived health, and disease during the past year. A limitation of this study includes potential recall bias. The authors state that it has been shown that episodes of low back pain are poorly remembered. Another limitation is the cross-sectional design. Causality cannot be determined from cross-sectional studies since outcome and exposure are measured simultaneously.

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

Appendix Q2

Case-Control Studies of Other Health Effects

APPENDIX Q-2
CASE-CONTROL STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (N=2)

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. Cases were recruited via hospital-based neurology units as well as privately run neurology units in Sweden. All cases of MS were examined and diagnosed by a neurologist located at the unit in which the case was entered. For each potential case, 2 controls were randomly selected from the national population register. Information on exposure was collected by questionnaire. The type of snuff used in this population is Swedish snus.	<u>Tobacco Use</u> **Smoking** Never-smoker 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) Never-tobacco Current Snuff users < 5 (n=10) ≥ 5 (n=9) p value for trend Ever smokers (pack-years) Never-tobacco < 5 (n=57) ≥ 5 (n=30) p value for trend > 15y prior to disease onset (See Hedstrom et al. 2009 for additional analyses)	<u>Odds Ratio (95% CI)</u> 1.0 (reference) 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* 1.0 (reference) 0.8 (0.4-1.3) 0.4 (0.01-13) 0.4 (0.01-18) ---- 1.0 (reference) 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 Q-2
CASE-CONTROL STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION,	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Wolk et al. 2009 Stockholm, Sweden This study investigated the relationship between a variety of risk factors, including smoking and smokeless tobacco use and plaque psoriasis	Case-control study (population-based) Cases included 373 patients with first-time onset plaque psoriasis who were recruited from the Stockholm area between January 2001 and January 2006. Controls were matched for sex, age in one year intervals, postal code number and randomly selected from the Swedish Population Registry. Psoriasis activity was measured using the Psoriasis Area and Severity Index (PASI). All patients and controls answered a self-administered questionnaire. With respect to snuff use, participants were asked the following: regular current, occasional current, stopped within the last 12 months, stopped more than 12 months ago, or never snuff use. 15% (5% women, 27% men) of the cases and 16% of controls were snuff users. Snuff is not defined in this study but is assumed to be Swedish snus.	<u>Tobacco Use</u> **Snuff Use** Never snuff use Current snuff use **Smoking** Never-smokers Current smokers	<u>Odds Ratios (95% CI)</u> 1.0 (reference) 1.0 (0.6-1.9) 1.0 (reference) 1.6 (1.0-1.4)	**The authors concluded that smokeless tobacco use was not associated with plaque psoriasis.** Odds ratios were adjusted for age, sex, post code, body mass index, weight gain, alcohol and smoking. A strength of this study is the population-based design, and early inclusion of psoriasis patients from the source population. The response rate was 88%.

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

Appendix Q3

Cohort Studies of Other Health Effects

APPENDIX Q-3
COHORT STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (N=8)

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 all-cause mortality 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, ischemic heart disease and stroke are presented in Appendices G, 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, all causes 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>All-Cause Mortality By Tobacco Usage</u> **Among all subjects** Nonusers Smokeless tobacco (n=440) Smoker (< 15 cig/day) (n=900) Smoker (≥ 15 cig/day) (n=923) **Among ages 35-54 at study entry** Nonusers Smokeless tobacco (n=105) Smoker (< 15 cig/day) (n=317) Smoker (≥ 15 cig/day) (n=437) **Among ages 55-65 at study entry** Nonusers Smokeless tobacco (n=301) Smoker (< 15 cig/day) (n=496) Smoker (≥ 15 cig/day) (n=377)	<u>Relative Risk (95% CI) of death</u> 1.0 (reference) 1.4 (1.3-1.8)* 1.7 (1.6-1.9)* 2.2 (2.0-2.4)* 1.0 (reference) 1.9 (1.6-2.4)* 2.0 (1.7-2.3)* 2.6 (2.3-3.0)* 1.0 (reference) 1.2 (1.0-1.3) 1.6 (1.5-1.8)* 2.0 (1.8-2.2)*	**The authors presented results that show that both smokeless tobacco users and smokers face a higher risk of dying from any cause compared to nonusers of tobacco, although the risk is lower for smokeless tobacco users than for smokers.** 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 Q-3
COHORT STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Brattwall et al. 2010 Sweden This study investigated the potential effects of different tobacco administration routes on pain and post-operative nausea and vomiting (PONV) following three common day surgical procedures.	Cohort study (multi-center) Subjects were 355 hospital patients (134 males and 170 females) scheduled for one of three different elective surgical procedures from autumn 2006 through spring 2008. Patients filled out a questionnaire that included questions about background demographic variables and pre-operative nicotine habits. Trained nurses registered parameters such as pain, PONV and possible side effects in the recovery room and at the time of discharge. Patients also received a questionnaire at discharge. 20% of the patients were smokers and 14% were snuffers. 32% reported smoking or snuff use. "Snuff" is not defined in this study but is assumed to be Swedish snus.	Tobacco use was found to significantly reduce the occurrence of PON during the stay in hospital (21% vs. 34% for non-tobacco users; $P < 0.05$), with approximately equal effects on PON of both snuffing (20% PONV) and smoking (21% PONV). However, in these rather small subgroups of tobacco users, the reduction in vomiting did not reach statistical significance. In all patients, 11% of tobacco users and 16% of nontobacco users vomited on one or more occasions. No significant impact of regular tobacco use on the incidence of post-operative pain was observed.		**The authors concluded that the use of snuff has the same effect in reducing early PONV as smoking. An impact on post-operative pain from smoking or snuffing was not observed.** Though this was a prospective study that investigated the incidence of post-operative symptoms, it has some limitations. The number of patients included with regular tobacco use was not sufficient for further sub-group analysis. The authors were also unable to analyze the impact of individual patient therapy.

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

APPENDIX Q-3
COHORT STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Carlens et al. 2010 Sweden This study investigated the potential relationship between chronic inflammatory diseases (including rheumatoid arthritis (RA), sarcoidosis, and multiple sclerosis (MS)) and the use of "Swedish moist snuff" (snus). See Appendix L-3 for results on ulcerative colitis and Crohn's disease.	Cohort study Subjects were 277,777 male Swedish construction workers who underwent regular health check-ups and had at least one visit from 1978-1993, when information on smoking and snus was obtained through personal interviews with nurses. Subjects were followed until date of diagnosis, death, emigration or December 31, 2004, whichever occurred first. Follow-up was carried out through linkage with nationwide death, emigration, and Swedish Hospital Discharge registries. Adjusted relative risks were derived from Cox proportional hazards regression models. Categories of use included various smoked tobacco as well as snuff use status (never, former, current). Amount of snuff use was also reported (< 22g or ≥ 22g). 13% of the subjects were current or former snus users (among never-smokers). "Snuff" is defined as oral Swedish moist snuff (snus) in this study.	Tobacco Usage **Risk of Rheumatoid Arthritis Among all Cohort Members** Never-users of tobacco Ever-smoker (n=641) Ever-user of snuff (n=168) **Risk of Rheumatoid Arthritis Among Pure or Dual Tobacco Users** Never-users of tobacco Ever-smoker (n=500) Ever-user of snuff (n=27) Ever-smoker/ever-snuff (n=141) **Risk of Sarcoidosis Among all Cohort Members** Never-users of tobacco Ever-smoker (n=135) Ever-user of snuff (n=103) **Risk of Sarcoidosis Among Pure or Dual Tobacco Users** Never-users of tobacco Ever-smoker (n=94) Ever-user of snuff (n=62) Ever-smoker/ever-snuff (n=41)	Relative Risk (95% CI) 1.0 (reference) 2.1 (1.7-2.5)* 1.0 (0.9-1.2) 1.0 (reference) 2.3 (1.9-2.7)* 1.2 (0.8-1.8) 2.0 (1.6-2.6)* 1.0 (reference) 0.5 (0.4-0.5)** 1.1 (0.9-1.4) 1.0 (reference) 0.5 (0.4-0.6)** 1.1 (0.8-1.5) 0.5 (0.4-0.8)**	**The authors concluded that they found no evidence of any significant association between use of moist snuff and the risk for any of these chronic inflammatory diseases with the exception of a borderline increased risk for MS with snuff use among never-smokers.** The authors suggested that inhaled nonnicotinic components of cigarette smoke are more important than nicotine itself in the etiology of these diseases. The authors note that none of the diseases exhibited a dose-response relationship with the amount of snus used (data not shown). Relative risks were adjusted for age, smoking or snus use (among all cohort members), and region of residence. The study population of this cohort was large with prospectively collected data, had a high prevalence of exposure and had high power. However, there were some limitations: Tobacco habits were assessed only at study entry; changes in tobacco habits over time could influence the results. Other exposures among construction workers and selection into the cohort ("healthy worker effect") may limit the generalizability of the results.

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

APPENDIX Q-3
COHORT STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
		Risk of Multiple Sclerosis Among all Cohort Members		
		Never-users of tobacco	1.0 (reference)	
		Ever-smoker (n=150)	1.9 (1.4-2.6)*	
		Ever-user of snuff (n=64)	1.0 (0.8-1.4)	
		Risk of Multiple Sclerosis Among Pure or Dual Tobacco Users		
		Never-users of tobacco	1.0 (reference)	
		Ever-smoker (n=113)	2.5 (1.7-3.6)*	
		Ever-user of snuff (n=27)	1.8 (1.1-2.9)*	
		Ever-smoker/ever-snuff (n=37)	1.9 (1.2-3.1)*	

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

APPENDIX Q-3
COHORT STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (continued)

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>Tobacco usage</u> Non-tobacco use Pure snuff dipping Mixed snuff dipping/smoking Pure smoking	<u>Relative Risk (95% CI)</u> 1.00 (reference) 0.6 (0.3-1.5) 0.9 (0.6-1.4) 0.7 (0.5-1.1)	**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; 69 among pure smokers).

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

APPENDIX Q-3
COHORT STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Heir and Eide 1997 Norway This study examined the association between snuff use and proneness to musculoskeletal injury during training.	Prospective study Subjects were 480 male military conscripts who participated in a 10-week basic training. Prior to the training, data on past activity, and demographic and lifestyle characteristics were collected via questionnaire. Injuries were registered as they occurred by doctors attached to the training camp. An injury was defined as pain, inflammation or functional disorder which (a) involved the musculoskeletal or soft tissues; (b) was serious enough for the conscript to seek and obtain a medical consultation; and (c) could have occurred entirely or in part as a consequence of an external trauma or strain sustained during training. 15% of subjects were snuff users. Snuff is defined as "moist snuff" in this study and is assumed to be Swedish snus.	<u>Univariate</u> **Snuff** No Yes **Smoking** 0 1-10 > 10 <u>Multivariate</u> **Snuff** No Yes	<u>Relative Risk (95% CI)</u> 1.00 (0.76-1.31) 1.75 (1.18-2.58)* 1.00 (0.70-1.43) 1.07 (0.68-1.68) 1.53 (1.06-2.21)* <u>Odds Ratio (95% CI)</u> 1.00 (reference) 2.31 (1.34-3.99)*	**The authors concluded that snuff-users had, when adjustments were made for other risk factors, a twofold increase in the risk of injury.** The authors note that causality could hypothetically be attributed to a number of different conditions, such as the inhibiting effect of nicotine on neuromuscular transmission, reduced regional blood flow to the working tissues, dysregulation of the microcirculation, changes in metabolism, or the acting of free radicals on vulnerable tissue. The adjusted risk of injury among snuff users was controlled for age and fitness.

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

APPENDIX Q-3
COHORT STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Lindström et al. 2007 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. Snus is defined as Swedish oral moist snuff in this study.	Snus Use **Any Complication** Never user of snus Ever user of snus **Mean Length of Hospital Stay** Never user of snus Ever user of snus Smoking **Any Complication** Never smoking Current smoking **Mean Length of Hospital Stay** Never smoking Current smoking	Odds Ratio (95% CI) 1.00 (reference) 0.93 (0.71-1.22) Coefficient (95% CI) ---- (reference) 0.02 (0.00-0.04) *p=0.15* Odds Ratio (95% CI) 1.00 (reference) 1.34 (1.04-1.72)* Coefficient (95% CI) ---- (reference) 0.01 (-0.01-0.03) *p=0.42*	**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-3
COHORT STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
Nordenvall et al. 2012 Sweden This study aimed to investigate the relationship between tobacco use and death from all-causes and those other than cancer among individuals diagnosed with cancer. [Subjects were selected from the same overall study population as Bolinder et al. 1992 and Bolinder et al. 1994] Results on all-cause mortality and death from causes other than cancer (among those diagnosed with cancer) are presented in Appendix H.	Cohort study Subjects were 336,381 male Swedish construction workers 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, 2007, whichever occurred first. Cancer-specific death had to be the same as the first cancer diagnosis (i.e. the cause of death was cancer at the same site as the primary cancer). 40,230 new first cancers were diagnosed during follow up (who did not die the same day as diagnosis). Of these cases, 14,533 died from the primary cancer and 9,716 died from other causes. Cause of death was ascertained through the year 2007 by record linkage with nationwide cancer and death registries. Survival was investigated among never-smoking snus users, never-snus-using smokers, and combined users (currently or in sequence). Snus is defined as Scandinavian moist snuff (snus) in this study.	<u>Among Those Diagnosed with Cancer</u> **Overall Death** Never-users of any tobacco Ever tobacco users Pure snus users Pure smokers Combined users **Death From Causes Other Than Cancer** Never-users of any tobacco Ever tobacco users Pure snus users Pure smokers Combined users <u>Among Those Diagnosed with Smoking-Related Cancer</u> **Overall Death** Never-users of any tobacco Ever tobacco users Pure snus users Pure smokers Combined users **Death From Causes Other Than Cancer** Never-users of any tobacco Ever tobacco users Pure snus users Pure smokers Combined users	<u>Hazard ratio (95% CI)</u> 1.00 (reference) 1.19 (1.15-1.23)* 1.13 (1.05-1.20)* 1.21 (1.17-1.25)* 1.17 (1.12-1.22)* 1.00 (reference) 1.25 (1.19-1.32)* 1.12 (1.01-1.25)* 1.26 (1.19-1.32)* 1.29 (1.21-1.38)* 1.00 (reference) 1.19 (1.14-1.23)* 1.15 (1.06-1.24)* 1.20 (1.15-1.25)* 1.15 (1.09-1.21)* 1.00 (reference) 1.24 (1.17-1.32)* 1.11 (0.98-1.26) 1.25 (1.17-1.33)* 1.27 (1.18-1.37)*	**The authors concluded that smokers had an increased risk of deaths that were not cancer-specific, which was not as evident for exclusive snus users.** The authors further stated that their data provide little guidance in regard to the mechanisms, which warrant further investigations, but suggest that their results concerning snus users seem to narrow in on nicotine as a conceivable culprit. Hazard ratios were adjusted for age at cancer diagnosis, calendar period of diagnosis, cancer site, and BMI at entry. This study has several strengths. It was a large, prospective study with almost complete follow-up and accurate identification of outcomes using national registers. The authors also state that confounding due to occupation or socioeconomic status was unlikely given the homogeneous population of construction workers. A limitation of this study is that 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 also state that the results may not be generalizable to women, and a healthy worker effect cannot be disregarded. In an earlier study of the same population (Nordenvall et al. 2010), the authors noted that there were no data on other possible confounders such as diet, alcohol intake and physical activity.

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

APPENDIX Q-3
COHORT STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (continued)

CITATION, LOCATION	STUDY TYPE, POPULATION	SNUS USE	MEASURE OF EFFECT	AUTHORS' CONCLUSIONS REGARDING SNUFF USE AND COMMENTS
		Overall Death by Comorbidity Among Those Diagnosed with Cancer	Hazard ratio (95% CI)	
		No Comorbidity		
		Never-users of any tobacco	1.00 (reference)	
		Ever tobacco users	1.22 (1.10-1.36)*	
		Pure snus users	1.10 (0.86-1.40)	
		Pure smokers	1.24 (1.11-1.38)*	
		Combined users	1.20 (1.04-1.40)*	
		Chronic Pulmonary / Cerebrovascular Disease / Myocardial Infarction		
		Never-users of any tobacco	1.00 (reference)	
		Ever tobacco users	1.13 (1.04-1.23)*	
		Pure snus users	1.08 (0.89-1.29)	
		Pure smokers	1.16 (1.06-1.26)*	
		Combined users	1.07 (0.96-1.20)	
		Other Comorbidity		
		Never-users of any tobacco	1.00 (reference)	
		Ever tobacco users	1.17 (1.12-1.23)*	
		Pure snus users	1.15 (1.04-1.27)*	
		Pure smokers	1.19 (1.13-1.25)*	
		Combined users	1.12 (1.05-1.19)*	

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

APPENDIX Q-3
COHORT STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (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 all-cause mortality and respiratory death. Results on smoke-related cancers and any cancer are presented in Appendix H, head and neck cancers in Appendix C, and cardiovascular diseases in Appendix J-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 9,976 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. Snus is defined as Scandinavian moist snuff in this study.	Tobacco Usage **All-Cause Mortality** Snus use Never daily use Ever daily use (n=641) Restricted to never smokers Snus use Never daily use Ever daily use Smoking Never daily use Age < 75 Age 75+ **Respiratory Death** Snus use, age < 80 Never daily use Ever daily use Among never-smokers Snus use, age 80+ Never daily use Ever daily use Among never-smokers Smoking Never daily use Ever daily use	Hazard Ratio (95% CI) 1.00 (reference) 1.10 (1.01-1.21)* 1.00 (reference) 1.23 (1.09-1.40)* 1.00 (reference) 1.63 (1.45-1.83)* 1.26 (1.15-1.38)* 1.0 (reference) 0.8 (0.4-1.6) 0.8 (0.2-3.0) 1.0 (reference) 1.8 (1.2-2.7)* 2.0 (1.2-3.4)* 1.0 (reference) 1.7 (1.2-2.3)*	**The authors concluded that snus use was associated with a statistically significant, albeit small, risk elevation for death of any cause. A statistically significant excess risk of respiratory death among snus users was noted in the older stratum but not in the younger.** 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 Q-3
COHORT STUDIES OF OTHER HEALTH EFFECTS AMONG SWEDISH SNUS USERS (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 concluded that the use of snuff does not have the negative effects-such as delayed bone healing 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 VI

Relative Risk among Snus Users and Smokers

Compared to Nontobacco Users

Appendix (VI) to Chapter 5: Human Health Effects of Snus: Relative Risks among Snus Users and Smokers Compared to Nontobacco Users

This appendix summarizes the available data on the health risks associated with the use of Swedish snus compared to those from smoking. The available evidence was analyzed using three distinct sets of data:

1. Relative risk estimates from epidemiology studies of Swedish tobacco use in which estimates for snus and for smoking, each compared to a reference population (usually nontobacco users), are presented. The results of these studies collectively provide evidence of lower, and often no, increased risks of smoking-related health endpoints from use of Swedish snus compared to the substantially increased risks from smoking.

2. Relative risk estimates from meta-analyses and large cohort studies of Swedish snus users and cigarette smokers, in which risk estimates were used for two purposes: additional presentation of relative risks for snus and for cigarettes, and to provide context to the data from the individual epidemiology studies. Though the summary relative risks and risk estimates from large cohorts are not as directly comparable to each other or as relevant as the first dataset, these data largely support the findings in the individual studies.

3. Attributable risk data of smoking mortality in the United States was used to estimate potential excess mortality among smokers compared to users of Swedish snus. The health outcomes included in this analysis, combined with nonmalignant respiratory diseases known to be caused by smoking, account for nearly all, approximately 90%, of smoking-related deaths. For these same mortality causes, Swedish snus presents no apparent risk or at least a very large reduction in risk.

The adverse health outcomes causally related to smoking were first confirmed in the 1960s, and have been well studied since that time (USDHHS Surgeon General 2010). These include lung and other cancers, noncancer pulmonary outcomes, such as emphysema and chronic obstructive pulmonary disease (COPD), cardiovascular diseases, and reproductive and developmental effects. The estimated disease mortality burden that smoking poses in the US has been quantified using relative risk estimates from the American Cancer Society's Cancer Prevention Study II (CPS-II) data, and is presented in (Table A VI-A) (CDC 2008), ranked by the highest number of deaths among smokers attributed to that health outcome. More recently, the Food and Drug Administration revised the estimates of smoking-attributable mortality using updated relative risks based on National Health Interview Survey data (Rostron 2012). In the updated analysis, the estimated attributable fractions of smoking-related deaths were very similar to those presented in the CDC (2008) analysis (see Table A VI-B). There were, however, fewer disease-specific categories; therefore, the original CDC (2008) estimates were used in the following analysis for all outcomes of interest except lung cancer, ischemic heart disease (IHD), other heart disease and stroke, for which the updated Rostron (2012) estimates were able to be used.

Table A VI-A: Estimated Number of Outcome-Specific Deaths and Attributable Fraction among All Smokers, 2000-2004			
Rank (by # of deaths)	**Outcome**	**Smoking Deaths**	**Attributable Fraction***
1	**Lung Cancer**	125,522	32.0%
2	**IHD**	80,005	20.4%
3	COPD	78,988	20.1%
4	**Other heart disease**	21,004	5.3%
5	**Stroke**	15,922	4.1%
6	Bronchitis, Emphysema	13,927	3.5%
7	Pneumonia, influenza	10,423	2.7%
8	**Esophageal Cancer**	8,592	2.2%
9	**Aortic Aneurysm**	8,419	2.1%
10	**Pancreatic Cancer**	6,683	1.7%
11	Urinary Bladder Cancer	4,983	1.3%
12	**Oral Cancer**	4,893	1.2%
13	Kidney Cancer	3,043	0.8%
14	Laryngeal Cancer	3,009	0.8%
15	**Stomach Cancer**	2,484	0.6%
16	**Atherosclerosis**	1,893	0.5%
17	Other circulatory disease	1,254	0.3%
18	AML	1,192	0.3%
19	Cervical Cancer	447	0.1%
*Among a total estimate of 392,683 smoking-related deaths (males and females combined) **Bolded** outcomes were those analyzed in this appendix Reference: CDC 2008 (Based on CPS-II data)			

Table A VI-B: Estimated Number of Outcome-Specific Deaths and Attributable Fraction (AF) among All Smokers, 2004			
Rank (by # of deaths)	**Outcome**	**Smoking Deaths**	**Attributable Fraction***
1	Lung Cancer	118,950	31.5%
2	COPD	91,045	24.1%
3	IHD	88,525	23.4%
4	Other heart disease	16,113	4.3%
5	Stroke	14,692	3.9%
6	Pneumonia, influenza	10,444	2.8%
*Among a total estimate of 377,521 smoking-related deaths (males and females combined) Reference: Rostron (FDA) 2012 (Based on NHIS data)			

In the following analysis, the relative risks for smoking-related adverse health outcomes are compared among smokers and among Swedish snus users, in the epidemiological studies that provide both of these estimates in a common study population, relative to nontobacco users in

the study population. The health outcomes examined were those with the highest number of deaths attributable to smoking, as well as several additional health outcomes, as provided in the epidemiological studies.

Based on the results presented in this appendix, it is clear that the use of snus presents a much lower risk, if any risk at all, of the smoking-related diseases that lead to the highest number of deaths among smokers (lung cancer, CVD, stroke). The health outcomes included in this analysis, combined with nonmalignant respiratory diseases known to be caused by smoking, account for nearly all, approximately 90%, of all smoking-related deaths. Use of Swedish snus as an alternative to smoking presents no risk of respiratory disease (COPD, bronchitis and emphysema, and pneumonia and influenza) or lung cancer, and at least, a very large reduction in risk from CVD.

Methodology: Study and Relative Risk Estimate Selection

Using the pool of epidemiological studies of potential health risks among snus users identified in the literature search methods described in the Introduction to this report, relative risk estimates were extracted from studies that provided these estimates for snus users and for cigarette smokers within the same study population. The overall relative risk estimates, and those from subanalyses such as age- or dose (exposure)-groups, were plotted to compare pictorially the relative risks for tobacco-related disease among users of these two tobacco types (Figures A VI-1 – 10). A summary document for each of the health outcomes that lists the selected relative risk estimates, as well as other relevant study details for the epidemiological studies included in this analysis, is provided in Tables A VI-1 – 10.

The usual (and desirable) comparison group for each tobacco group was lifelong nontobacco users, though this was not always provided in each study analysis. Relative risk estimates that were not controlled for tobacco use in other categories (nonuse, smoking, or snus use), either by stratification or using other statistical methods, are also listed and described in Tables A VI-1 – 10, but are not used in the Figures. Relative risk estimates that are stratified by or adjusted for current tobacco use only, which may not account for past tobacco use, are included in the forest plots. When multiple relative risk estimates were available to compare, the following is the order of preference used to select the most valid comparisons:

- Preference was given to relative risk estimates in which a common reference group (e.g. never-users of tobacco) and common exposure groups (e.g. ever smokers vs. ever snus users) was provided;
- Preference was given to relative risk estimates in which the exposures were defined similarly, such as ever users or current users, over other subanalyses (or whichever exposure groups were presented commonly for both smokers and snus users);
- Relative risk estimates from multivariate analyses in which potential confounders were included in the model were selected over relative risk estimates from univariate analyses, where possible (after the above priorities were met); and
- In dose/response analyses, the relative risk estimate for the highest tobacco use group was selected (for both snus and cigarette users).

This order of selection was followed if the preferred relative risk estimates were available; however, there were instances where more than one relative risk estimate was included (e.g. multivariate in addition to relative risk estimates stratified by tobacco use, dose and/or duration groups, gender, mortality and incidence, age groups, etc.). In situations where the preferred analyses were carried out for only one tobacco type, i.e., smoking but not for snus, relative risk estimates meeting the lower-priority criteria were selected for the plot so that the health risks of snus and cigarettes were more comparable. Relative risk estimates selected for each health outcome include both morbidity and mortality endpoints.

In addition to relative risks from the individual epidemiology studies, the summary data in the Tables and in the Figures for each health outcome also include results of recently-published meta-analyses or large cohorts identified using the literature search methods described earlier in this report. The results from the meta-analyses of snus studies may be overlapping (i.e., were meta-analyses conducted on the same or similar set of studies). Boffetta et al. (2008) conducted a meta-analysis for four cancer outcomes (esophageal, lung, oral and pancreatic). Boffetta and Straif (2009) conducted a meta-analysis of cardiovascular disease (CVD) and stroke. Lee and Hamling (2009) also published a meta-analysis of cancer outcomes. These were also presented by Lee (2011), which additionally presented summary relative risk estimates for CVD and stroke. Lee (2007) presented a summary relative risk specifically for ischemic heart disease (IHD), myocardial infarction (MI) and stroke, which other meta-analyses did not present; however, additional epidemiological studies of these outcomes were published subsequent to Lee (2007) (e.g., Hansson et al. 2009).

For smoking-related morbidity and mortality, several targeted literature searches were conducted using methods similar to those described earlier. Three meta-analyses for specific outcomes (diabetes, pancreatic cancer, and stroke) among smokers compared to nonsmokers were identified. Additionally, we identified, and included on the plots, relative risk estimates for the specific health outcomes among smokers compared to nonsmokers from three large US cohorts and one large, international case control study. These include CPS-II (SAMMEC and Surgeon General 1989), the Kaiser Permanente cohort (Friedman et al. 1997), the U.S. Veterans cohort (McLaughlin et al. 1995) and the large INTERHEART case-control study of myocardial infarction (Teo et al. 2006).

Forest plots of relative risk estimates for each of nine health outcomes are presented in Figures A VI-1 – 10, and study summaries are presented in Tables A VI-1 – 10.

Results

Below are the results for each of the health outcomes that were analyzed for snus users and cigarette smokers. Health outcomes that represent the highest attributable fraction of smoking-related deaths are presented first, followed by health outcomes that provide a smaller fraction, or for which no smoking-related attributable risk estimates were identified (e.g., diabetes).

For each health outcome, the summary of results presents a brief description of the literature (e.g., the number and type of studies available), a summary of the relative risk estimates as selected for and presented in the Figures (given the potential limitations in the available data, as discussed), a discussion of the study qualities, and an overall conclusion for that outcome.

Lung Cancer (Table A VI-1, Figure A VI-1)

- Two cohort studies reported risk estimates for both snus users and smokers in the same population.
- None of the relative risks from the individual studies or the summary estimates (from two meta-analyses) were significantly increased among snus users. Almost all of the point estimates were below 1.0, and one study reported a significantly reduced risk of lung cancer among snus users. As expected, the risk estimates among smokers were all significantly increased, with risk estimates ranging from 7.2 to 30.6 in the individual studies and from 8.1 to 22.3 among the large US cohorts.
- The two available studies of Swedish snus users and smokers used a common reference group and comparable exposure groups for smokers and snus users.
- The results indicate that snus users are at no more risk of developing lung cancer than non- or never-users of tobacco, while smokers are 7 to 30 times more likely to develop lung cancer based on two studies of the large Swedish Construction Worker cohort (Bolinder et al. 1994; Luo et al. 2007).

Cardiovascular Disease (CVD): (ischemic heart disease (IHD), coronary heart disease (CHD), myocardial infarction (MI) and Overall CVD) (Table A VI-2, Figure A VI-2)

- Six cohort, four case-control, and one cross-sectional study reported relative risk estimates for both snus users and smokers in the same population. Only Janzon and Hedblad (2009) was excluded because this study did not provide a smoking relative risk estimate that was adjusted or controlled to exclude the potential effects of snus use. Additionally, the cross-sectional study conducted by Bolinder et al. (1992) was not included in the plot as the Bolinder et al. (1994) study presented a prospective analysis of this same cohort.
- Among snus users, relative risk estimates included in this analysis were not significantly increased for the individual studies, with the exception of increased risks of IHD and overall CVD observed in the Construction Workers cohort study reported by Bolinder et al. (1994). Pooled risk estimates from meta- and pooled-analyses combining studies of snus users were generally consistent, showing no increased risk, except for a small, statistically significant increase in fatal MI reported by Boffetta and Straif (2009). The more recent meta-analysis (Lee 2011) and pooled analysis (Hansson et al. 2012) found no increased summary risk of any or fatal MI. Among smokers, all but one point estimate (Huhtasaari et al. 1992: ≤10 cigarettes/day), were significantly increased, which is consistent with the risk estimates from the large US CPS-II cohort and a case-control study of 52 countries (Teo et al. 2006). Most of the relative risk estimates for CVD among Swedish smokers extracted from the individual studies generally ranged from 1.5 to 3.6.
- Only Roosaar and colleagues (2008) did not use a common reference group for smokers and snus users. Control for confounders varied by study, but generally included several important risk factors for cardiovascular disease. Outcome definitions varied from study to study, though most include MI or IHD and include similar International Classification of Disease (ICD) code definitions, though the Bolinder et al. (1994) and Roosaar et al. (2008) studies included a broader spectrum of cardiovascular events not included in the other

studies. Three of the studies used in the forest plots compared risk estimates based on different exposure groups (Bolinder et al. 1994; Roosaar et al. 2008; Johansson et al. 2005).

- Overall, the results indicate that, consistent with what is known about smoking and CVD risk, the observed increased risk is generally 1.5 to 3 times that observed among nontobacco users. Overall, CVD risk was not increased among snus users. In particular, the study conducted by Hansson et al. (2009) among over 16,000 participants within the Swedish Twin Registry, provided convincing evidence that snus use (at any intensity), is not significantly associated with an increased risk of overall CVD or IHD, while an increased risk among smokers was observed as expected. Furthermore, this study controlled for important potential confounders, such as age, sex, diabetes, blood pressure, and cholesterol levels, while tobacco use categories included exclusive snus users or smokers. Similar exposure groups also allowed for a valid comparison of the risks of snus users and smokers. Note that the Lee (2007) summary risk estimate for IHD did not include Hansson et al. (2009) as it was not published at the time. There are known differences in exposures among snus users that may account for the observed difference in risk of CVD between snus users and cigarette smokers. Though snus users and smokers are both exposed to nicotine, which has known acute effects on the cardiovascular system, cigarette smokers are also exposed to other cardiovascular toxicants including carbon monoxide and fine particulate matter. Pope et al. (2009) concluded that relatively low levels of fine particulate exposure from secondhand cigarette smoke are sufficient to induce adverse biological responses increasing the risk of cardiovascular disease mortality.

Stroke (Table A VI-3, Figure A VI-3)

- Two case-control and four cohort studies reported relative risk estimates for stroke among both snus users and smokers in the same population. One case-control (Koskinen and Blomstedt 2006) and one cohort study (Janzon and Hedblad 2009) were excluded from the forest plots because the authors did not control for tobacco use among either snus users, smokers, or both.
- Among snus users, risk estimates from the individual studies and summary estimates from meta-analyses were not significantly increased. Among smokers, risk estimates from most of the individual studies were significantly increased and where increased, generally ranged from 1.4 to 3.0. Meta-analyses and large US cohorts were generally consistent with the results from the individual studies.
- Among the individual studies, all used a common reference group for smokers and snus users, and only one study (Bolinder et al. 1994) did not use comparable exposure groups. Outcome definitions for stroke were also similar among the studies. Three of the four studies controlled for high blood pressure or hypertension in the analysis, an important risk factor for stroke.
- Overall, the stroke risk among snuff users appears to be no different than that of non-users of tobacco, while the risk is consistently increased, at least 40% greater, among smokers compared to non-users of tobacco. Of the four studies, which included two cohort studies of the Swedish Construction Workers and Swedish Twin Registry, none reported any significantly increased risks of stroke among snus users, while all four reported a significantly increased risk among smokers. Hansson et al. (2009) (Swedish Twin Registry),

in particular, provided relative risks that were adjusted for high blood pressure, a major potential confounder.

Respiratory Disease

- Nonmalignant respiratory disease is a major cause of smoking-related death. These diseases include chronic obstructive pulmonary disease (COPD), bronchitis, emphysema, pneumonia, and influenza, which account for 103,338 (26.3%) smoking-related deaths annually (CDC 2008).
- Though no studies are available that investigated the relationship between the use of snus and any of these nonmalignant respiratory diseases, one study did investigate the effects of snus use and smoking on respiratory death in general. Roosaar and colleagues (2008) reported a significantly increased risk of respiratory death among smokers (RR = 1.7; 95% CI: 1.2-2.3) and snus users over the age of 80 years (RR = 1.8; 95% CI: 1.2-2.7). No increased risk of respiratory death among snus users was observed among those younger than age 80 (RR = 0.8; 95% CI: 0.4-1.6).
- Because there is no known mechanism by which snus could cause respiratory disease, the significant excess risk of respiratory death among those over age 80 could be due to confounding by other factors or to exposure misclassification. The SCENIHR working group stated that "there is no consistent evidence that any smokeless tobacco products cause any of these major respiratory diseases. Complete substitution of smokeless tobacco products for tobacco smoking would thus ultimately prevent nearly all deaths from respiratory disease currently caused by smoking (SCENIHR 2008)."

Esophageal Cancer (Table A VI-4, Figure A VI-4)

- Two case-control studies and one cohort study reported risk estimates for both snus users and smokers in the same population.
- Among snus users, two studies did not observe an association between snus use and esophageal cancer risk while one study reported a significant excess for one esophageal cancer subtype, squamous-cell carcinoma (Zendehdel et al. 2008); (RR = 3.5; 95% CI: 1.6-7.6). Esophageal cancer risks were nearly universally increased for smokers in these studies, with the exception of adenocarcinoma among current smokers and high intensity smokers in the Lagergren et al. (2000) study. Point estimates for esophageal cancer risk among smokers ranged from 1.6 to 2.9 for adenocarcinoma and 7.6 to 9.3 for squamous cell carcinoma. Lewin et al. (2000) reported a relative risk estimate of 5.2 for smokers for all subtypes of esophageal cancer combined.
- Two meta-analyses of snuff users are consistent with the overall results for esophageal cancer among Swedish snus users: Lee and Hamling (2009) and Lee (2011) reported no significant increase of esophageal cancer for all subtypes combined. In the only study that examined risk among never smokers, the relative risk estimate for esophageal cancer was borderline significant (RR = 1.92; 95% CI: 1.0-3.68; Zendehdel et al. 2008). Boffetta and colleagues (2008) reported a significantly increased summary estimate because they used the higher, squamous cell risk estimates from Lagergren et al. (2000) and Zendehdel et al. (2008) to combine further with the risk estimate for any subtype of esophageal cancer from Lewin et al. (1998). By comparison to snus, relative risks for esophageal cancer among

smokers from the large cohorts were all significantly increased, and were generally consistent with relative risk estimates from the individual studies, ranging from 3.3 to 10.3 among current smokers.

- Only Zendehdel et al. (2008) used common reference groups for snus and smoking risk estimates (never-users of any tobacco). Lewin et al. (1998) reported the risk only for combined subtypes. The number of cases among snus users in the Zendehdel et al. (2008) study was small, especially for adenocarcinoma, and was the only study that did not control for potential confounding from alcohol.
- Overall, three of the four studies of esophageal cancer did not find an increased risk among snus users. One study did find an increased risk but did not control for alcohol (Zendehdel et al. 2008), and a dose-response was suggested in another study (Lagergren et al. 2000). Even if the finding of Boffetta et al. (2008) of a summary relative risk estimate among snus users of 1.6 is real, compared to the risk of esophageal cancer among smokers, the increased risk among snus users would be at least several fold lower compared to that among current smokers.

Pancreatic Cancer (Table A VI-5, Figure A VI-5)

- Three cohort studies reported risk estimates for both snus users and smokers, however, only the Luo et al. (2007) study was included in this analysis due to limitations in the analyses of the additional two studies. Boffetta et al. (2005) did not provide an analysis among smokers that accounted for snus use, and Heuch et al. (1983), which was updated by the Boffetta et al. (2005) analysis, was excluded because the authors did not include confidence intervals in the multivariate analysis, and the tobacco type used among participants of the study is unclear.
- In the one study that was available, and the one analysis within that study that allowed for a comparison of risks between snus users and smokers, the risk of pancreatic cancer among ever-users of snus (adjusted for smoking) was similar to never-users of any tobacco, while the risk among smokers (adjusted for snus use) was significantly increased (RR = 2.8; 95% CI: 2.1-3.7). While the authors also reported the relative risk of pancreatic cancer among never-smoking snus users, they did not do a comparable analysis among smokers (among never-users of snus). The risk estimates used in the forest plot include common reference and exposure groups.
- Consistent with the known association between smoking and risk of pancreatic cancer, the relative risks of pancreatic cancer among smokers from the large US cohorts were elevated, and generally ranged from 1.4 to 2. Most of the point estimates from meta-analyses of snus users generally hovered around 1.0 with a few significant excesses observed, depending on the risk estimate selection criteria employed by the authors. For example, Boffetta et al. (2008) selected the higher relative risks from the Boffetta et al. (2005) and Luo et al. (2007) studies (smoking-adjusted from Boffetta et al. and the relative risk among never-smokers from Luo et al. 2007), while Lee (2011) combined similar analyses and presented the smoking-adjusted and never-smoking summary estimates separately.
- Though uncertainties and inconsistencies exist as to whether the risk of pancreatic cancer among snus users is increased, pancreatic cancer is consistently increased among smokers, as reported in multiple studies and meta-analyses (Bertuccio et al. 2011; Boffetta

et al. 2008; Friedman et al. 1997; Iodice et al. 2008; Lee et al. 2011; McLaughlin et al. 1995; Sponsiello-Wang et al. 2008; US Surgeon General 1989). A recent pooled-analysis of studies of cigarette and Western population smokeless tobacco users (though likely not snus) from 11 international case-control studies, performed by Bertuccio and colleagues (2011), also reported an increased risk of pancreatic cancer among smokers (RR=1.5, 95% CI: 1.4-1.6), but found no increased risk of pancreatic cancer among smokeless tobacco users (RR = 0.62, 95% CI: 0.37-1.04). Though not specific to snus, this finding for smokeless tobacco generally suggests that it is unlikely that Swedish snus poses a risk for pancreatic cancer given that the smokeless tobacco used by participants in these studies likely contained higher levels of TSNAs compared to Swedish snus, the principal component of tobacco thought to be associated with the development of pancreatic cancer (Boffetta et al. 2008).

Oral Cancer (Table A VI-6, Figure A VI-6)

- Three case-control studies and two cohort studies reported risk estimates for both Swedish snus users and smokers in the same population. One case-control study was excluded from the forest plot because it is unclear whether the smoking estimates were adjusted for snuff use (Rosenquist et al. 2005).
- Among snus users, risk estimates from individual studies and summary estimates from meta-analyses were not significantly increased, with only one significant excess observed in a single study. In this study, the increased risk was observed among ever-users of snus (adjusted for smoking), though this excess disappeared when analyzed among never-smokers (Roosaar et al. 2008). Among current or ever smokers, significantly increased risk of oral cancer was observed in most studies, ranging from 1.7 to 4.9, and all of the relative risk estimates from the large US cohorts were significantly increased (ranging from 2.6 to 27).
- Most of the studies controlled for alcohol consumption, a known risk factor for oral cancer, except for Luo et al. (2007). Luo et al. (2007) was also the only study that used a common reference group for smokers and snus users. Most of the studies included in this analysis used comparable exposure groups, except for Roosaar et al. (2008), who stratified smokers by age, but did not provide a similar analysis for snus users. In addition, the ICD codes included in the definition of oral cancer varied by study.
- Overall, relative risks for snus users do not suggest a relationship between snus and oral cancer and indicate that snus users are at no more risk of developing oral cancer than non- or never-users of tobacco. This conclusion is based on evidence from various Swedish populations in four different case-control and cohort studies (including the Swedish Construction Worker cohort; the only cohort not adjusted for alcohol consumption). In these same studies plus large US cohorts, risk of oral cancer morbidity and mortality is consistently increased among the smokers, with risk estimates ranging from 1.7 to 27 (Lewin et al. 1998; Luo et al. 2007; McLaughlin et al. 1995;Roosaar et al. 2008; Schildt et al. 1998; US Surgeon General 1989).

Stomach Cancer (Table A VI-7, Figure A VI-7)

- Three case-control studies and one cohort study reported risk estimates for both snus users and smokers in the same population.
- Among snus users, risk estimates from the individual studies and summary estimates from meta-analyses were not increased, with the one exception of a significant excess observed for the noncardia stomach cancer subtype (RR = 1.4; 95% CI: 1.1-1.9) (Zendehdel et al. 2008). Among smokers, almost all of the risk estimates among the individual studies were significantly increased (ranging from 1.4 to 2.3). Summary estimates from meta-analyses and relative risks from large US cohorts were consistent with the results from the individual studies among snus users and smokers, respectively.
- As described in Table A VI-7, which provides details for the individual studies, the comparability among studies was somewhat limited. The type of stomach cancers included in the four studies differed. Two of the four studies used common reference groups (Zendehdel et al. 2008; Ye et al. 1999), and only one study used comparable exposure groups (Ye et al. 1999).
- Overall, the risk of stomach cancer among smokers was clearly increased, while the evidence consistently suggests that the risk of stomach cancer among snus users appears to be no different than non-users of tobacco (Lee and Hamling 2009; Lee 2011).

Diabetes (Table A VI-8, Figure A VI-8)

- Two cross-sectional studies, a third cross-sectional study with follow-up, and two cohort studies reported risk estimates for diabetes among snus users and smokers in the same population. One cohort study was excluded from the forest plot because it was unclear if other forms of tobacco use were controlled for among snus users and smokers (Hilding et al. 2005), however the same study population was examined in a more recent study (Ostenson et al. 2012).
- Of the four studies included in the forest plot, one cross-sectional study reported a significantly increased prevalence of diabetes among current exclusive snus users (Persson et al. 2000), and Ostenson et al. (2012) reported a significant association between high consumption (>5 boxes/week) and type 2 diabetes, but not among consistent snus users adjusted for smoking, or consistent exclusive snus use. Though this study adjusts for most of the important potential confounders with the exception of any dietary variables, it presents many limitations (described in greater detail in Appendix V – M4), including the exclusion of diabetes cases discovered during follow-up, which may have differed with respect to tobacco use characteristics compared to cases ascertained at the final follow-up point. For smokers, the cross-sectional study with follow-up (Eliasson et al. 2004) was the only study that reported an increased prevalence and incidence of diabetes (consistent with the meta-analysis among smokers by Willi et al. 2007, see below).
- Though few of the important potential confounders were accounted for, only Eliasson and colleagues (2004) reported risk estimates using a common reference group (never-users of tobacco) for snus users and smokers, however, all four studies reported risk estimates using comparably-defined tobacco exposure groups. Confidence intervals were imprecise for

many of the risk estimates among snus users and smokers, due to the small number of cases.

- No published meta-analyses that presented pooled estimates of diabetes risk among snus users were identified; however, a meta-analysis of smoking and diabetes was available, and reported a significantly increased risk of incident diabetes among active smokers (RR = 1.44; 95% CI: 1.31-1.58) (Willi et al. 2007). A US cohort study did not observed an increased risk of mortality due to diabetes among smokers (Friedman et al. 1997); the risk estimates had imprecise confidence intervals, due to few observed cases (only three cases among women and one case among men were observed, and may be due to difficulties with identifying diabetes as a cause of death on death certificates (McEwen et al. 2006)).
- Overall, it is unclear whether the risk of diabetes among snus users is different from those who do not use tobacco, though the only prospective analysis of the four studies that examined all incident cases of diabetes, conducted by Eliasson et al. (2004), observed no incident cases of diabetes among consistent exclusive snus users and an increased risk of diabetes among exclusive smokers who participated in the Northern Sweden MONICA cohort. A clear association between diabetes and smoking was also observed in a meta-analysis (Willi et al. 2007).

Metabolic Syndrome (Table A VI-9, Figure A VI-9)

- Two cross-sectional analyses reported risk estimates for both Swedish snus users and smokers in the same population. An additional cohort study (Norberg et al. 2006) was excluded from the forest plot because it appears that the authors did not control for tobacco use among snus users and smokers.
- None of the risk estimates among current snus users were significantly increased while a significant increase was observed for former smokers only.
- It is unclear which reference groups were used in the two studies, though both use comparable exposure groups to determine risk of metabolic syndrome among snus users and smokers. Both studies used the International Diabetes Federation (IDF) criteria to define metabolic syndrome.
- Based on the limited number of studies available, the results suggest that the prevalence of metabolic syndrome is not significantly increased among snus users (Gustafsson et al. 2011; Wandell et al. 2008). An increased prevalence observed among former smokers may be related to weight gain following smoking cessation, and illustrates the importance of controlling for current and former smoking.

All-Cause Mortality (Table A VI-10, Figure A VI-10)

- Two cohort studies reported risk estimates for both snus users and smokers in the same population.
- A significant increase in all-cause mortality was observed in these studies among smokers and snus users. For smokers, the relative risk point estimate for all-cause mortality in the individual studies (ranging from 1.2 to 2.2) was only slightly greater than that observed among snus users (ranging from 1.1 to 1.4). Mortality risks among smokers observed in the

three large US cohorts were consistent with those observed in the two individual studies that reported smoking relative risk estimates.

- One out of the two head-to-head studies used a common reference group and the authors of both studies used different exposure groups for the snus and smoking risk estimates. Few potential confounding factors were considered, with only one of the studies controlling for alcohol consumption.
- Per the outcome-specific results above, the results from all-cause mortality from the two available studies (Bolinder et al. 1994; Roosaar et al. 2008) are inconsistent with the findings for the major smoking-related causes of death, which show significantly lower risks among snus users compared to smokers. Many health outcomes have been examined and updated for the Swedish Construction Worker cohort in several publications since the Bolinder et al. (1994) study was published, however, updated results for all-cause mortality have not been presented in any of these publications. With respect to the Roosaar et al. (2008) study, a significant excess risk of all-cause mortality among snus users may be due to confounding by other factors, such as smoking, or to exposure misclassification. As mentioned previously, a significant excess risk of respiratory death among snus users over age 80 was also observed in this cohort even though there is no known mechanism by which snus could cause respiratory disease. Regarding all-cause mortality, Lee (2011) stated, “more evidence is clearly needed.”

Discussion

The forest plots used to present relative risk estimates for outcomes from individual epidemiologic studies of snus users and smokers provide a means to graphically summarize and compare disease risks from these two tobacco exposures. Additional risk estimates from meta-analyses and large cohort studies provides context to relative risks from individual studies. Based on the results presented in this section, it is clear that the use of snus presents a much lower risk, if any risk at all, of the smoking-related diseases that result in the highest number of deaths among smokers (lung cancer, CVD, stroke).

Numerous epidemiology studies provided relative risk estimates for snus users and cigarette smokers in the same study populations, which were used for comparing risks of major health outcomes compared to nonusers of tobacco. In addition, risk estimates from meta-analyses that combine risk estimates of snus users and cigarette smokers and those from other large cohorts studies for smoker, were extracted and plotted as a reference to the results of the more-variable individual studies of snus users and smokers. Plots of the available relative risk estimates for snus users and smokers show that relative to nontobacco users, the expected increased risks among smokers were observed in the epidemiology studies conducted in Sweden and other Scandinavian countries. Among snus users, very few, if any, increased risks of these same health outcomes, were observed, and were not consistently increased among snus users compared to nontobacco users.

Several limitations were identified that affect the comparability of the risk estimates both within individual studies and when comparing risk estimates across studies. First, as noted for each health outcome, there were several individual studies used in this analysis for which the same reference groups (ideally, nontobacco users) were not used for generating the risk estimates for

the two types of tobacco users. There were also examples of studies in which there were differences in exposure groups or subgroup analyses, for example, where snus users were not stratified by age whereas smokers were; for these studies, exact risk comparisons were not possible. For some comparisons, different inclusion criteria were applied for snus users than were used for cigarette smokers, for example, current snus users vs. ever smokers. In some studies, risk estimates stratified by exclusive tobacco use were reported for one tobacco user group, but not the other, and thus risk estimates from multivariate analyses in which tobacco use was adjusted in the model (and possibly other potential confounders) were used in the plots, or in other instances, the only comparable risk estimates available to extract were from univariate, unadjusted analyses.

Between-study differences included different tobacco use definitions for snus and smoking (e.g., ever or current user, varying definitions of current use, such as daily or occasional, stratification by different dose groups, etc.). There were also differences in inclusion and exclusion criteria across disease outcomes, for example, oral cancers including/excluding pharyngeal cancer, stroke and stomach cancer subtypes, and other differences in CVD outcomes. Studies differed in consideration of and control for important confounders, and quality of control for confounders. Some studies provided dose (risk/duration)-response analyses where others did not. In addition, the studies and meta-analyses varied as to whether they considered morbidity versus mortality, which were both included in the forest plots. For many of the cancer-related outcomes, where mortality is high, most cases of the outcome are captured, but for outcomes such as oral cancer, diabetes, and cardiovascular disease outcomes, incident cases may be missed and risk estimates biased for these outcomes. It should be noted, also, that the snus and smoking risk estimates from the individual studies are from Swedish and other Scandinavian populations, whereas the risk estimates from large cohorts provided for comparison are based on mostly on US populations. Though there may be moderate differences in disease risks between populations, control for potential confounders in the multivariate analyses helps minimize potential population differences.

As detailed in the Summary Table for each of the health outcomes examined, the number of relevant studies of snus users that were excluded from this analysis because they lacked relative risk estimates for both snus users and smokers ranged from 0-2 studies depending on the outcome. The relative risk estimates for snus users from these excluded studies are, however, accounted for in the plots where they were included in the meta-analyses by Boffetta et al. (2008), Lee et al. (2007; 2011).

Harm Reduction Potential of Snus

As discussed previously, the US Centers for Disease Control (CDC) has detailed the number of smoking related deaths in the US (Table A VI-A). Lung cancer, cardiovascular diseases (ischemic heart disease, other heart disease, atherosclerosis, aortic aneurysm), and stroke account for 252,765 (approximately 64% of smoking-related deaths) deaths annually due to smoking in the US (CDC 2008).

Though accounting for significantly fewer smoking-related deaths compared to some of the outcomes presented in Table A VI-A, other outcomes were included in this chapter for a variety of reasons. Pancreatic cancer was included in this section due to ongoing controversy within

the scientific community, though it accounts for only 1.7% of smoking-related deaths in the US annually. Although not confirmed as a smoking-related outcomes by the US Surgeon General (2010), diabetes and metabolic syndrome were also included due to the significant burden of morbidity in the population, and high interest as potentially tobacco-related outcomes within the scientific community. Oral cancer was included because it is commonly misperceived, by the general public and some within the scientific community, as an outcome related to Swedish snus, though numerous epidemiological studies and scientific reviews have now confirmed that no such association exists. In the CDC (2008) analysis, oral cancer accounted for 1.2% of smoking-related deaths annually in the US. Uncertainty about the possible relationship with snus remains for two other health outcomes presented in this section, notably esophageal cancer and stomach cancer, which account for 2.2% and 0.6% of annual smoking-related deaths, respectively. As with oral cancer, the results presented in this section generally suggest that the risk of stomach and esophageal cancer among snus users is no different than non-users of tobacco, and certainly much higher among smokers.

The health outcomes included in this analysis, combined with nonmalignant respiratory diseases known to be caused by smoking, account for nearly all, approximately 90%, of all smoking-related deaths. Use of Swedish snus as an alternative to smoking presents no risk of respiratory disease (COPD, bronchitis and emphysema, and pneumonia and influenza) or lung cancer, and at least, a very large reduction in risk from CVD.

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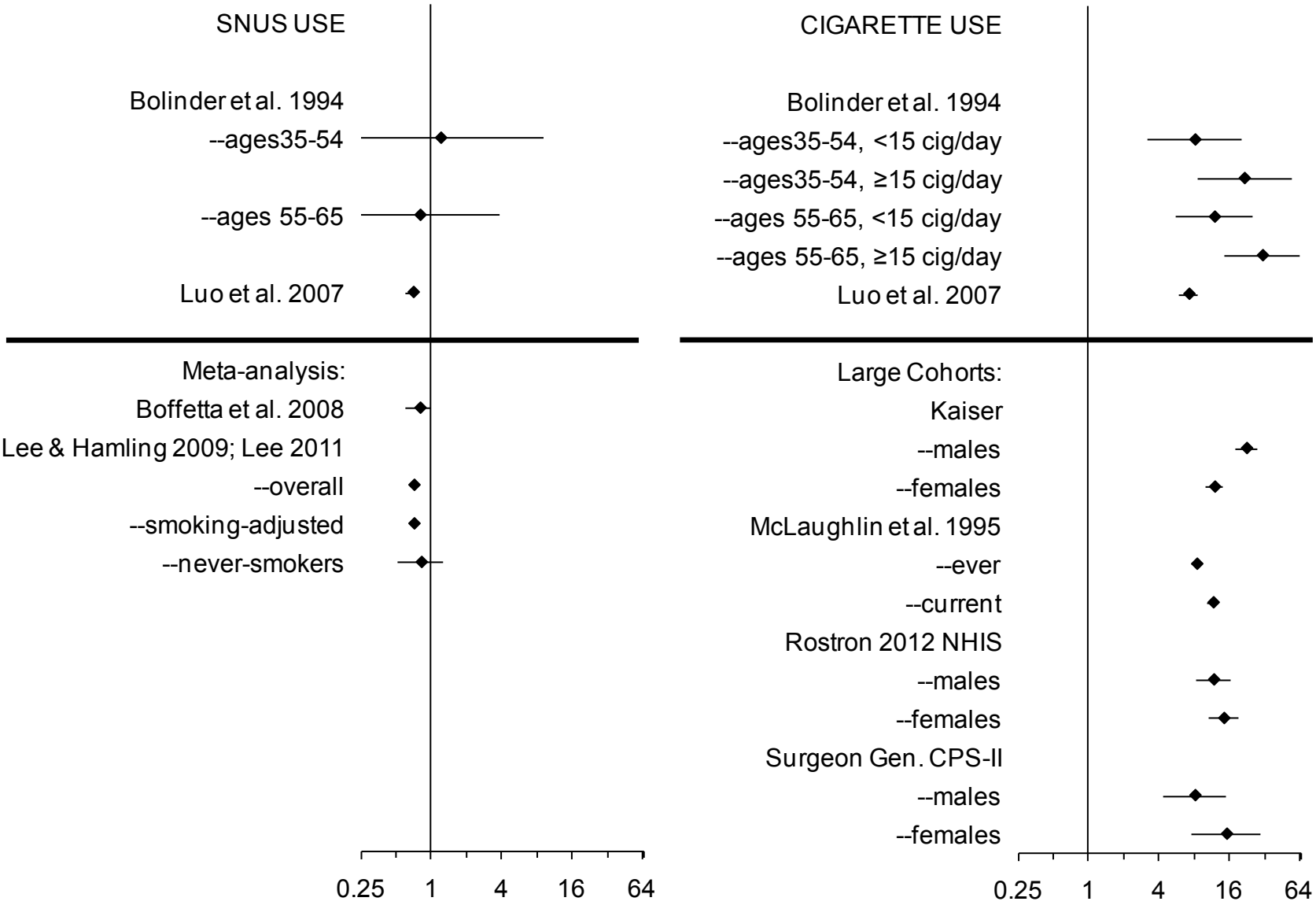
Figure A VI-1: Lung Cancer



ENVIRON

Table A VI-1: Lung Cancer

Risk factors: Age, air pollution, asbestos exposure, family history, and radon (ACS 2013a).

Smoking-attributable mortality in the US (2000-2004): Overall: 80%, Males: 87%, Females: 70%, Among Smokers only (overall): 32%

ICD Codes: ICD-8: 162, ICD-9: 162, ICD-10: C33-C34

Head-to-Head Study Comparisons
Bold: statistically significant
Highlight: risk estimates used in forest plots
(n="# of exposed cases")

Study Information	Snus Use	Cigarette Use
Bolinder et al. 1994 Cohort study Swedish Construction Worker cohort 1971 – 1974 through 1985 Risk estimates adjusted for age, sex and region of origin. Tobacco use categories were exclusive.	Among those aged 35-54 years Non-users of tobacco: 1.0 (reference) Snuff users (n=1): 1.2 (0.2-9.1) Among those aged 55-65 years Non-users of tobacco: 1.0 (reference) Snuff users (n=2): 0.8 (0.1-3.9) Among all cohort members Never-users of tobacco: 1.0 (reference) Snuff users (n=3): 0.9 (0.2-3.0)	Among those aged 35-54 years Non-users of tobacco: 1.0 (reference) <15 cig/day (n=16): **8.1 (3.2-20.4)** ≥15 cig/day (n=43): **21.4 (8.5-54.1)** Ex-smokers, 1-5 years (n=7): **6.7 (2.3-19.7)** Ex-smokers, >5 years (n=3): 1.2 (0.3-4.5) Among those aged 55-65 years Non-users of tobacco: 1.0 (reference) <15 cig/day (n=36): **11.9 (5.5-25.6)** ≥15 cig/day (n=57): **30.6 (14.6-64.1)** Ex-smokers, 1-5 years (n=14): **9.4 (3.9-22.3)** Ex-smokers, >5 years (n=12): 2.3 (1.0-5.7)
Luo et al. 2007 Cohort study Swedish Construction Worker cohort 1978 – 1992 All risk estimates adjusted for age and BMI. Men only. Snus and smoking estimates among all cohort members adjusted for respective tobacco use.	Among all cohort members Never-users of any tobacco 1.0 (reference) Ever-users of snus: **0.7 (0.6-0.7)** Among never-smokers Never-users of any tobacco: 1.0 (reference) Ever-users of snus (n=18): 0.8 (0.5-1.3) Ex-users (n=3): 0.9 (0.3-3.0) Current users (n=15): 0.8 (0.4-1.3) 1-9 g/day (n=7): 1.0 (0.5-2.1) ≥10 g/day (n=10): 0.7 (0.4-1.3)	Among all cohort members Never-users of any tobacco: 1.0 (reference) Ever-smokers (n=2062): **7.2 (6.0-8.5)** Ex-smokers (n=329): **2.6 (2.2-3.2)** Current smokers (n=1733): **10.2 (8.6-12.2)**

Snus & Smoking Review Summary Estimates

Study Information	Summary Estimate
Cigarette Use	
Friedman et al. 1997 Kaiser Population 1979-1986, followed through 1987	Males Current smoker (n=53): **8.1 (4.4-15.0)** Females Current smoker (n=54): **15.1 (7.7-29.7)**

Study Information	Summary Estimate
Age-adjusted relative risk of death of current smokers compared to never-smokers.	
McLaughlin et al. 1995 Cohort study US veterans who held government life insurance policies active at the end of 1953. Followed through 1980. Mortality	Use Status Ever smoker: **8.4 (7.5-9.4)** Current smoker: **11.6 (10.4-13.0** Former smoker: **3.6 (3.1-4.1)** Smoking Dose (cigs/day) 1-9: **3.7 (3.1-4.5)** 10-20: **9.9 (8.8-11.2)** 31-39: **16.9 (15.0-19.0)** 40+: **22.9 (19.8-26.6)** **P<0.01**
Rostron 2012 National Health Interview Survey (NHIS) – Linked Mortality Files 1997 – 2004, followed through 2006 Mortality	Males Current smoker: **11.71 (8.30-16.53)** Former smoker: **3.85 (2.80-5.31)** Females Current smoker: **14.30 (10.67-19.15)** Former smoker: **6.01 (4.53-7.97)**
SAMMEC CPS II Population 1982 – 1988 Relative risk of death among adults aged 35 and older.	Males Current smoker: 23.26 Former smoker: 8.70 Females Current smoker: 12.69 Former smoker: 4.53
US Surgeon General 1989 CPS II Population 1982 – 1986 Relative risk of death among adults aged 35 and older.	Males Current smoker: **22.36 (17.77-28.13)** Former smoker: **9.36 (7.43-11.77)** Females Current smoker:**11.94 (9.99-14.26)** Former smoker: **4.69 (3.86-5.70)**
Snus Use	
Lee & Hamling 2009; Lee 2011 Snus meta-analysis (incidence) Combined and presented estimates for smoking-adjusted and never-smokers separately from Boffetta et al. 2005 and Luo et al. 2007.	Overall data: 0.71 (0.66-0.76) Smoking-adjusted: 0.71 (0.66-0.76) Never smokers: 0.82 (0.52-1.28)
Boffetta et al. 2008 Snus meta-analysis (incidence) for Nordic countries. RR from Boffetta et al. 2005 (never-smokers) and Luo et al. 2007 (never-smokers).	Ever use: 0.8 (0.6-1.0)

Summary of Study Quality & Comparability

Study design: Two cohort studies.

Reference group comparability: Both studies used common reference groups for snus and smoking risk estimates; Luo et al. (2007): never-users of any tobacco; Bolinder et al. (1994): nonusers of tobacco.

Confounding: Both studies controlled for age, gender and tobacco use. Luo et al. (2007) controlled for BMI.

Outcome comparability: Luo et al. (2007) used ICD-7 code 162 for lung cancer, while Bolinder et al. (1994) used lung cancer deaths (ICD-8) identified from the National Cause of Death Register in Sweden.

Exposure comparability (intra-study): The two studies use comparable exposure groups.

Dose-risk or duration-risk relationship:

Bolinder et al. (1994)
Smokers: no analysis.
Snus users: no analysis.

Luo et al. (2007)
Smokers: no analysis.
Snus users: no significant dose-response observed.

Studies with no smoking comparison:
Boffetta et al. 2005

Figure A VI-2: Cardiovascular Disease (IHD, CHD, MI and Overall CVD)

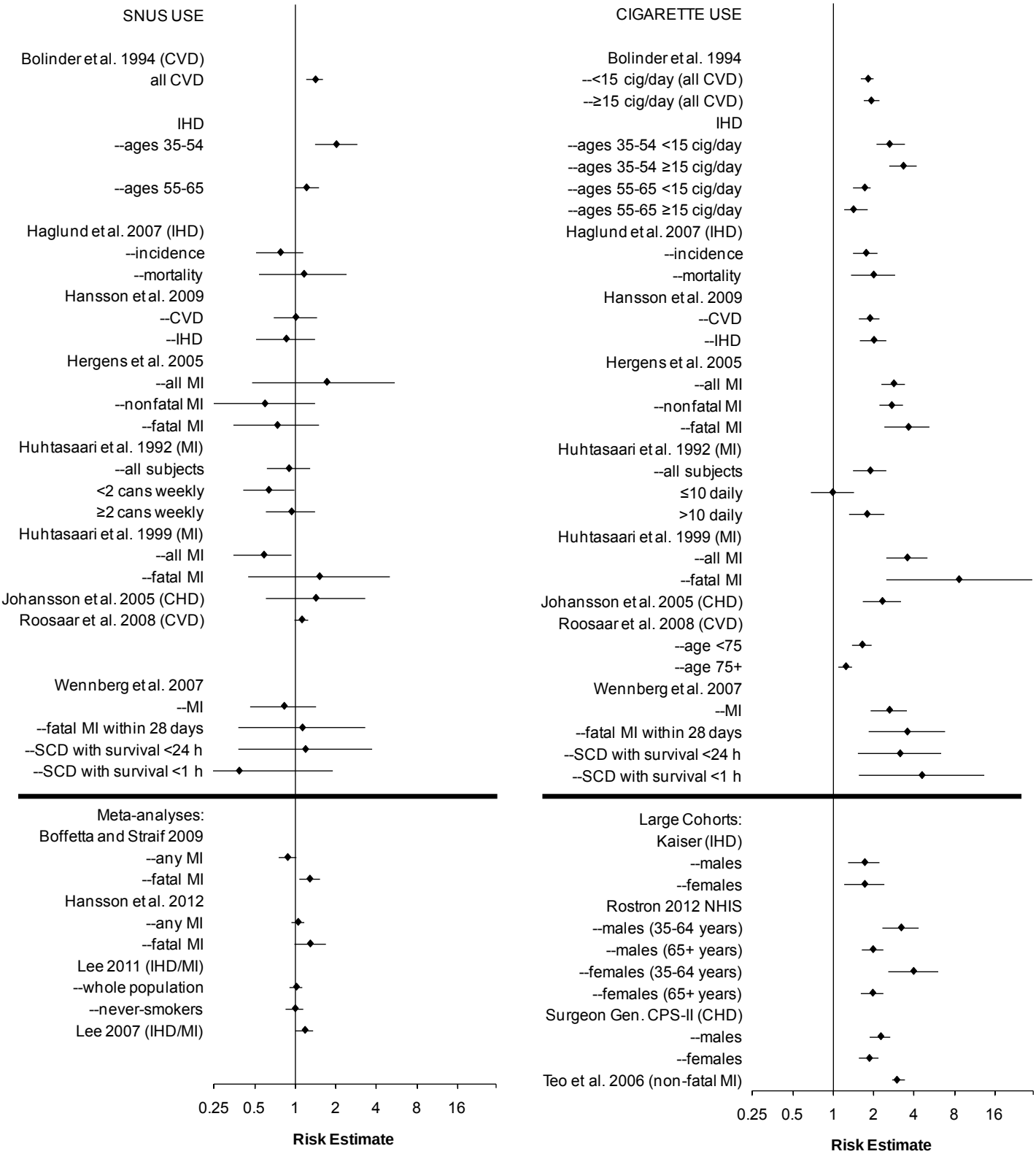


Table A VI-2: Cardiovascular Disease (IHD, CHD, MI and Overall CVD)

Risk factors: Age, male gender, high cholesterol, physical inactivity, high blood pressure, obesity, diabetes, stress, alcohol consumption (AHA 2012).

Smoking-attributable mortality in the US (IHD) (2000-2004): Overall: 16%, Males: 20%, Females: 12%, Among Smokers only: 20.4%, Among Smokers only (overall CVD): 32.7%

ICD Codes: IHD, CHD, MI: ICD-8: 410-414, ICD-9: 410-414, ICD-10: I20-I25. Overall CVD: ICD-8,9: 390-458, ICD-10: I00-I99

Head-to-Head Study Comparisons
Bold: statistically significant
Highlight: risk estimates used in forest plots
(n="# of exposed cases")

Study Information	Snus Use	Cigarette Use
Bolinder et al. 1992 Cross-sectional study Swedish Construction Worker cohort 1971 – 1974 Risk estimates adjusted for age, sex (men only). Tobacco use categories were exclusive. Cardiovascular diagnosis Excluded from forest plot because same population was analyzed prospectively in the Bolinder et al. 1994 study.	Cardiovascular diagnosis Ages 46-55 Never-users of tobacco: 1.0 (reference) Snuff users (n=8): 1.6 (0.7-3.5) Cardiovascular diagnosis Ages 56-65 Never-users of tobacco: 1.0 (reference) Snuff users (n=69): **1.5 (1.1-1.9)**	Cardiovascular diagnosis Ages 46-55 Never-users of tobacco: 1.0 (reference) ≥15 cig/day (n=22): **2.2 (1.3-3.9)** Cardiovascular diagnosis Ages 56-65 Never-users of tobacco: 1.0 (reference) ≥15 cig/day (n=33): 1.3 (0.9-1.9)
Bolinder et al. 1994 Cohort study Swedish Construction Worker cohort 1971 – 1974 through 1985 Risk estimates adjusted for age, sex (men only) and region of origin, however, high blood pressure, diabetes, BMI, region, blood pressure meds, and previous cardiac symptoms were considered and not found to significantly alter RR.	All CVD Mortality Never-users of tobacco: 1.0 (reference) Snuff users (n=220): **1.4 (1.2-1.6)** All CVD Mortality Ages 35-54 Never-users of tobacco: 1.0 (reference) Snuff users (n=44): **2.1 (1.5-2.9)** All CVD Mortality Ages 55-65 Never-users of tobacco: 1.0 (reference) Snuff users (n=174): 1.1 (1.0-1.4)	All CVD Mortality Never-users of tobacco: 1.0 (reference) <15 cig/day (n=450): **1.8 (1.6-2.0)** ≥15 cig/day (n=381): **1.9 (1.7-2.2)** Ex-smokers, 1-5 years (n=169): **1.4 (1.1-1.6)** Ex-smokers, >5 years (n=402): 1.1 (0.9-1.2) All CVD Mortality Ages 35-54 Never-users of tobacco: 1.0 (reference) <15 cig/day (n=164): **2.7 (2.2-3.4)** ≥15 cig/day (n=199): **3.2 (2.6-3.9)** Ex-smokers, 1-5 years (n=46): 1.4 (1.0-2.0) Ex-smokers, >5 years (n=83): 1.1 (0.9-1.5) All CVD Mortality Ages 55-65 Never-users of tobacco: 1.0 (reference) <15 cig/day (n=272): **1.5 (1.3-1.7)** ≥15 cig/day (n=167): **1.5 (1.3-1.7)**

Study Information	Snus Use	Cigarette Use
Tobacco use categories were exclusive. ICD8: 390-458 (All CVD), 410-414 (IHD)	 IHD Mortality Ages 35-54 Never-users of tobacco: 1.0 (reference) Snuff users (n=35): **2.0 (1.4-2.9)** IHD Mortality Ages 55-65 Never-users of tobacco: 1.0 (reference) Snuff users (n=137): 1.2 (1.0-1.5)	Ex-smokers, 1-5 years (n=120): **1.3 (1.1-1.6)** Ex-smokers, >5 years (n=317): 1.0 (0.9-1.2) IHD Mortality Ages 35-54 Never-users of tobacco: 1.0 (reference) <15 cig/day (n=128): **2.6 (2.1-3.4)** ≥15 cig/day (n=162): **3.3 (2.6-4.2)** Ex-smokers, 1-5 years (n=37): 1.4 (1.0-2.1) Ex-smokers, >5 years (n=67): 1.2 (0.9-1.6) IHD Mortality Ages 55-65 Never-users of tobacco: 1.0 (reference) <15 cig/day (n=225): **1.7 (1.4-1.9)** ≥15 cig/day (n=122): **1.4 (1.2-1.8)** Ex-smokers, 1-5 years (n=89): **1.3 (1.1-1.6)** Ex-smokers, >5 years (n=248): 1.1 (0.9-1.2)
Haglund et al. 2007 Cohort study Swedish population 1988 – 1989 through 2003 All risk estimates adjusted for age, sex (men only), socioeconomic status, residential area, self-reported health, number of longstanding illnesses, and physical activity. Tobacco use categories were exclusive (but may include former smokers/snuff users). ICD9: 410-414; ICD10: I20-I25 (IHD)	IHD Incidence No tobacco: 1.0 (reference) Snuff (n=28): 0.77 (0.51-1.15) IHD Mortality No tobacco: 1.0 (reference) Snuff (n=8): 1.15 (0.54-2.41)	IHD Incidence No tobacco: 1.0 (reference) Smoke (n=153): **1.74 (1.41-2.14)** Smoke & Snuff (n=15): 1.64 (0.96-2.79) IHD Mortality No tobacco: 1.0 (reference) Smoke (n=52): **1.98 (1.35-2.91)** Smoke & Snuff (n=3): 1.69 (0.52-5.46)
Hansson et al. 2009 Cohort study Swedish Twin Registry Born 1928 – 1958 & followed through 2003 for mortality and 2005 for hospitalization. All risk estimates adjusted for age, sex, diabetes, high blood pressure, and high cholesterol. Tobacco use categories were exclusive. All CVD; ICD10: I20-I21, I24-I25 [excluding I25.2] (IHD: MI or coronary revascularization procedures)	All CVD Among never-smokers Never-tobacco: 1.0 (reference) Former (n=19): 1.21 (0.75-1.97) Current (n=32): 1.00 (0.69-1.46) IHD Among never-smokers Never-tobacco: 1.0 (reference) Former (n=11): 1.07 (0.56-2.03) Current (n=18): 0.85 (0.51-1.41)	All CVD Among never-snus users Never-tobacco: 1.0 (reference) Former (n=318): 1.17 (1.00-1.38) Current (n=230): **1.86 (1.56-2.22)** IHD Among never-snus users Never-tobacco: 1.0 (reference) Former (n=229): 1.34 (1.10-1.64) Current (n=155): **1.99 (1.59-2.50)**
Hergens et al. 2005	All Cases among never-smokers Never-tobacco: 1.0 (reference)	All Cases among never snus users Never-tobacco: 1.0 (reference)

Study Information	Snus Use	Cigarette Use
Case-control study Residents of Stockholm county, Sweden 1992 – 1994 All risk estimates adjusted for age, sex, hospital catchment area, diabetes, hyperlipidemia, hypertension, overweight, physical inactivity, and job strain. Tobacco use categories were exclusive. MI	Former: 1.2 (0.46-3.1) Current: 0.73 (0.35-1.5) Nonfatal cases among never-smokers Never-tobacco: 1.0 (reference) Former: 1.2 (0.43-3.2) Current: 0.59 (0.25-1.4) Fatal cases among never-smokers Never-tobacco: 1.0 (reference) Former: 1.7 (0.21-13.6) Current: 1.7 (0.48-5.5)	Former: **1.3 (1.1-1.6)** Current: **2.8 (2.3-3.4)** Nonfatal cases among never snus users Never-tobacco: 1.0 (reference) Former: 1.2 (0.98-1.5) Current: **2.7 (2.2-3.3)** Fatal cases among never snus users Never-tobacco: 1.0 (reference) Former: **1.7 (1.6-2.6)** Current: **3.6 (2.4-5.2)**
Huhtasaari et al. 1992 Case-control study Northern Sweden MONICA project: Norrbotten and Vasterbotten provinces. 1989 – 1991 All risk estimates adjusted for age and sex (men only). Tobacco use categories were exclusive (but may include former smokers/snuff users). MI	Among ages 35-54 years No tobacco: 1.0 (reference) Snuff dipping: 0.96 (0.56-1.67) Among ages 55-64 years No tobacco: 1.0 (reference) Snuff dipping: 1.24 (0.67-2.30) All subjects No tobacco: 1.0 (reference) Snuff dipping: 0.89 (0.62-1.29) <2 cans weekly: **0.63 (0.41-0.98)** ≥2 cans weekly: 0.93 (0.61-1.41)	Among ages 35-54 years No tobacco: 1.0 (reference) Smoking: **3.11 (2.09-4.63)** Among ages 55-64 years No tobacco: 1.0 (reference) Smoking: 1.35 (0.87-2.10) All subjects No tobacco: 1.0 (reference) Smoking: **1.87 (1.40-2.48)** ≤10 daily: 0.98 (0.68-1.42) >10 daily: **1.77 (1.31-2.39)** Among ages 35-54 years Snuff use: 1.0 (reference) Smoking: **3.22 (1.82-5.70)** Among ages 55-64 years Snuff use: 1.0 (reference) Smoking: 1.09 (0.55-2.16) All subjects Snuff use: 1.0 (reference) Smoking: **2.09 (1.39-3.15)**
Huhtasaari et al. 1999 Case-control study Northern Sweden MONICA project: Norrbotten and Vasterbotten provinces. 1991 – 1993 Multivariate estimates adjusted for age (matched) and sex (men only), hypertension, diabetes, high cholesterol, family history of early cardiac death, low education level, and marital status. Tobacco use categories were	Univariate: Among no current smoking Never used tobacco: 1.0 (reference) Current snuff user: 0.96 (0.65-1.41) Multivariate: Fatal and nonfatal acute MI Never used tobacco: 1.0 (reference) Regular use of snuff: **0.58 (0.35-0.94)** Fatal acute MI only Regular use of snuff: 1.50 (0.45-5.03)	Univariate Among no current snuff use Never used tobacco: 1.0 (reference) Current smoker: **3.65 (2.67-4.99)** Multivariate: Fatal and nonfatal acute MI Never used tobacco: 1.0 (reference) Regular smoking: **3.53 (2.48-5.03)** Fatal acute MI only Regular smoking: **8.57 (2.48-30.3)**

Study Information	Snus Use	Cigarette Use
exclusive (but may include former smokers/snuff users). ICD:410-414 (MI)		
Johansson et al. 2005 Cohort study Random sample from Swedish population: SALLS survey. Interviewed in 1988 and 1989, and followed through 2000 All risk estimates adjusted for age, sex (men only), BMI, physical activity, diabetes, and hypertension. Risk estimates did not change much when socioeconomic status was considered. Tobacco use categories were exclusive (but smoking category may include former snuff users). ICD9: 410-414; ICD10: I20-I25 (CHD event)	Among never-smokers Never-smoker: 1.0 (reference) Daily snuffer: 1.41 (0.61-3.28)	May include former snuff users Never-smoker: 1.0 (reference) Daily smoker: **2.30 (1.66-3.19)**
Roosaar et al. 2008 Cohort study Uppsala County, central Sweden Exposure information collected 1973-1974 and followed through 2002 All risk estimates adjusted for age, sex (men only), calendar period (attained), area of residence, alcohol consumption and smoking or snus use. ICD8,9: 390-458; ICD10: I00-I99 (CVD death)	Smoking adjusted Never snus user: 1.0 (reference) Snus use (ever): 1.11 (0.98-1.25) Among never-smokers Never snus user: 1.0 (reference) Snus use (ever): 1.15 (0.97-1.37)	Snus adjusted Never-smoker: 1.0 (reference) Smoking (ever) age <75: **1.63 (1.37-1.93)** Smoking (ever) age 75+: **1.23 (1.09-1.38)**
Wennberg et al. 2007 Prospective incident case-referent study Nested in northern Sweden MONICA cohort: Norrbotten and Vasterbotten provinces. Cases obtained from 1985	MI *Among never-smokers* Never used tobacco: 1.0 (reference) Former snuff user (n=11): 0.66 (0.32-1.34) Current snuff user (n=21): 0.82 (0.46-1.43) Fatal MI within 28 days	MI *Among never snuff users:* Never used tobacco: 1.0 (reference) Former smoker (n=58): 1.18 (0.82-1.70) *Among no current snuff users:* Current smoker (n=136): **2.60 (1.91-3.54)** Fatal MI within 28 days *Among never snuff users:* Never used tobacco: 1.0 (reference)

Study Information	Snus Use	Cigarette Use
– 1999 All risk estimates adjusted for age, sex, BMI, leisure time physical activity, educational level and cholesterol level. Tobacco use categories were exclusive, but smoking category may have included some past snuff users. ICD9: 410-414, 429.2; ICD10: I20-I25 (MI, fatal MI, Sudden cardiac death (SCD))	*Among never-smokers* Never used tobacco: 1.0 (reference) Former snuff user (n=2): 0.64 (0.13-3.18 Current snuff user (n=7): 1.12 (0.38-3.29) SCD with survival <24 h *Among never-smokers* Never used tobacco: 1.0 (reference) Former snuff user (n=2): 0.70 (0.14-3.64) Current snuff user (n=7): 1.18 (0.38-3.70) SCD with survival <1 h *Among never-smokers* Never used tobacco: 1.0 (reference) Former snuff user (n=1): 0.35 (0.03-4.56) Current snuff user (n=4): 0.38 (0.08-1.89)	Former smoker (n=11): 1.02 (0.45-2.31) *Among no current snuff users:* Current smoker (n=37): **3.53 (1.83-6.84)** SCD with survival <24 h *Among never snuff users:* Never used tobacco: 1.0 (reference) Former smoker (n=7): 0.74 (0.28-1.97) *Among no current snuff users:* Current smoker (n=31): **3.12 (1.53-6.33)** SCD with survival <1 h *Among never snuff users:* Never used tobacco: 1.0 (reference) Former smoker (n=4): 0.35 (0.07-1.78) *Among no current snuff users:* Current smoker (n=21): **4.54 (1.55-13.25)**

Studies not included due to insufficient control for tobacco use

Study Information	Snus Use	Cigarette Use
Janzon and Hedblad 2009 Cohort study Male residents of Malmö, Sweden. 1991 – 1996 through 2004 All risk estimates adjusted for age, sex (men only), BMI, smoking habits, diabetes, hypertension, physical activity, marital status, and occupation. ICD9: 410-414 (MI)	MI Nontobacco users: 1.0 (reference) Snuff user, never-smoker: 0.75 (0.3-1.8)	MI Nontobacco users: 1.0 (reference) Smoker, snuff user: 1.31 (0.8-2.0)

Snus & Smoking Review Summary Estimates

Study Information	Summary Estimate
Cigarette Use	
Friedman et al. 1997 Kaiser Population 1979-1986, followed through 1987 Age-adjusted relative risk of IHD death (ICD-9 410-414) of current smokers compared to never-smokers.	Males Current smoker (n=109): **1.7 (1.3-2.2)** Females Current smoker (n=50): **1.7 (1.2-2.4)**
Rostron 2012 National Health Interview	Males 35-64 Current smoker: **3.18 (2.34-4.33)** Former smoker: **1.59 (1.11-2.27)**

Study Information	Summary Estimate
Survey (NHIS) – Linked Mortality Files 1997 – 2004, followed through 2006 IHD Mortality	 Males 65+ Current smoker: **1.96 (1.62-2.37)** Former smoker: **1.16 (1.01-1.34)** Females 35-64 Current smoker: **3.93 (2.56-6.05)** Former smoker: 1.48 (0.82-2.64) Females 65+ Current smoker: **1.95 (1.60-2.37)** Former smoker: 1.09 (0.85-1.41)
SAMMEC CPS II Population 1982 – 1988 Relative risk of IHD death among adults aged 35 and older.	Males 35-64 Current smoker: 2.80 Former smoker: 1.64 Males 65+ Current smoker: 1.51 Former smoker: 1.21 Females 35-64 Current smoker: 3.08 Former smoker: 1.32 Females 65+ Current smoker: 1.60 Former smoker: 1.20
Teo et al. 2006 Case-control study 52 Countries Odds ratio for non-fatal acute MI compared with never-smokers (male and female).	Current Smokers Overall: **2.95 (2.77-3.14)** 1-9 cigs/day: **1.63 (1.45-1.82)** 10-19 cigs/day: **2.59 (2.35-2.85)** >20 cigs/day: **4.59 (4.21-5.00)**
US Surgeon General 1989 CPS II Population 1982 – 1986 Relative risk of CHD death (ICD-9 410-414) among adults aged 35 and older.	Males >35 Current smoker: **1.94 (1.80-2.08)** Former smoker: **1.41 (1.33-1.50)** Males 35-64 Current smoker: **2.81 (2.49-3.18)** Former smoker: **1.75 (1.55-1.99)** Males 65+ Current smoker: **1.62 (1.48-1.77)** Former smoker: **1.29 (1.20-1.38)** Females >35 Current smoker: **1.78 (1.62-1.97)** Former smoker: **1.31 (1.19-1.44)** Females 35-64 Current smoker: **3.00 (2.50-3.59)** Former smoker: **1.43 (1.15-1.77)** Females 65+ Current smoker: **1.60 (1.42-1.80)** Former smoker: **1.29 (1.16-1.43)**

Study Information	Summary Estimate
Snus Use	
Boffetta and Straif 2009 Snus meta-analysis (any and fatal MI)	Any MI Sweden: 0.87 (0.75-1.02) Fatal MI Sweden: **1.27 (1.07-1.52)**
Hansson et al. 2012 Acute myocardial infarction Snus pooled analysis	Any MI: 1.04 (0.93-1.17) Fatal MI (28 day): 1.28 (0.99-1.68)
Lee 2011 IHD/MI Snus meta-analysis	Whole population: 1.01 (0.91-1.12) Never-smokers: 0.99 (0.85-1.14)
Lee 2007 IHD/AMI Snus (Sweden) meta-analysis (incidence)	Current use (never-smokers): **1.17 (1.01-1.35)**

Summary of Study Quality & Comparability

Study design:

Study Design	Cross-sectional	Case-control	Cohort
Author	Bolinder et al. 1992	Hergens et al. 2005 Huhtasaari et al. 1992 Huhtasaari et al. 1999 Wennberg et al. 2007	Bolinder et al. 1994 Haglund et al. 2007 Hansson et al. 2009 Janzon and Hedblad 2009 Johansson et al. 2005 Roosaar et al. 2008

Reference group comparability: 10 studies used common reference groups for snus and smoking risk estimates, while one study did not (Roosaar et al. 2008). Roosaar et al. used never snus users and never smokers as the reference groups for snus users and smokers respectively.

Confounding:

	Age	Sex	Weight/ BMI	Diabetes	Blood pressure, Hyperten.	Phys. act.	Ed level, SES	Cholest. level, hyperlip.	Alcohol	Stress /Job strain	Fam Hist	Snus, Cigs.
Bolinder et al. 1992	X	X										X
Bolinder et al. 1994	X	X	X	X	X							X
Haglund et al. 2007	X	X				X	X					X
Hansson et al. 2009	X	X		X	X			X				X
Hergens et al. 2005	X	X	X	X	X	X		X		X		X
Huhtasaari et al. 1992	X	X										X
Huhtasaari et al. 1999	X	X		X	X		X				X	X
Janzon and Hedblad 2009	X	X	X	X	X	X	X					
Johansson et al. 2005	X	X	X	X	X	X	X					X
Roosaar et al. 2008	X	X							X			X
Wennberg et al. 2007	X	X	X			X	X	X				X

Outcome comparability:

Author	Outcome
Bolinder et al. 1992	Cardiovascular diagnosis
Bolinder et al. 1994	ICD8: 390-458 (All CVD); ICD8: 410-414 (IHD)
Haglund et al. 2007	ICD9: 410-414; ICD10: I20-I25 (IHD)
Hansson et al. 2009	All CVD; ICD10: I20-I21, I24-I25 [excluding I25.2] (IHD: MI or coronary revascularization procedures)
Hergens et al. 2005	MI
Huhtasaari et al. 1992	MI
Huhtasaari et al. 1999	ICD:410-414 (MI)
Janzon and Hedblad 2009	ICD9: 410-414 (MI)
Johansson et al. 2005	ICD9: 410-414; ICD10: I20-I25 (CHD event)
Roosaar et al. 2008	ICD8,9: 390-458; ICD10: I00-I99 (CVD death)
Wennberg et al. 2007	ICD9: 410-414, 429.2; ICD10: I20-I25 (MI, fatal MI, Sudden cardiac death (SCD))

Exposure comparability (intra-study): Five of the 11 studies compared different exposure groups (Bolinder et al. 1992, 1994; Janzon and Hedblad 2009; Roosaar et al. 2008; Johansson et al. 2005). Bolinder et al. (1992, 1994) compared smokeless tobacco use with smoking dose. Janzon and Hedblad compare snuff use among never smokers with dual users. Johansson et al. (2005) compare daily snuffers (never-smoking) with daily smokers (including some who may have used snuff), while Roosaar et al. (2008) compare snus users with smokers stratified by age (<75 years and 75+).

Dose-risk or duration-risk relationship:

Bolinder et al. 1992
Smokers: no analysis.
Snus users: no analysis.

Bolinder et al. (1994)
Smokers: a dose-response relationship was observed (significance not reported).
Snus users: no analysis.

Haglund et al. (2007)
Smokers: no analysis.
Snus users: no analysis.

Hansson et al. (2009)
Smokers: no analysis.
Snus users: no dose response relationship observed (significance not reported).

Hergens et al. 2005
Smokers: no analysis.
Snus users: no analysis.

Huhtasaari et al. 1992
Smokers: a dose-response relationship was not observed (significance not reported).
Snus users: a dose-response relationship was not observed (significance not reported).

Huhtasaari et al. 1999
Smokers: no analysis.
Snus users: no analysis.

Janzon and Hedblad (2009)
Smokers: no analysis.
Snus users: no analysis.

Johansson et al. 2005
Smokers: no analysis.
Snus users: no analysis.

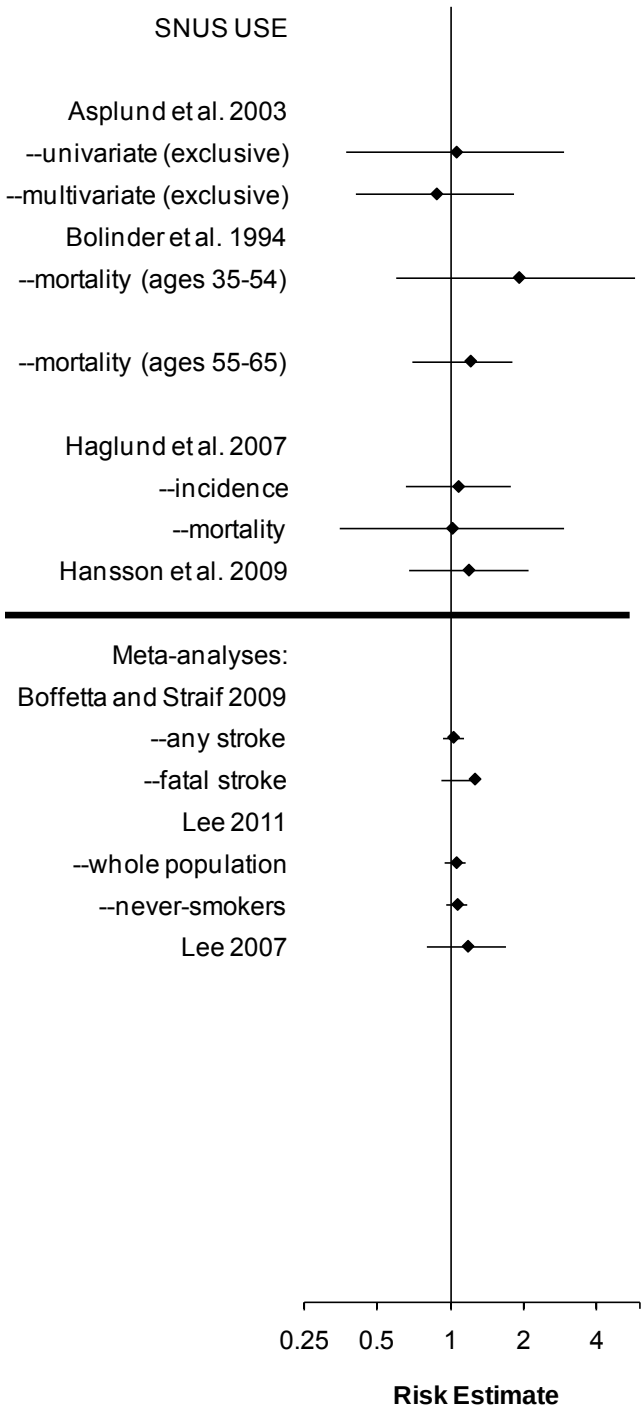
Roosaar et al. 2008
Smokers: no analysis.
Snus users: no analysis.

Wennberg et al. 2007
Smokers: no analysis.
Snus users: no analysis.

Studies with no smoking comparison:

Arefalk et al. 2011
Hergens et al. 2007

Figure A VI-3: Stroke



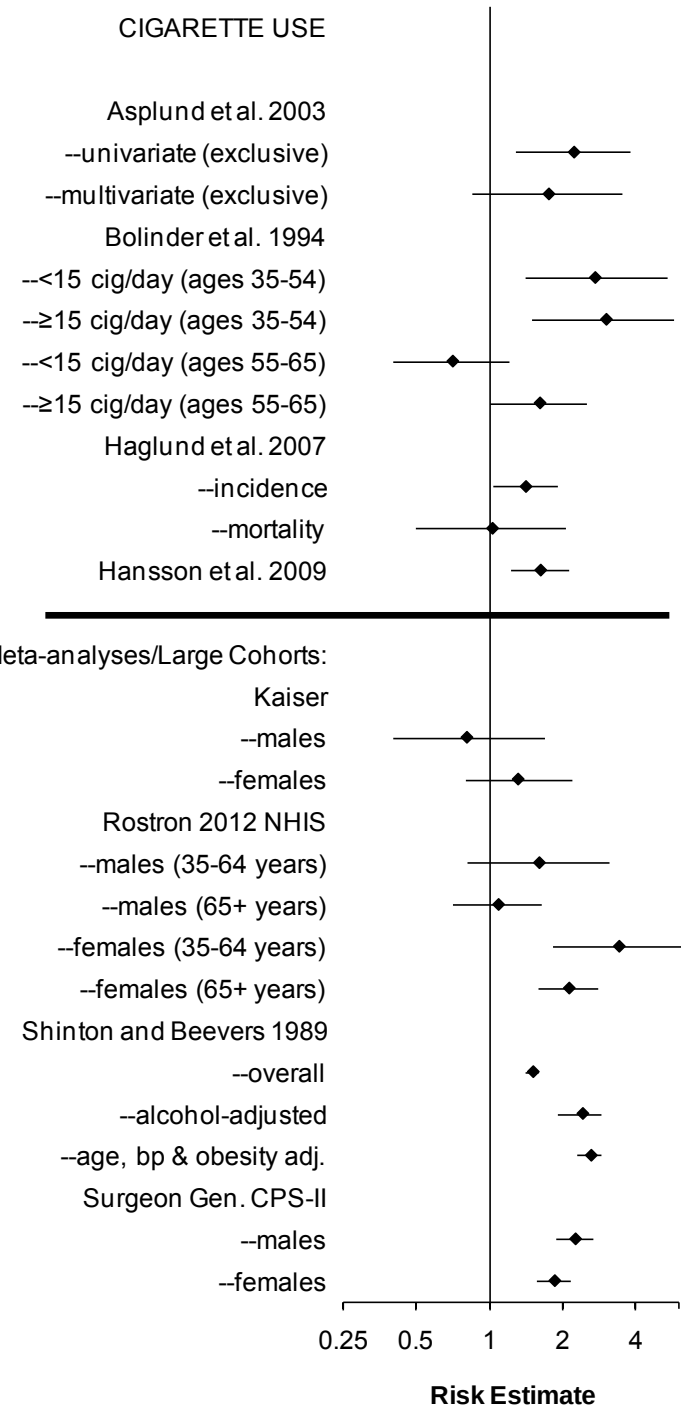


Table A VI-3: Stroke

Risk factors: Age, blood pressure, obesity, female gender (Shinton and Beevers 1989). Hypertension (US Surgeon General 2004).

Smoking-attributable mortality in the US (2000-2004): Overall: 10%, Males: 13%, Females: 8%, Among Smokers only: 4.1%.

ICD Codes: ICD-8: 430-438, ICD-9: 430-438, ICD-10: I60-I69

Head-to-Head Study Comparisons
Bold: statistically significant
Highlight: risk estimates used in forest plots
(n="# of exposed cases")

Study Information	Snus Use	Cigarette Use
Asplund et al. 2003 Case-control study Cases and controls from 2 cohorts: Northern Sweden MONICA Project and the Västerbotten Intervention Project. 1985 – 2000 Matching based on age, sex, geographical area, year of baseline exam, and cohort. Risk estimates from conditional logistic regression were adjusted for elevated blood pressure, low level of education, not married or cohabitant, diabetes, and serum cholesterol. Reference group for these analyses not defined. Exclusive and adjusted snuff user/smoker groups may have included former users. First ever fatal or nonfatal stroke.	Univariate Analysis Never users of tobacco: 1.00 (reference) All snuff users (n=30): 1.16 (0.60-2.22) Exclusive snuff users: 1.05 (0.37-2.94) Conditional Logistic Regression Exclusive snuff users: 0.87 (0.41-1.83)	Univariate Analysis Never users of tobacco: 1.00 (reference) All smokers (n=67): **1.86 (1.13-3.05)** Exclusive smokers: **2.21 (1.29-3.79)** Conditional Logistic Regression Exclusive smokers: 1.74 (0.85-3.54)
Bolinder et al. 1994 Cohort study Swedish Construction Worker cohort 1971 – 1974 through 1985 Risk estimates adjusted for age and sex only, however, high blood	Stroke Mortality Ages 35-54 Never-users of tobacco: 1.0 (reference) Snuff users (n=4): 1.9 (0.6-5.7) Stroke Mortality Ages 55-65 Never-users of tobacco: 1.0 (reference) Snuff users (n=26): 1.2 (0.7-1.8)	Stroke Mortality Ages 35-54 Never-users of tobacco: 1.0 (reference) <15 cig/day (n=17): **2.7 (1.4-5.4)** ≥15 cig/day (n=19): **3.0 (1.5-5.7)** Ex-smokers, 1-5 years (n=4): 1.2 (0.4-3.7) Ex-smokers, >5 years (n=5): 0.7 (0.2-1.9) Stroke Mortality Ages 55-65 Never-users of tobacco: 1.0 (reference) <15 cig/day (n=19): 0.7 (0.4-1.2) ≥15 cig/day (n=25): 1.6 (1.0-2.5)

Study Information	Snus Use	Cigarette Use
pressure, diabetes, BMI, region, blood pressure meds, and previous cardiac symptoms were considered and not found to significantly alter RR. Tobacco use categories were exclusive. ICD8: 430-438 (stroke/cerebrovascular disorders)		Ex-smokers, 1-5 years (n=20): 1.5 (0.9-2.5) Ex-smokers, >5 years (n=35): 0.8 (0.5-1.2)
Haglund et al. 2007 Cohort study Swedish population 1988 – 1989 through 2003 All risk estimates adjusted for age, socioeconomic status, residential area, self-reported health, number of longstanding illnesses, and physical activity. Tobacco use categories were exclusive (but may include former smokers/snuff users). ICD9: 430-438; ICD10: I60-I69 (stroke)	Stroke Incidence No tobacco: 1.0 (reference) Snuff (n=19): 1.07 (0.65-1.77) Stroke Mortality No tobacco: 1.0 (reference) Snuff (n=4): 1.01 (0.35-2.92)	Stroke Incidence No tobacco: 1.0 (reference) Smoke (n=66): **1.40 (1.03-1.91)** Smoke & Snuff (n=9): 1.98 (1.00-3.95) Stroke Mortality No tobacco: 1.0 (reference) Smoke (n=12): 1.02 (0.50-2.05) Smoke & Snuff (n=3): **4.30 (1.22-15.1)**
Hansson et al. 2009 Cohort study Swedish Twin Registry Born 1928 – 1958 & followed through 2003 for mortality and 2005 for hospitalization. All risk estimates adjusted for age, sex, diabetes, high blood pressure, and high cholesterol. Tobacco use categories were exclusive. ICD10: I60-I61, I63-I64, G45; ICD9: 430-431, 433-436 (stroke)	Among never-smokers Never-tobacco: 1.0 (reference) Former (n=8): 1.35 (0.65-2.82) Current (n=14): 1.18 (0.67-2.08)	Among never-snus users Never-tobacco: 1.0 (reference) Former (n=115): 1.01 (0.78-1.30) Current (n=81): **1.61 (1.22-2.13)**

Studies not included due to insufficient control for tobacco use

Study Information	Snus Use	Cigarette Use
Janzon and Hedblad 2009 Cohort study Male residents of Malmö, Sweden. 1991 – 1996 through 2004 All risk estimates adjusted for age, BMI, smoking habits, diabetes, hypertension, physical activity, marital status, and occupation. ICD9: 430, 431, 434, 436 (stroke)	First ever stroke Nontobacco users: 1.0 (reference) Snuff user, never-smoker (n=4): 0.59 (0.2-1.5)	First ever stroke Nontobacco users: 1.0 (reference) Smoker, snuff user (n=13): 1.13 (0.6-2.0)
Koskinen and Blomstedt 2006 Case-control study Northern Sweden (Umea University Hospital) 1997-1998 Risk estimates were not adjusted for potential confounders. Subarachnoid haemorrhage	Among Men Reference not defined: 1.0 (reference) Snuff use: 0.48 (0.17-1.30) Among Women Reference not defined: 1.0 (reference) Snuff use: 1.30 (0.33-5.18)	Among Men Reference not defined: 1.0 (reference) Smoking: **2.63 (1.20-5.72)** Among Women Reference not defined: 1.0 (reference) Smoking: **2.26 (1.69-3.01)**

Snus & Smoking Review Summary Estimates

Study Information	Summary Estimate
Cigarette Use	
Friedman et al. 1997 Kaiser Population 1979-1986, followed through 1987 Age-adjusted relative risk of death of current smokers compared to never-smokers.	Males Current smoker (n=11): 0.8 (0.4-1.7) Females Current smoker (n=20): 1.3 (0.8-2.2)
Rostron 2012 National Health Interview Survey (NHIS) – Linked Mortality Files 1997 – 2004, followed through 2006 Stroke Mortality	Males 35-64 Current smoker: 1.59 (0.81-3.11) Former smoker: 1.07 (0.50-2.26) Males 65+ Current smoker: 1.08 (0.71-1.64) Former smoker: 1.00 (0.75-1.33) Females 35-64

Study Information	Summary Estimate
	Current smoker: **3.39 (1.81-6.33)** Former smoker: 2.07 (0.85-5.07) Females 65+ Current smoker: **2.11 (1.59-2.81)** Former smoker: 1.09 (0.85-1.41)
SAMMEC CPS II Population 1982 – 1988 Relative risk of death among adults aged 35 and older.	Males 35-64 Current smoker: 3.27 Former smoker: 1.04 Males 65+ Current smoker: 1.63 Former smoker: 1.04 Females 35-64 Current smoker: 4.00 Former smoker: 1.30 Females 65+ Current smoker: 1.49 Former smoker: 1.03
Shinton and Beevers 1989 Stroke Smoking meta-analysis (32 studies)	Overall: **1.5 (1.4-1.6)** Alcohol-adjusted: **2.4 (1.9-2.9)** Age, blood pressure & obesity adjusted: **2.6 (2.3-2.9)** Stroke subtype: Cerebral infarction: **1.92 (1.71-2.16)** Haemorrhagic stroke: 1.01 (0.81-1.26) Intracerebral haemorrhage: **0.74 (0.56-0.98)** Subarachnoid haemorrhage: **2.93 (2.48-3.46)** Sex: Men: **1.43 (1.35-1.52)** Women: **1.72 (1.59-1.86)** Age: <55: **2.94 (2.40-3.59)** 55-74: **1.75 (1.56-1.97)** ≥75: 1.11 (0.96-1.28) Cigarettes/day: Low (mainly <10): **1.37 (1.24-1.52)** Intermediate (mainly 10-20): **1.45 (1.33-1.57)** High (>20): **1.82 (1.70-1.96)**
US Surgeon General 1989 CPS II Population 1982 – 1986 Relative risk of death among adults aged 35 and older.	Males >35 Current smoker: **2.24 (1.88-2.67)** Former smoker: **1.29 (1.10-1.51)** Males 35-64 Current smoker: **3.67 (2.51-5.36)** Former smoker: 1.38 (0.91-2.07) Males 65+ Current smoker: **1.94 (1.58-2.38)** Former smoker: **1.27 (1.07-1.50)** Females >35 Current smoker: **1.84 (1.56-2.16)** Former smoker: 1.06 (0.88-1.27)

Study Information	Summary Estimate
	Females 35-64 Current smoker: **4.80 (3.52-6.54)** Former smoker: 1.41 (0.94-2.13) Females 65+ Current smoker: **1.47 (1.19-1.81)** Former smoker: 1.01 (0.83-1.24)
Snus Use	
Boffetta and Straif 2009 Snus meta-analysis (any and fatal stroke)	Any stroke Sweden: 1.02 (0.93-1.13) Fatal stroke Sweden: 1.25 (0.91-1.70)
Lee 2011 Stroke Snus meta-analysis (incidence)	Whole population: 1.05 (0.95-1.15) Never-smokers: 1.06 (0.96-1.17)
Lee 2007 Snus (Sweden) meta-analysis (incidence)	Current use (never-smokers): 1.17 (0.80-1.70)

Summary of Study Quality & Comparability

Study design: Two case-control studies (Asplund et al. 2003; Koskinen and Blomstedt 2006) and four cohort studies (Bolinder et al. 1994; Haglund et al. 2007; Hansson et al. 2009; Janzon and Hedblad 2009).

Reference group comparability: Five of the six studies used common reference groups (never-users of any tobacco) for snus and smoking risk estimates, while the sixth study (Koskinen and Blomstedt 2006) did not identify reference groups.

Confounding:

	Age	Sex	Physical activity	Diabetes	High Cholest -erol	SES	BMI	High Blood pressure/hy-pertension	Snus, Cigs
Asplund et al. 2003	X	X		X	X	X		X	X
Bolinder et al. 1994	X	X		X			X	X	X
Haglund et al. 2007	X	X	X			X			X
Hansson et al. 2009	X	X		X	X			X	X
Janzon and Hedblad 2009	X	X	X	X		X	X	X	
Koskinen and Blomstedt 2006									

Outcome comparability:

Author	Outcome
Asplund et al. 2003	First ever fatal or nonfatal stroke
Bolinder et al. 1994	Stroke/cerebrovascular disorders. ICD8: 430-438: 430 Subarachnoid haemorrhage 431 Cerebral haemorrhage 432 Occlusion of precerebral arteries 433 Cerebral thrombosis 434 Cerebral embolism 435 Transient cerebral ischaemia 436 Acute but ill-defined cerebrovascular disease 437 Generalised ischaemic cerebrovascular disease 438 Other and ill-defined cerebrovascular disease
Haglund et al. 2007	Stroke. ICD9: 430-438; ICD10: I60-I69 ICD9: 430 Subarachnoid haemorrhage 431 Intracerebral haemorrhage 432 Other and unspecified intracranial haemorrhage 433 Occlusion and stenosis of precerebral arteries 434 Occlusion of cerebral arteries 435 Transient cerebral ischaemia 436 Acute but ill-defined cerebrovascular disease 437 Other and ill-defined cerebrovascular disease 438 Late effects of cerebrovascular disease ICD10: I60 Subarachnoid haemorrhage I61 Intracerebral haemorrhage I62 Other nontraumatic intracranial haemorrhage I63 Cerebral infarction I64 Stroke, not specified as haemorrhage or infarction I65 Occlusion and stenosis of precerebral arteries, not resulting in cerebral infarction I66 Occlusion and stenosis of cerebral arteries, not resulting in cerebral infarction I67 Other cerebrovascular diseases I68 Cerebrovascular disorders in diseases classified elsewhere I69 Sequelae of cerebrovascular disease
Hansson et al. 2009	Stroke. ICD10: I60-I61, I63-I64, G45; ICD9: 430-431, 433-436 ICD9: 430 Subarachnoid haemorrhage 431 Intracerebral haemorrhage 433 Occlusion and stenosis of precerebral arteries 434 Occlusion of cerebral arteries 435 Transient cerebral ischaemia 436 Acute but ill-defined cerebrovascular disease ICD10: I60 Subarachnoid haemorrhage I61 Intracerebral haemorrhage I63 Cerebral infarction I64 Stroke, not specified as haemorrhage or infarction G45 Transient cerebral ischaemic attacks and related syndromes
Janzon and Hedblad 2009	Stroke. ICD9: 430 (Subarachnoid haemorrhage), 431 (Intracerebral haemorrhage), 434 (Occlusion of cerebral arteries), 436 (Acute but ill-defined cerebrovascular disease)
Koskinen and Blomstedt 2006	Subarachnoid haemorrhage

Exposure comparability (intra-study): Only Bolinder et al. (1994) and Janzon and Hedblad (2009) compare different exposure groups within each study. Bolinder et al. compares smokeless tobacco use with smoking dose, while Janzon and Hedblad compare snuff use among never smokers with dual users.

Dose-risk or duration-risk relationship:

Asplund et al. (2003)
Smokers: no analysis.
Snus users: no analysis.

Bolinder et al. (1994)
Smokers: a dose-response relationship was observed (significance not reported).
Snus users: no analysis.

Haglund et al. (2007)
Smokers: no analysis.
Snus users: no analysis.

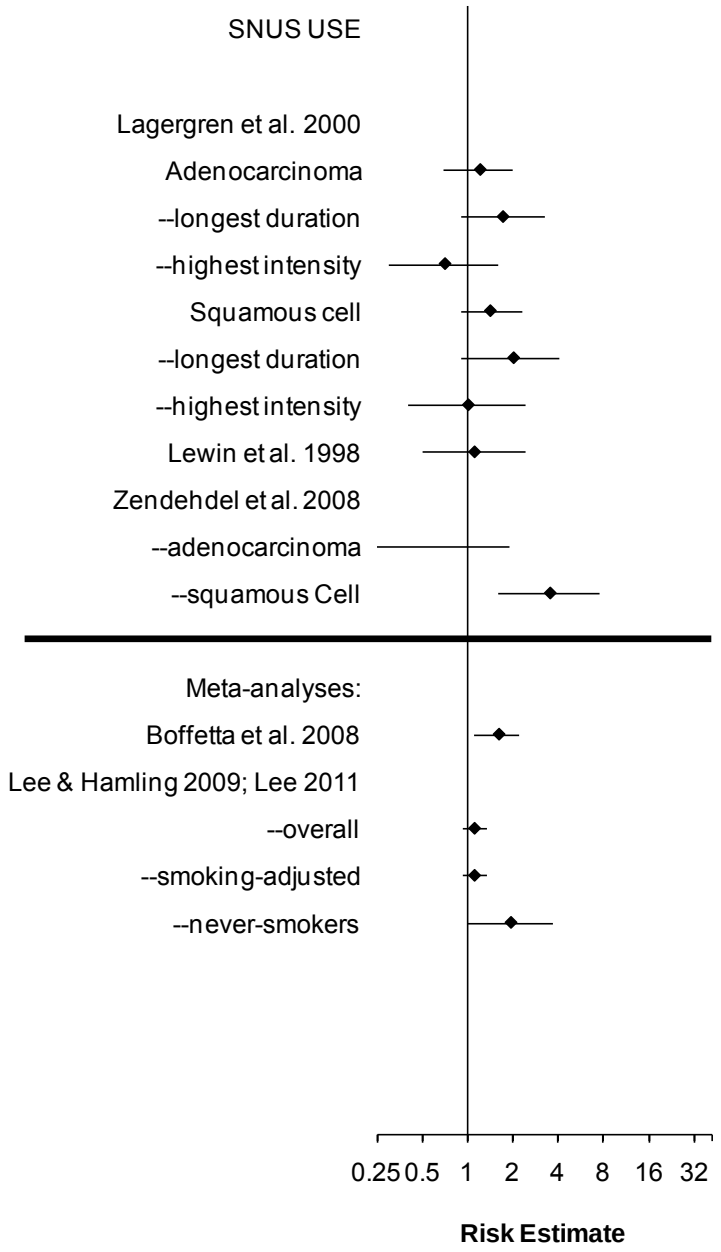
Hansson et al. (2009)
Smokers: no analysis.
Snus users: risks were higher (nonsignificant) among higher dose groups but not for duration (significance not reported).

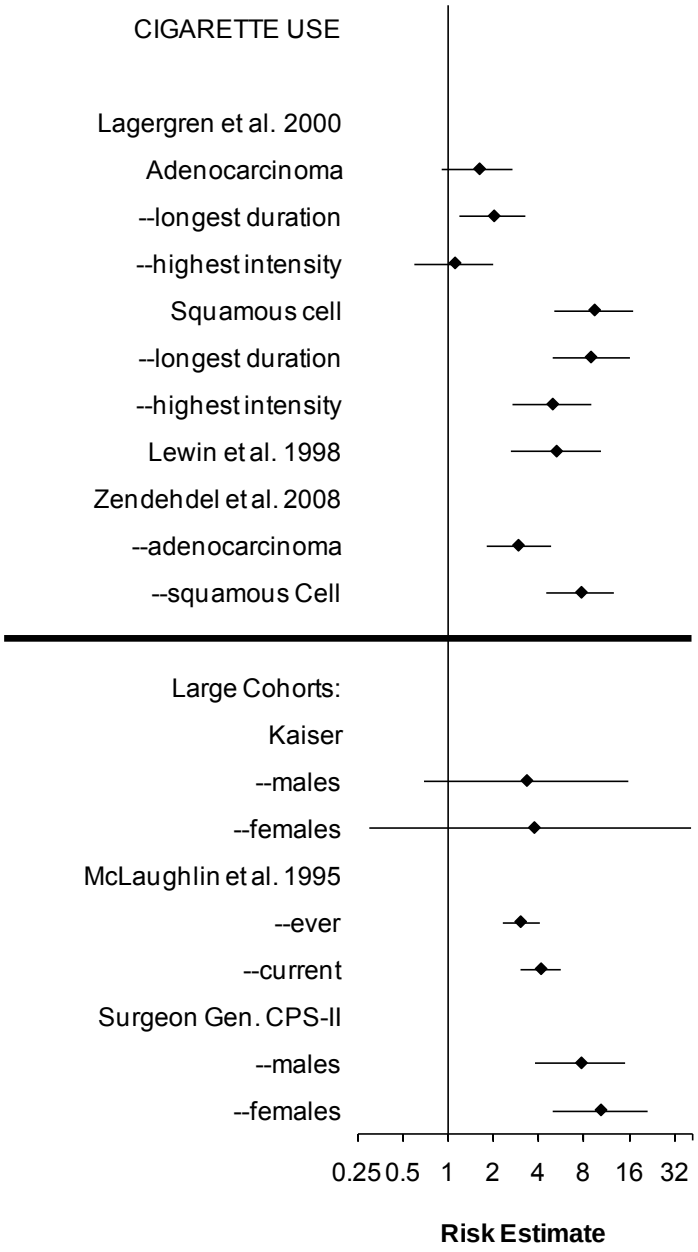
Janzon and Hedblad (2009)
Smokers: no analysis.
Snus users: no analysis.

Koskinen and Blomstedt (2006)
Smokers: no dose-response relationship observed (significance not reported).
Snus users: no analysis.

Studies with no smoking comparison:
Hergens et al. 2008

Figure A VI-4: Esophageal Cancer





ENVIRON

Table A VI-4: Esophageal Cancer

Risk factors: Alcohol use, a diet high in fruits and vegetables are linked to a lower risk, obesity (adenocarcinoma), male (3x higher than women), age (most cases occur in those 65 and older), gastroesophageal reflux disease (GERD- adenocarcinoma), Barrett's esophagus (adenocarcinoma), exposure to solvents used for dry cleaning, people who have had treatment to rid the stomach of *H. pylori*, and people who have had other cancers such as lung, mouth and throat cancer are at higher risk (ACS 2012).

Smoking-attributable mortality in the US (2000-2004): Overall: 68%, Males: 72%, Females: 56%, Among Smokers only: 2.2%.

ICD Codes: ICD-8: 150, ICD-9: 150, ICD-10: C15

Head-to-Head Study Comparisons
Bold: statistically significant
Highlight: risk estimates used in forest plots
(n="# of exposed cases")

Study Information	Snus Use	Cigarette Use
Lagergren et al. 2000 Case-control study Swedish population-1995 – 1997 All risk estimates are multivariate-adjusted for age, gender, alcohol use, educational level, BMI, reflux symptoms, intake of fruit and vegetables, energy intake and physical activity, and mutual adjustments for tobacco smoking or snuff use.	Adenocarcinoma *Use Status* Never user of snuff: 1.0 (reference) Ever (n=35): 1.2 (0.7-2.0) *Duration of use (years)* Never user of snuff: 1.0 (reference) 1-10 (n=10): 1.0 (0.5-2.1) 11-25 (n=10): 1.0 (0.5-2.0) >25 (n=15): 1.7 (0.9-3.3) *Intensity of use (quids/week)* Never user of snuff: 1.0 (reference) 1-14 (n=11): 1.0 (0.5-2.1) 15-35 (n=17): **2.2 (1.2-4.1)** >35 (n=7): 0.7 (0.3-1.6) Squamous cell carcinoma *Use Status* Never user of snuff: 1.0 (reference) Ever (n=33): 1.4 (0.9-2.3) *Duration of use (years)* Never user of snuff: 1.0 (reference) 1-10 (n=11): 1.2 (0.5-2.5) 11-25 (n=8): 0.9 (0.4-2.1) >25 (n=14): 2.0 (0.9-4.1)	Adenocarcinoma *Use Status* Never-smoker: 1.0 (reference) Previous (n=89): **1.9 (1.2-2.9)** Current (n=43): 1.6 (0.9-2.7) *Duration of smoking (years)* Never-smoker: 1.0 (reference) 1-20 (n=42): **1.8 (1.1-3.1)** 21-35 (n=37): 1.5 (0.9-2.6) >35 (n=53): **2.0 (1.2-3.3)** *Smoking dose (cigs/day)* Never-smoker: 1.0 (reference) 1-9 (n=32): 1.2 (0.7-2.2) 10-19 (n=46): 1.7 (1.0-2.9) >19 (n=41): 1.1 (0.6-2.0) *Years since cessation* Never-smoker: 1.0 (reference) 0-2 (n=40): 1.7 (1.0-3.0) 3-10 (n=20): **2.4 (1.2-4.8)** 11-25 (n=29): 1.6 (0.9-2.5) >25 (n=30): 1.6 (0.9-2.8) Squamous cell carcinoma *Use Status* Never-smoker: 1.0 (reference) Previous (n=44): **2.5 (1.4-4.7)** Current (n=101): **9.3 (5.1-17)** *Duration of smoking (years)* Never-smoker: 1.0 (reference) 1-20 (n=21): **2.3 (1.1-4.6)** 21-35 (n=27): **2.9 (1.5-5.8)** >35 (n=97): **8.8 (4.9-16.1)**

Study Information	Snus Use	Cigarette Use
	Intensity of use (quids/week) Never user of snuff: 1.0 (reference) 1-14 (n=10): 1.2 (0.5-2.5) 15-35 (n=15): 2.1 (1.0-4.4) >35 (n=7): 1.0 (0.4-2.4)	*Smoking dose (cigs/day)* Never-smoker: 1.0 (reference) 1-9 (n=28): **2.8 (1.5-5.2)** 10-19 (n=54): **3.9 (2.2-6.9)** >19 (n=7): **4.9 (2.7-9.0)** *Years since cessation* Never-smoker: 1.0 (reference) 0-2 (n=93): **10.3 (5.6-19.1)** 3-10 (n=18): **5.2 (2.4-11.3)** 11-25 (n=15): 2.1 (1.0-4.7) >25 (n=13): 1.9 (0.8-4.0)
Lewin et al. 1998 Case-control study Stockholm county or Southern healthcare region of Sweden 1988 – 1990 All risk estimates adjusted for age, region, tobacco intake and alcohol intake. Head and neck cancer estimates including esophageal cancer included in study but not provided here.	Never used: 1.0 Ever used (n=19): 1.2 (0.7-2.2) Current users (n=10): 1.1 (0.5-2.4) Ex-users (n=9): 1.3 (0.6-3.1)	Never smoked: 1.0 Current: **5.2 (2.6-10.3)** ≥45 years: **5.4 (2.7-11.0)**
Zendehdel et al. 2008 Cohort study Swedish Construction Worker cohort 1971 – 1993 and followed through 2004 All risk estimates adjusted for attained age, BMI and smoking (among ever smokers or entire cohort). "Snus user only" estimate was among never-smokers and had a reference group that matched that of the smoking reference group (never-user of any tobacco). Smokers were never users of snus.	<u>Adenocarcinoma</u> Never-users of any tobacco: 1.0 (reference) Snus user only (n=1): 0.2 (0.0-1.9) Never-users of snus: 1.0 (reference) Snus user (full cohort)(n=27): 1.0 (0.6-1.5) Snus user (ever-smoke)(n=26): 1.3 (0.8-2.0) <u>Squamous cell carcinoma</u> Never-users of any tobacco: 1.0 (reference) Snus user only (n=10): **3.5 (1.6–7.6)** Never-users of snus: 1.0 (reference) Snus user (full cohort)(n=50): 1.0 (0.8-1.4) Snus user (ever-smoke)(n=40): 1.2 (0.8-1.7)	<u>Adenocarcinoma</u> Never-users of any tobacco: 1.0 (reference) Ever smoker: **2.3 (1.4–3.7)** Current smoker: **2.9 (1.8–4.8)** <10 g/day: 1.8 (0.9–3.2) 10-19 g/day: **3.8 (2.1–6.7)** ≥20 g/day: **4.7 (2.5–9.0)** <u>Squamous cell carcinoma</u> Never-users of any tobacco: 1.0 (reference) Ever smoker: **5.2 (3.1–8.6)** Current smoker: **7.6 (4.5–12.7)** <10 g/day: **6.9 (4.0–11.8)** 10-19 g/day: **6.3 (3.5–11.1)** ≥20 g/day: **11.2 (6.2–20.2)**

Snus & Smoking Review Summary Estimates

Study Information	Summary Estimate
Cigarette Use	
Friedman et al. 1997 Kaiser Population 1979-1986, followed through 1987 Age-adjusted relative risk of death of current smokers compared to never-smokers.	Males Current smoker (n=4): 3.3 (0.7-15.8) Females Current smoker (n=1): 3.7 (0.3-41.1)
McLaughlin et al. 1995 Cohort study US veterans who held government life insurance policies active at the end of 1953. Followed through 1980. Mortality	Use Status Ever smoker: **3.0 (2.3-4.1)** Current smoker: **4.1 (3.0-5.6)** Former smoker: 1.5 (1.0-2.2) Smoking Dose (cigs/day) 1-9: 1.4 (0.7-2.7) 10-20: **3.3 (2.4-4.7)** 31-39: **6.7 (4.7-9.4)** 40+: **6.1 (3.5-10.7)** **P<0.01**
SAMMEC CPS II Population 1982 – 1988 Relative risk of death among adults aged 35 and older.	Males Current smoker: 6.76 Former smoker: 4.46 Females Current smoker: 7.75 Former smoker: 2.79
US Surgeon General 1989 CPS II Population 1982 – 1986 Relative risk of death among adults aged 35 and older.	Males Current smoker: **7.60 (3.81-15.17)** Former smoker: **5.83 (3.02-11.25)** Females Current smoker: **10.25 (4.94-21.27)** Former smoker: **3.16 (1.45-6.85)**
Snus Use	
Lee & Hamling 2009; Lee 2011 Snus meta-analysis (incidence) Adenocarcinoma and squamous cell carcinoma risk estimates combined for Lagergren et al. 2000 and Zendehdel et al. 2008.	Overall data: 1.10 (0.92-1.33) Smoking-adjusted: 1.10 (0.92-1.33) Never smokers: 1.92 (1.00-3.68)
Boffetta et al. 2008 Snus meta-analysis (incidence) for Nordic countries. Uses squamous cell carcinoma estimates only (higher estimates) from Lagergren et al. 2000 and Zendehdel et al. 2008.	Ever use: **1.6 (1.1-2.2)**

Summary of Study Quality & Comparability

Study design: Two case-control studies and one cohort study (Zendehdel et al. 2008).

Reference group comparability: Only one of the three studies use common reference groups (never-users of any tobacco) for snus and smoking risk estimates (Zendehdel et al. 2008). Reference groups for the other two studies include never users of snus and never-smokers.

Confounding:

	Age	Sex	Alcohol	Bodyweight/ BMI	Physical Activity	Diet	Gastroesophageal Reflux Disease	Educational Level	Snus, Cigs
Lagergren et al. 2000	X	X	X	X	X	X	X	X	X
Lewin et al. 1998	X	X	X						X
Zendehdel et al. 2008	X	X		X					

Outcome comparability: Lagergren et al. (2000) and Zendehdel et al. (2008) report outcomes of both adenocarcinoma and squamous cell carcinoma, while the Lewin et al. (1998) study population included those with head and neck cancer, however subsite risk estimates are reported, including esophageal cancer in general (combined subsites). Only Zendehdel et al. report using the ICD-7 code 150 for esophageal cancer and WHO/HS/CANC/24.1 histology codes for the subsites: 096 for adenocarcinoma and 146 for squamous cell carcinoma.

Exposure comparability (intra-study): Only Lewin et al. (1998) compare the same exposure groups (current snus users vs. current smokers). The other studies differ in use status (e.g. ever use vs. current use), duration and other exposure descriptions. It is unclear whether the exposure group "user of snus only" is comparable to the "ever-smokers" group in the Zendehdel et al. (2008) study.

Dose-risk or duration-risk relationship:

Lagergren et al. (2000)
Smokers: significant dose and duration response for squamous cell carcinoma.
Snus users: no dose-response observed.

Lewin et al. (1998)
Smokers: no analysis.
Snus users: no analysis.

Zendehdel et al. (2008)
Smokers: significant does response for adenocarcinoma.
Snus users: no dose-response observed.

Studies with no smoking comparison:
Boffetta et al. 2005

Figure A VI-5: Pancreatic Cancer

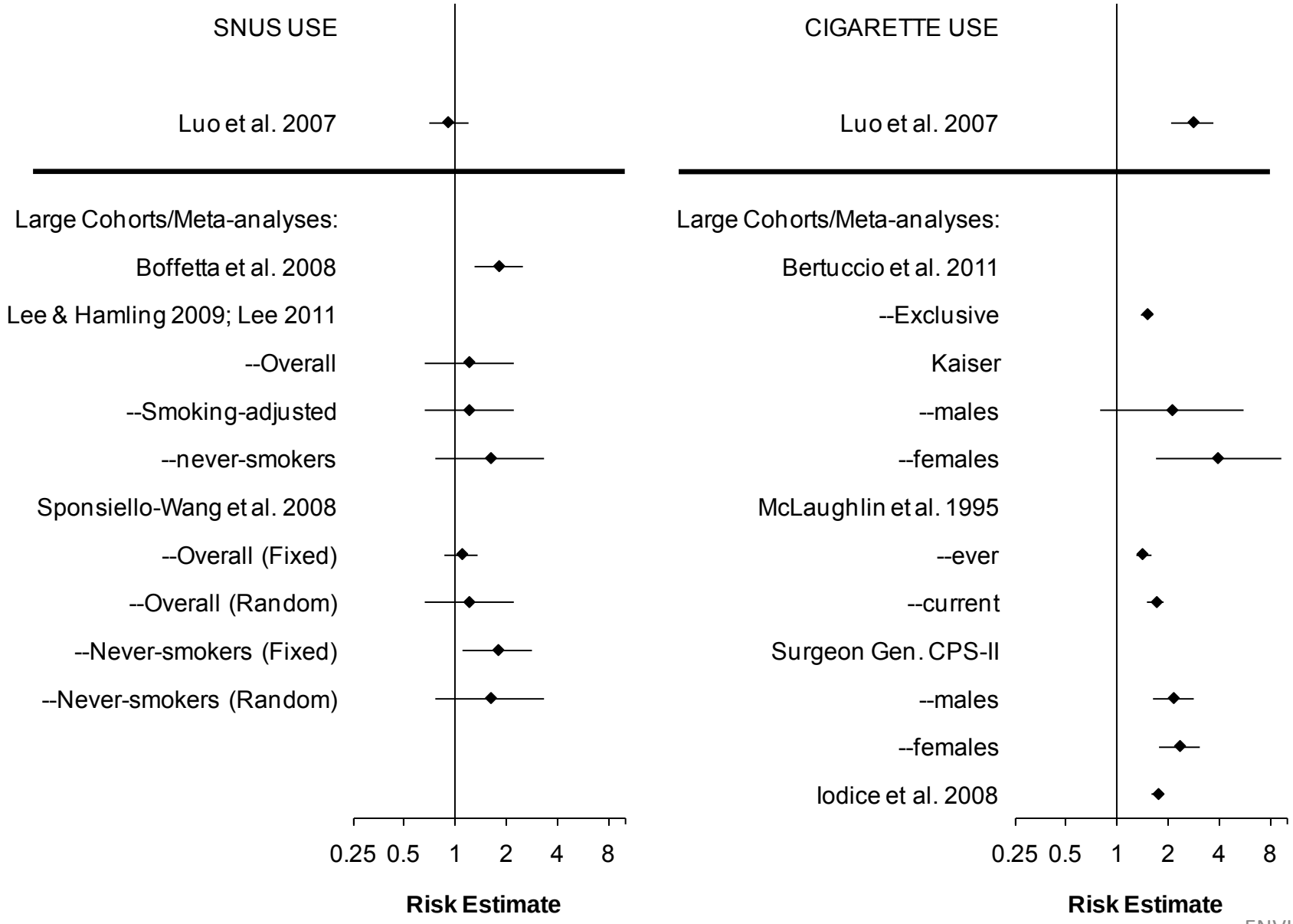


Table A VI-5: Pancreatic Cancer

Risk factors: Age (60 and older), diabetes, male gender, African American ethnicity, family history, chronic pancreatitis (NCI 2010), alcohol use (US Surgeon General 2004).

Smoking-attributable mortality in the US (2000-2004): Overall: 22%, Males: 21%, Females: 23%, Among Smokers only: 1.7%.

ICD Codes: ICD-7: 157, ICD-8: 157, ICD-9: 157, ICD-10: C25

Head-to-Head Study Comparisons
Bold: statistically significant
Highlight: risk estimates used in forest plots
(n="# of exposed cases")

Study Information	Snus Use	Cigarette Use
Heuch et al. 1983 Cohort study General Norwegian population, relatives of Norwegian migrants to the US and male and female spouses of the siblings of individuals interviewed in a case-control study of gastrointestinal cancer. 1964, 1967 – 1968 All risk estimates adjusted for region, urban/rural place of residence, age and sex. Some analyses adjusted for tobacco habit and alcohol. Tobacco type unclear and population followed up by Boffetta et al. 2005.	All cases of pancreatic cancer (regular use vs. never used) Among all individuals with chewing data (n=12): 1.34 (p=0.21) Histologically-verified cases only (regular use vs. never used) Among all individuals with chewing data (n=9): **2.20 (p=0.045)** Among men with alcohol, cigarette and chewing data (n=6): 2.31 (p=0.067) Among men with alcohol, cigarette and chewing data, with adjustment for alcohol use and cigarette smoking (n= 6): 2.85 (p=0.060)	All cases of pancreatic cancer (≥10 cigs/day vs. never smoked) Among men with cigarette data (n=6): 1.13 (p=0.35) Histologically-verified cases only (≥10 cigs/day vs. never smoked) Among men with cigarette data (n=5): 2.04 (p=0.087) Among men with alcohol, cigarette and chewing data (n=4): 1.88 (p=0.13) Among men with alcohol, cigarette and chewing data, with adjustment for alcohol use and tobacco chewing (n=4): 2.13 (p=0.12)
Luo et al. 2007 Cohort study Swedish Construction Worker cohort 1978 – 1992 All risk estimates adjusted for age and BMI. Snus and smoking estimates among all cohort members adjusted for respective tobacco use.	Among all cohort members Never-users of any tobacco 1.0 (reference) Ever-users of snus: 0.9 (0.7-1.2) Among never-smokers Never-users of any tobacco: 1.0 (reference) Ever-users of snus (n=20): **2.0 (1.2-3.3)** Ex-users (n=2): 1.4 (0.4-5.9) Current users (n=18): **2.1 (1.2-3.6)** 1-9 g/day (n=6): 1.9 (0.8-4.3) ≥10 g/day (n=13): **2.1 (1.1-3.8)**	Among all cohort members Never-users of any tobacco: 1.0 (reference) Ever-smokers (n=385): **2.8 (2.1-3.7)** Ex-smokers (n=105): **1.8 (1.3-2.4)** Current smokers (n=280): **3.5 (2.6-4.6)**

Studies not included due to insufficient control for tobacco use

Study Information	Snus Use	Cigarette Use
Boffetta et al. 2005 Cohort study Two sources: General Norwegian population (1960 census) and relatives of Norwegian migrants to the US (1964-1967 questionnaire). All risk estimates adjusted for age, sex and smoking (smoking estimates are among snuff users).	Among all cohort members Never user of snus: 1.0 (reference) Ever user of snus (n=45): **1.67 (1.12-2.50)** Former user (n=18): **1.80 (1.04-3.09)** Current user of snus(n=27): 1.60 (1.00-2.55) Among never-smokers Never user of snus: 1.0 (reference) Ever user of snus (n=3): 0.85 (0.24-3.07)	Among ever-users of snus according to smoking status Never user of snus: 1.0 (reference) Never smokers (n=3): 0.85 (0.24-3.07) Former smokers (n=14): 1.37 (0.59-3.17) Current smokers (n=28): **1.86 (1.13-3.05)**

Snus & Smoking Review Summary Estimates

Study Information	Summary Estimate
Cigarette Use	
Bertuccio et al. 2011 Pooled-analysis of 11 case-control studies (international) Adjusted for center, race, sex, age, education, history of diabetes, body mass index and total alcohol consumption.	Never tobacco user: 1.00 (reference) Cigarette-only smokers: **1.50 (1.39-1.62)**
Friedman et al. 1997 Kaiser Population 1979-1986, followed through 1987 Age-adjusted relative risk of death of current smokers compared to never-smokers.	Males Current smoker (n=8): 2.1 (0.8-5.6) Females Current smoker (n=12): **3.9 (1.7-9.3)**
Iodice et al. 2008 Cigarette meta-analysis (82 studies)	Current smoker: **1.74 (1.61-1.87)** Former smoker: 1.2 (1.11-1.29)
McLaughlin et al. 1995 Cohort study US veterans who held government life insurance policies active at the end of 1953. Followed through 1980. Mortality	Use Status Ever smoker: **1.4 (1.3-1.6)** Current smoker: **1.7 (1.5-1.9)** Former smoker: 1.1 (0.9-1.3) Smoking Dose (cigs/day) 1-9: **1.4 (1.1-1.8)** 10-20: **1.7 (1.4-1.9)** 31-39: **1.8 (1.5-2.2)** 40+: **1.6 (1.1-2.3)** **P<0.01**

Study Information	Summary Estimate
SAMMEC CPS II Population 1982 – 1988 Relative risk of death among adults aged 35 and older.	Males Current smoker: 2.31 Former smoker: 1.15 Females Current smoker: 2.25 Former smoker: 1.55
US Surgeon General 1989 CPS II Population 1982 – 1986 Relative risk of death among adults aged 35 and older.	Males Current smoker: **2.14 (1.62-2.85)** Former smoker: 1.12 (0.86-1.45) Females Current smoker: **2.33 (1.77-3.08)** Former smoker: **1.78 (1.37-2.30)**
Snus Use	
Bertuccio et al. 2011 Meta-analysis of 11 case-control studies (international – non-snus) Adjusted for center, race, sex, age, education, history of diabetes, body mass index and total alcohol consumption.	Never tobacco user: 1.00 (reference) Ever smokeless tobacco: 0.98 (0.75-1.27) Smokeless tobacco-only: 0.62 (0.37-1.04)
Boffetta et al. 2008 Snus meta-analysis (incidence) for Nordic countries. Chose higher RR of smoking-adjusted and never-smoking estimates from Boffetta et al. 2005 (smoking-adjusted) and Luo et al. 2007 (never-smokers).	Ever use: **1.8 (1.3-2.5)**
Lee & Hamling 2009; Lee 2011 Snus meta-analysis (incidence) Combined and presented estimates for smoking-adjusted and never-smokers separately from Boffetta et al. 2005 and Luo et al. 2007.	Overall data: 1.2 (0.66-2.20) Smoking-adjusted: 1.2 (0.66-2.20) Never smokers: 1.61 (0.77-3.34)
Sponsiello-Wang et al. 2008 Snus meta-analysis (incidence)	Sweden or Norway Overall (never-smokers used if overall estimate not available) Fixed-effect: 1.09 (0.87-1.36) Random-effects: 1.20 (0.66-2.20) Never-smokers (overall used if never-smokers estimate not available) Fixed-effect: **1.78 (1.11-2.85)** Random-effects: 1.61 (0.77-3.34)

Summary of Study Quality & Comparability

Study design: Three cohort studies.

Reference group comparability: Two of the three studies used common reference groups (never-users of any tobacco) for snus and smoking risk estimates (Luo et al. 2007; Boffetta et al. 2005). Reference groups used in the other study include never users of snus and never-smokers.

Confounding: All three studies controlled for age and gender. Heuch et al. (1983) controlled for alcohol use, while Luo et al. (2007) controlled for BMI. Neither Boffetta et al. or Luo et al. controlled for alcohol use.

	Age	Sex	Alcohol	BMI	Race	Diabetes	Family History	Chronic Pancreatitis	Snus, Cigs
Heuch et al. 1983	X	X	X						X
Boffetta et al. 2005	X	X							
Luo et al. 2007	X	X		X					X

Outcome comparability: Luo et al. (2007) and Boffetta et al. (2005) used ICD-7 code 157 for pancreatic cancer, while Heuch et al. (1983) used new cases of pancreatic cancer identified from the Cancer Registry of Norway.

Exposure comparability (intra-study): Boffetta et al. (2005) did not use comparable exposure groups since the smoking estimates were among only ever users of snus. The remaining two studies use comparable exposure groups. However, in the Heuch et al. study it is unclear how much of the smokeless tobacco includes snus since it is referred to as "chewing tobacco". Also, the type of tobacco is likely different from today's Swedish snus.

Dose-risk or duration-risk relationship:

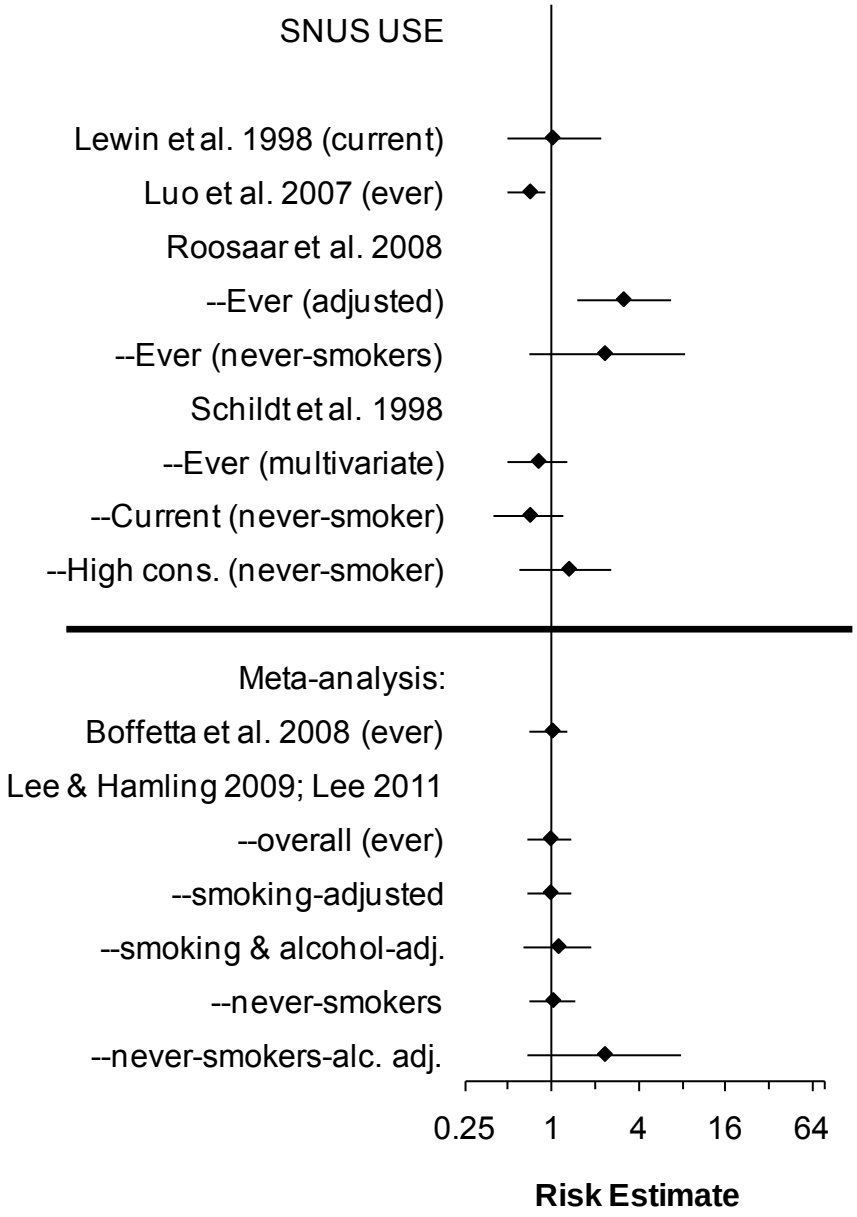
Boffetta et al. (2005)
Smokers: no analysis.
Snus users: no analysis.

Luo et al. (2007)
Smokers: no analysis.
Snus users: significant dose-response observed.

Heuch et al. (1983)
Smokers: no analysis.
Snus users: no analysis.

Studies with no smoking comparison:
None

Figure A VI-6: Oral Cancer



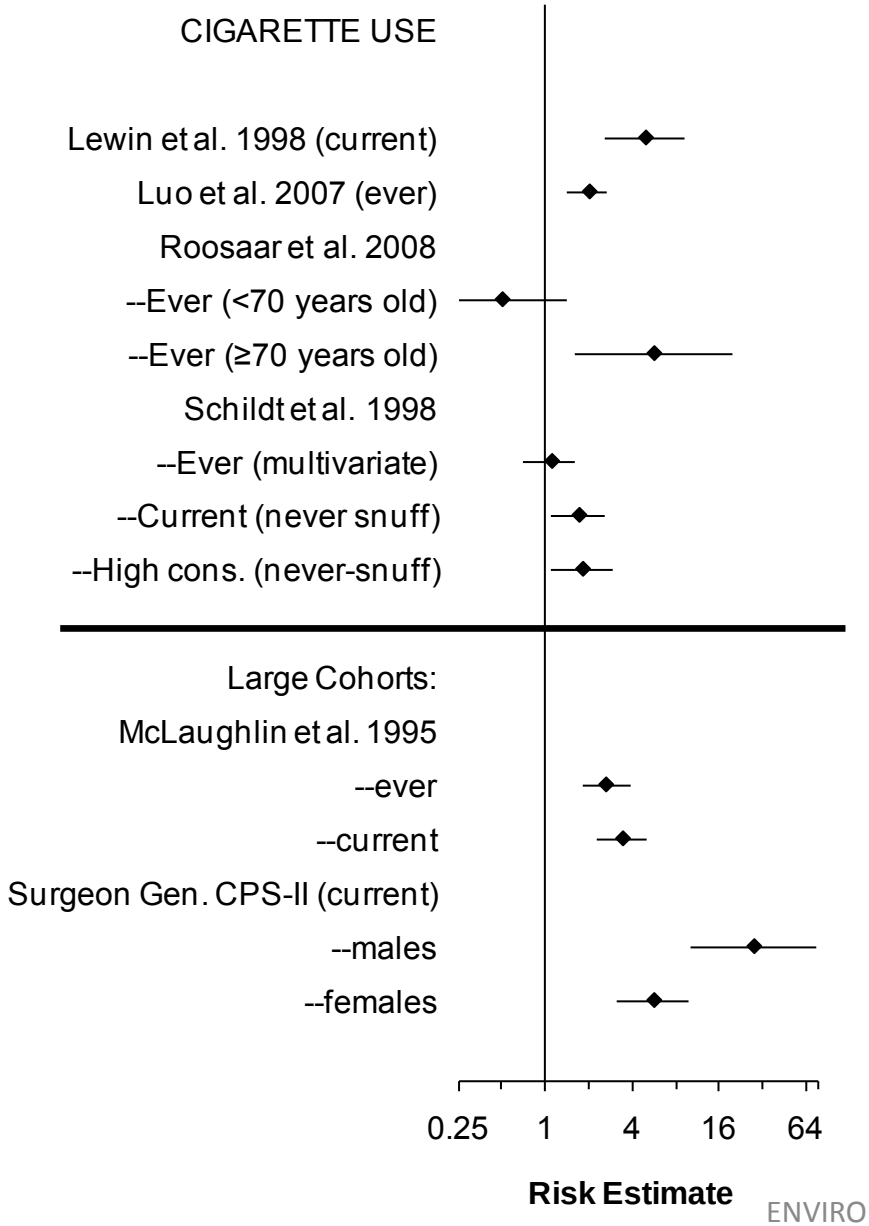


Table A VI-6: Oral (oropharyngeal) Cancer

Risk factors: Heavy alcohol use, HPV infection, excessive sun exposure (lip), diet (lack of fruits and vegetables) (NCI 2009a).

Smoking-attributable mortality in the US (2000-2004): Overall: 64%, Males: 73%, Females: 46%, Among Smokers only: 1.2%.

ICD Codes: ICD-7: 140-148, ICD-8: 140-149, ICD-9: 140-149, ICD-10: C00-C14

Head-to-Head Study Comparisons
Bold: statistically significant
Highlight: risk estimates used in forest plots
(n="# of exposed cases")

Study Information	Snus Use	Cigarette Use
Lewin et al. 1998 Case-control study Stockholm county or Southern healthcare region of Sweden 1988 – 1990 All risk estimates adjusted for age, region, tobacco intake and alcohol intake.	Oral cavity Never used: 1.0 (reference) Ever used (n=25): 1.4 (0.8-2.4) Current users (n=10): 1.0 (0.5-2.2) Ex-users (n=15): 1.8 (0.9-3.7) Pharynx Never used: 1.0 (reference) Ever used (n=15): 0.7 (0.4-1.3) Current users (n=8): 0.7 (0.3-1.5) Ex-users (n=7): 0.8 (0.3-1.9)	Oral cavity Never smoked: 1.0 (reference) Current: **4.9 (2.6-9.2)** ≥45 years: **6.3 (3.2-12.4)** Pharynx Never smoked: 1.0 (reference) Current: **8.5 (4.0-18.2)** ≥45 years: **10.1 (4.6-22.1)**
Luo et al. 2007 Cohort study Swedish Construction Worker cohort 1978 – 1992, and followed through 2004 All risk estimates adjusted for age and BMI. Snus and smoking estimates among all cohort members adjusted for respective tobacco use.	Among all cohort members Never-users of any tobacco 1.0 (reference) Ever-users of snus: **0.7 (0.5-0.9)** Among never-smokers Never-users of any tobacco: 1.0 (reference) Ever-users of snus (n=10): 0.8 (0.4-1.7) Ex-users (n=1): 0.7 (0.1-5.0) Current users (n=9): 0.9 (0.4-1.8) 1-9 g/day (n=2): 0.7 (0.2-2.8) ≥10 g/day (n=8): 0.9 (0.4-2.0)	Among all cohort members Never-users of any tobacco: 1.0 (reference) Ever-smokers (n=198): **2.0 (1.4-2.7)** Ex-smokers (n=48): 1.1 (0.8-1.7) Current smokers (n=150): **2.5 (1.7-3.5)**
Roosaar et al. 2008 Cohort study Uppsala County, Central Sweden 1973 – 1974, and followed through 2002 All risk estimates adjusted for age, gender (males only), area of residence, smoking or snus use and alcohol consumption.	Never daily use: 1.0 (reference) Ever daily use (n=11): **3.1 (1.5-6.6)** Restricted to never smokers Never daily use: 1.0 (reference) Ever daily use (n=5): 2.3 (0.7-8.3)	Never daily use: 1.0 (reference) Ever daily use Age <70 years Never: 1.0 (reference) Ever (n=5): 0.5 (0.1-1.4) Age ≥70 years Never: 1.0 (reference) Ever (n=18): **5.6 (1.6-19.6)**

Study Information	Snus Use	Cigarette Use
Oral and pharyngeal		
Schildt et al. 1998 Case-control study 4 Northernmost counties of Sweden 1980 – 1989 Matched for gender, age and county and multivariate estimates adjusted for alcohol, snuff use and smoking.	Univariate analysis Never snuff user: 1.0 (reference) Active (n=39): 0.7 (0.4-1.1) Ex-user (n=28): 1.5 (0.8-2.9) Ever user (n=67): 0.9 (0.6-1.4) Among never smokers Never snuff user: 1.0 (reference) Ex-user of snuff (n=9): 1.8 (0.9-3.5) Active snuff user (n=19): 0.7 (0.4-1.2) Among ex-smokers Never snuff user: 0.9 (0.6-1.4) Ex-user of snuff (n=16): 1.6 (0.8-3.4) Active snuff user (n=15): 0.6 (0.3-1.3) Among active smokers Never snuff user: **1.7 (1.1-2.6)** Ex-user of snuff (n=3): **3.1 (1.4-6.8)** Active snuff user (n=10): 1.2 (0.6-2.4) Multivariate analysis Snuff (ever): 0.8 (0.5-1.3) Among never smokers Never snuff: 1.0 (reference) Low consumption: 0.8 (0.4-1.6) High consumption: 1.3 (0.6-2.6) Among low smoking consumption Never snuff: 1.2 (0.7-1.9) Low consumption (n=6): 1.0 (0.4-2.1) High consumption (n=7): 1.5 (0.6-3.5) Among high smoking consumption Never snuff: **1.8 (1.1-2.9)** Low consumption (n=10): 1.5 (0.6-3.3) High consumption (n=3): 2.3 (0.9-5.6)	Univariate analysis Never smoker: 1.0 (reference) Active (n=122): **1.8 (1.1-2.7)** Ex-smoker (n=80): 1.0 (0.6-1.6) Ever smoker (n=202): 1.3 (0.9-1.9) Among never snuff user Never smoker: 1.0 (reference) Ex-smoker (n=54): 0.9 (0.6-1.4) Active smoker (n=109): **1.7 (1.1-2.6)** Among ex-user of snuff Never smoker: 1.8 (0.9-3.5) Ex-smoker (n=16): 1.6 (0.8-3.4) Active smoker (n=3): **3.1 (1.4-6.8)** Among active snuff users Never smoker (n=): 0.7 (0.4-1.2) Ex-smoker (n=15): 0.6 (0.3-1.3) Active smoker (n=10): 1.2 (0.6-2.4) Multivariate analysis Smoking (ever): 1.1 (0.7-1.6) Among never snuff Never smokers (n=): 1.0 (reference) Low consumption (n=): 1.2 (0.7-1.9) High consumption: **1.8 (1.1-2.9)** Among low snuff consumption Never smokers: 0.8 (0.4-1.6) Low consumption (n=6): 1.0 (0.4-2.1) High consumption (n=10): 1.5 (0.6-3.3) Among high snuff consumption Never smokers: 1.3 (0.6-2.6) Low consumption (n=7): 1.5 (0.6-3.5) High consumption (n=3): 2.3 (0.9-5.6)

Studies not included due to insufficient control for tobacco use

Study Information	Snus Use	Cigarette Use
Rosenquist et al. 2005 Case-control study (population-based) Swedish population 2000 – 2004 All risk estimates adjusted for age, sex, alcohol use, HPV (data not shown), and county. Snuff estimates were adjusted for tobacco smoking, but it is unclear whether the smoking estimates were adjusted for snuff use.	Oral Snuff Use Never used: 1.0 (reference) Had used (n=20): 0.7 (0.3-1.3) Current user (n=13): 1.1 (0.5-2.5) Ex-user (n=7): **0.3 (0.1-0.9)** Type of Snuff Never Used: 1.0 (reference) Fermented (n=16): 0.7 (0.3-1.4) Non-fermented (n=4): 0.6 (0.2-1.9) Duration Never used: 1.0 (reference) <30 years (n=16): 0.6 (0.3-1.3) ≥30 years (n=4): 0.8 (0.2-2.8) Exposure Time Never used: 1.0 (reference) <10 hr/day (n=15): 0.7 (0.3-1.5) >10 hr/day (n=5): 0.5 (0.2-1.6) Consumption Never used: 1.0 (reference) 1-14 g/day (n=8): 0.9 (0.3-2.5) >14 g/day (n=5): 1.7 (0.5-5.7)	Cigarette consumption (cigs/day) Never smoked: 1.0 (reference) 1-10 (n=21): 1.1 (0.6-2.1) 11-20 (n=49): **2.4 (1.3-4.1)** >20 (n=20): **2.8 (1.3-6.1)** Total tobacco consumption (kg) Never smoked: 1.0 (reference) <125 (n=23): 1.1 (0.6-2.0) 125-250 (n=24): 1.8 (1.0-3.5) >250 (n=47): **4.7 (2.4-9.1)**

Snus & Smoking Review Summary Estimates

Study Information	Summary Estimate
Cigarette Use	
McLaughlin et al. 1995 Cohort study US veterans who held government life insurance policies active at the end of 1953. Followed through 1980. Mortality	Use Status Ever smoker: **2.6 (1.8-3.9)** Current smoker: **3.4 (2.3-5.0)** Former smoker: 1.5 (0.9-2.4) Smoking Dose (cigs/day) 1-9: 0.6 (0.2-2.1) 10-20: **2.5 (1.6-4.0)** 31-39: **5.4 (3.5-8.4)** 40+: **8.6 (4.7-15.7)** **P<0.01**
SAMMEC CPS II Population 1982 – 1988 Relative risk of death among adults aged 35 and older.	Males Current smoker: 10.89 Former smoker: 3.40 Females Current smoker: 5.08 Former smoker: 2.29
US Surgeon General 1989 CPS II Population 1982 – 1986 Relative risk of death among adults aged 35 and older.	Males Current smoker: **27.48 (9.96-75.83)** Former smoker: **8.80 (3.15-24.59)** Females Current smoker: **5.59 (3.15-9.91)** Former smoker: **2.88 (1.57-5.26)**

Study Information	Summary Estimate
Snus Use	
Lee & Hamling 2009; Lee 2011 Oropharynx Snus meta-analysis (incidence)	Overall data: 0.97 (0.68-1.37) Smoking-adjusted: 0.97 (0.68-1.37) Smoking and alcohol adjusted: 1.10 (0.64-1.90) Never smokers: 1.01 (0.71-1.45) Never smokers – alcohol adjusted: 2.30 (0.67-7.92)
Boffetta et al. 2008 Snus meta-analysis (incidence) for Nordic countries.	Ever use: 1.0 (0.7-1.3)

Summary of Study Quality & Comparability

Study design: Three case-control studies (Lewin et al. 1998; Rosenquist et al. 2005; Schildt et al. 1998) and two cohort studies (Luo et al. 2007; Roosaar et al. 2008).

Reference group comparability: Only one of the five studies use common reference groups (never-users of any tobacco) for snus and smoking risk estimates (Luo et al. 2007). Reference groups for the other studies included never-users of snus and never-smokers.

Confounding:

	Age	Sex	Sun exposure	Diet	Alcohol consumption	HPV	BMI	Snus, Cigs
Lewin et al. 1998	X	X			X			X
Luo et al. 2007	X	X					X	X
Roosaar et al. 2008	X	X			X			X
Rosenquist et al. 2005	X	X			X	X		
Schildt et al. 1998	X	X			X			X

Outcome comparability:

Author	Outcome
Lewin et al. 1998	head and neck cancer (squamous cell carcinoma of the oral cavity, oro- and hypopharynx, larynx, and esophagus – also oral cavity, larynx and pharynx analyzed separately
Luo et al. 2007	ICD-7: 140 (lip), 141 (tongue), 143 (floor of mouth), and 144 (oral cavity, not otherwise specified) (not including cancers of the salivary glands, pharynx, or larynx)
Roosaar et al. 2008	Combined category of oral and pharyngeal cancer: ICD-7: 140-148: 140 Malignant neoplasm of lip 141 Malignant neoplasm of tongue 142 Malignant neoplasm of salivary gland 143 Malignant neoplasm of floor of mouth 144 Malignant neoplasm of other parts of mouth, and of mouth, unspecified 145 Malignant neoplasm of oral mesopharynx 146 Malignant neoplasm of nasopharynx 147 Malignant neoplasm of hypopharynx 148 Malignant neoplasm of pharynx, unspecified
Rosenquist et al. 2005	Oropharyngeal squamous cell carcinoma (OOSCC). ICD-7: 141 (tongue), 143 (floor of mouth), 144 (oral cavity, not otherwise specified) and 145 (oropharynx)
Schildt et al. 1998	ICD-7: 140 (lip), 141 (tongue), 143 (floor of mouth), 144 (oral cavity, not otherwise specified), 145 (oropharynx)

Exposure comparability (intra-study): Only Lewin et al. (1998), Luo et al. (2007) and Schildt et al. (1998) compare the same exposure groups within each study. The other studies differ in use status (e.g. ever use vs. current use), duration, consumption, age group and other exposure descriptions.

Dose-risk or duration-risk relationship:

Lewin et al. (1998)
Smokers: no analysis.
Snus users: no analysis.

Luo et al. (2007)
Smokers: no dose-response observed.
Snus users: no analysis.

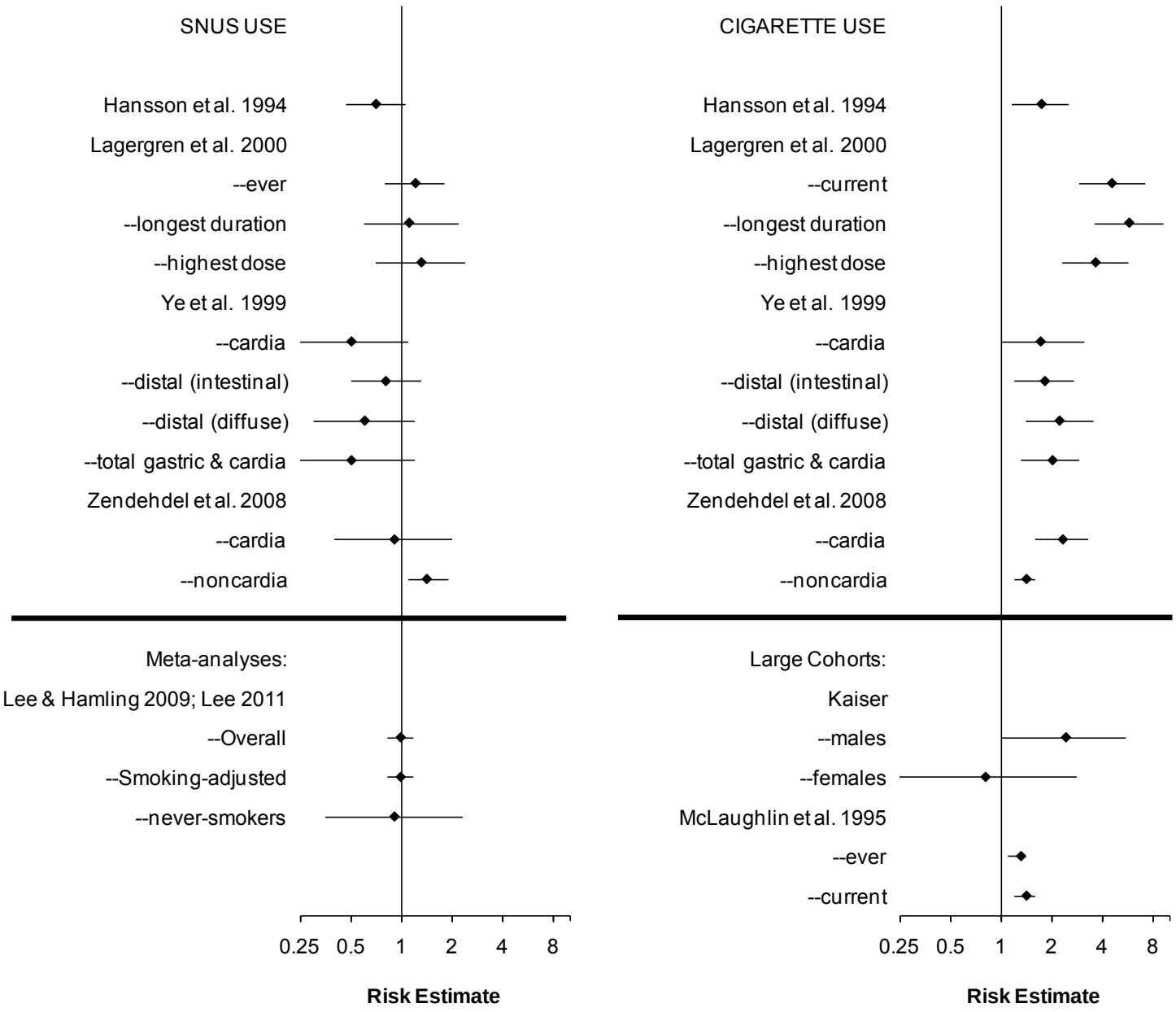
Rosenquist et al. (2005)
Smokers: significant dose-response relationship observed.
Snus users: no dose-response observed.

Schildt et al. (1998)
Smokers: significant dose-response relationship observed.
Snus users: no dose-response observed.

Roosaar et al. (2008)
Smokers: no analysis.
Snus users: no analysis.

Studies with no smoking comparison:
Boffetta et al. 2005

Figure A VI-7: Stomach Cancer



ENVIRON

Table A VI-7: Stomach Cancer

Risk factors: *H. pylori* infection, long term inflammation of the stomach, smoking, family history, poor diet (high in foods that are smoked, salted, or pickled), lack of physical activity, obesity, gender (more common in men), ethnicity (in the US: more common in Hispanic and African Americans, and most common in Asian/Pacific Islanders) and geography (most common in Japan, China, southern and Eastern Europe, and South and Central America) (NCI 2009b; ACS 2013b).

Smoking-attributable mortality in the US (2000-2004): Overall: 21%, Males: 27%, Females: 12%, Among Smokers only: 0.6%.

ICD Codes: ICD-7: 151, ICD-8: 151, ICD-9: 151, ICD-10: C16

Head-to-Head Study Comparisons
Bold: statistically significant
Highlight: risk estimates used in forest plots
(n="# of exposed cases")

Study Information	Snus Use	Cigarette Use
Hansson et al. 1994 Case-control (population-based) Swedish population (5 counties) 1989-1992 All risk estimates were adjusted for age, gender, SES, vegetable intake and other tobacco use. Gastric cancer	Snuff dipping: 0.70 (0.47-1.06) *(Reference group was not specified.)*	Non-users of tobacco: 1.0 (reference) Ex-smokers (n=85): 1.09 (0.75-1.59) Current smokers (n=78): **1.72 (1.16-2.54)**
Lagergren et al. 2000 Case-control study Swedish population-1995 – 1997 All risk estimates are multivariate-adjusted for age, gender, alcohol use, educational level, BMI, reflux symptoms, intake of fruit and vegetables, energy intake and physical activity, and mutual adjustments for tobacco smoking or snuff use. Gastric cardia adenocarcinoma	Use Status Never user of snuff: 1.0 (reference) Ever (n=53): 1.2 (0.8-1.8) Duration of use (years) Never user of snuff: 1.0 (reference) 1-10 (n=18): 1.0 (0.5-1.8) 11-25 (n=19): 1.1 (0.6-2.0) >25 (n=15): 1.1 (0.6-2.2) Intensity of use (quids/week) Never user of snuff: 1.0 (reference) 1-14 (n=19): 1.2 (0.6-2.1) 15-35 (n=15): 1.3 (0.7-2.5) >35 (n=18): 1.3 (0.7-2.4)	Use Status Never-smoker: 1.0 (reference) Previous (n=124): **3.4 (2.2-5.2)** Current (n=95): **4.5 (2.9-7.1)** Duration of smoking (years) Never-smoker: 1.0 (reference) 1-20 (n=38): **2.1 (1.2-3.4)** 21-35 (n=77): **3.9 (2.4-6.2)** >35 (n=104): **5.7 (3.6-9.1)** Smoking dose (cigs/day) Never-smoker: 1.0 (reference) 1-9 (n=46): **2.3 (1.4-3.7)** 10-19 (n=73): **3.1 (2.0-4.9)** >19 (n=86): **3.6 (2.3-5.7)** Years since cessation Never-smoker: 1.0 (reference) 0-2 (n=87): **5.0 (3.2-8.0)** 3-10 (n=35): **4.9 (2.8-8.7)** 11-25 (n=53): **4.2 (2.6-7.0)**

Study Information	Snus Use	Cigarette Use
		>25 (n=30): **2.1 (1.2-3.6)**
Ye et al. 1999 Case-control (population-based) Swedish population (5 counties) 1989-1995 Risk estimates for snuff use were adjusted for age, residence area, BMI, socio-economic status, and smoking. Odds ratios among smokers and exclusive tobacco groups were adjusted for age, gender, residence area, BMI, SES, use of smokeless tobacco, and use of beer, wine and liquor. Cardia cancer, intestinal-type gastric cancer, and diffuse-type gastric cancer.	Cardia cancer Never-users: 1.0 (reference) Ex-users (n=6): 0.8 (0.3-1.9) Current users (n=9): 0.5 (0.2-1.1) Ever-users (n=15): 0.6 (0.3-1.2) Distal gastric cancer-intestinal Never-users: 1.0 (reference) Ex-users (n=18): 0.9 (0.5-1.6) Current users (n=26): 0.8 (0.5-1.3) Ever-users (n=44): 0.8 (0.5-1.2) Distal gastric cancer-diffuse Never-users: 1.0 (reference) Ex-users (n=8): 0.7 (0.3-1.6) Current users (n=11): 0.6 (0.3-1.2) Ever-users (n=19): 0.7 (0.4-1.2) Total gastric and cardia cancer Never tobacco: 1.0 (reference) Ever user (never smoke) (n=11): 0.5 (0.2-1.2)	Cardia cancer Never-smokers: 1.0 (reference) Ex-smokers (n=25): 0.9 (0.5-1.6) Current smokers (n=31): 1.7 (1.0-3.1) Distal gastric cancer-intestinal Never-smokers: 1.0 (reference) Ex-smokers (n=101): 1.4 (1.0-2.0) Current smokers (n=67): **1.8 (1.2-2.7)** Distal gastric cancer-diffuse Never-smokers: 1.0 (reference) Ex-smokers (n=46): 1.3 (0.8-2.0) Current smokers (n=57): **2.2 (1.4-3.5)** Total gastric and cardia cancer Never tobacco: 1.0 (reference) Current smoker (never snus) (n=101): **2.0 (1.3-2.9)**
Zendehdel et al. 2008 Cohort study Swedish Construction Worker cohort 1971 – 1993 and followed through 2004 All risk estimates adjusted for attained age, BMI and smoking (among ever smokers or entire cohort). Only snus estimates among never smokers had a reference group that matched that of the smoking reference group (never-user of any tobacco). Smokers were never users of snus.	Cardia Never-users of any tobacco: 1.0 (reference) User of snus only (n=8): 0.9 (0.4-2.0) Never-users of snus: 1.0 (reference) User of snus (full cohort) (n=58): 1.0 (0.8-1.4) User of snus (ever-smokers) (n=50): 1.1 (0.8-1.6) Noncardia Never-users of any tobacco: 1.0 (reference) User of snus only (n=68): **1.4 (1.1-1.9)** Never-users of snus: 1.0 (reference) User of snus (full cohort) (n=253): 1.1 (1.0-1.3) User of snus (ever-smokers) (n=185): 1.0 (0.9-1.2)	Cardia Never-users of any tobacco: 1.0 (reference) Ever smoker: **2.1 (1.5-3.0)** Current smoker: **2.3 (1.6-3.3)** <10 g/day: **2.1 (1.4-3.1)** 10-19 g/day: **2.4 (1.6-3.7)** ≥20 g/day: **3.0 (1.8-5.0)** Noncardia Never-users of any tobacco: 1.0 (reference) Ever smoker: **1.3 (1.2-1.6)** Current smoker: **1.4 (1.2-1.6)** <10 g/day: **1.3 (1.1-1.6)** 10-19 g/day: **1.4 (1.2-1.8)** ≥20 g/day: **1.4 (1.1-1.9)**

Snus & Smoking Review Summary Estimates

Study Information	Summary Estimate
Cigarette Use	
Friedman et al. 1997 Kaiser Population 1979-1986, followed through 1987 Age-adjusted relative risk of death of current smokers compared to never-smokers.	Males Current smoker (n=12): 2.4 (1.0-5.5) Females Current smoker (n=3): 0.8 (0.2-2.8)
McLaughlin et al. 1995 Cohort study US veterans who held government life insurance policies active at the end of 1953. Followed through 1980. Mortality	Use Status Ever smoker: **1.3 (1.1-1.4)** Current smoker: **1.4 (1.2-1.6)** Former smoker: 1.0 (0.9-1.2) Smoking Dose (cigs/day) 1-9: 1.3 (1.0-1.7) 10-20: **1.4 (1.2-1.6)** 31-39: **1.4 (1.2-1.8)** 40+: **1.9 (1.3-2.7)** **P<0.01**
SAMMEC CPS II Population 1982 – 1988 Relative risk of death among adults aged 35 and older.	Males Current smoker: 1.96 Former smoker: 1.47 Females Current smoker: 1.36 Former smoker: 1.32
Snus Use	
Lee & Hamling 2009; Lee 2011 Snus meta-analysis (incidence)	Overall data: 0.98 (0.82-1.17) Smoking-adjusted: 0.98 (0.82-1.17) Never smokers: 0.90 (0.35-2.30)

Summary of Study Quality & Comparability

Study design: Three case-control studies and one cohort study (Zendehdel et al. 2008).

Reference group comparability: Two of the four studies use common reference groups (never-users of any tobacco) for snus and smoking risk estimates (Zendehdel et al. 2008; Ye et al. 1999). The reference groups used in the Lagergren et al. (2000) study include never users of snus and never-smokers. The reference group among snuff users in the Hansson et al. (1994) study is unspecified, however, it's likely that it was "non-users of tobacco" as was used for the smoking reference group.

Confounding:

	Age	Sex	Alcohol	Bodyweight / BMI	Physical Activity	Diet	Gastroesophageal Reflux Disease	SES	Snus, Cigs
Hansson et al. 1994	X	X				X		X	X
Lagergren et al. 2000	X	X	X	X	X	X	X	X	X
Ye et al. 1999	X	X	X	X				X	X
Zendehdel et al. 2008	X	X		X					X

Outcome comparability:

Author	Outcome
Hansson et al. 1994	Gastric cancer
Lagergren et al. 2000	Gastric cardia adenocarcinoma
Ye et al. 1999	Cardia cancer, intestinal-type gastric cancer, and diffuse-type gastric cancer
Zendehdel et al. 2008	ICD-7: Cardia (151.1) and Non-cardia (all other 151) cancer

Exposure comparability (intra-study): Only Ye et al. (1999) compare the same exposure groups (current snus users vs. current smokers), however, the authors did not use a common reference group for subtype comparisons (as was done for total gastric and cardia cases). The other studies differ in use status (e.g. ever use vs. current use), dose, duration and other exposure descriptions. It is unclear whether the exposure groups "user of snus only" and "snuff dipping" are comparable to "ever-smokers" or "current smokers" in the Zendehdel et al. (2008) and Hansson et al. (1994) studies, respectively.

Dose-risk or duration-risk relationship:

Hansson et al. (1994)
Smokers: significant duration-response trend, but not a dose-response was observed.
Snus users: no analysis.

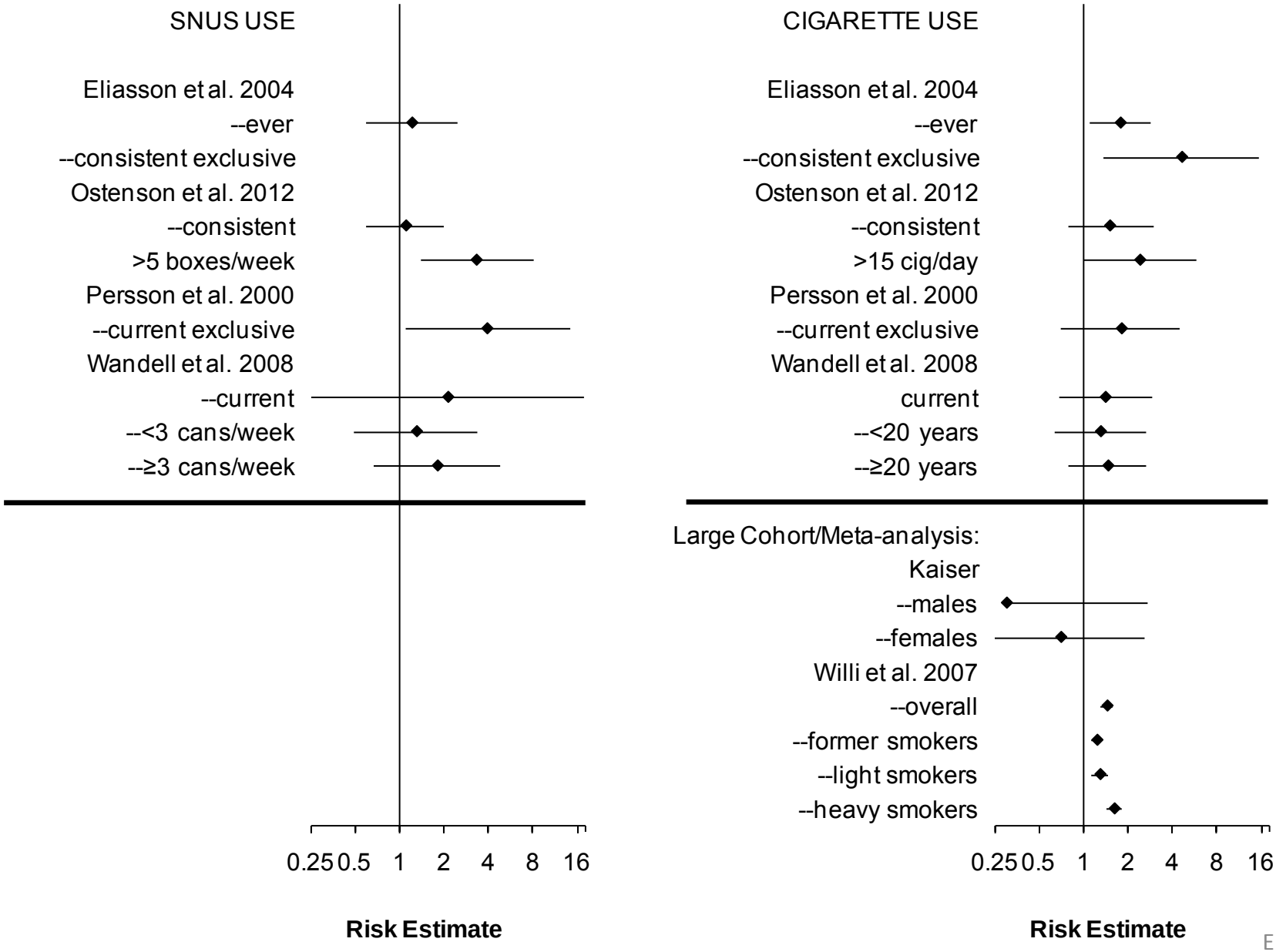
Lagergren et al. (2000)
Smokers: significant dose and duration response trends were observed.
Snus users: no significant dose- or duration- response trends were observed.

Ye et al. (1999)
Smokers: significant dose and duration response trends were observed for all subsites.
Snus users: no significant dose- or duration- response trends were observed.

Zendehdel et al. (2008)
Smokers: no significant dose response observed.
Snus users: no analysis.

Studies with no smoking comparison:
Boffetta et al. 2005

Figure A VI-8: Diabetes



ENVIRON

Table A VI-8: Diabetes

Risk factors: older age, obesity, family history of diabetes, history of gestational diabetes, impaired glucose metabolism, physical inactivity, and race/ethnicity (African Americans, Hispanic/Latino Americans, American Indians, and some Asian Americans and Native Hawaiians or Other Pacific Islanders) (CDC 2011).

Head-to-Head Study Comparisons
Bold: statistically significant
Highlight: risk estimates used in forest plots
(n="# of exposed cases")

Study Information	Snus Use	Cigarette Use
Eliasson et al. 2004 Cross-sectional and follow-up study Northern Sweden MONICA participants Prevalence from 1986, 1990, 1994 and 1999 surveys and followed for 5-13 years. Prevalence risk estimates adjusted for age, sex (men only) and waist circumference. Incidence estimates adjusted for age, follow-up and annual % weight gain between baseline and follow-up. Tobacco use categories were exclusive. Adjustment for physical activity did not change direction or significance of risk estimates. Stratified by exclusive tobacco use. Known type 2 diabetes.	Prevalence Never user of tobacco: 1.0 (reference) Ever snus use: 1.21 (0.59-2.49) Current snus user: 1.06 (0.43-2.64) Ex-snus user: 1.45 (0.54-3.87) Incidence Consistent no tobacco: 1.0 (reference) Consistent exclusive snus: 0 cases Ex-snus users: 1.72 (0.20-14.8)	Prevalence Never user of tobacco: 1.0 (reference) Ever smoking: **1.77 (1.10-2.87)** Current smoker: 1.62 (0.86-3.05) Ex-smoker: 1.87 (1.10-3.20) Incidence Consistent no tobacco: 1.0 (reference) Consistent exclus. smoker: **4.61 (1.37-15.5)** Ex-smoker: **3.13 (1.13-8.67)** Smokers switched to snus: 3.25 (0.78-13.6)
Ostenson et al. 2012 Prospective study Stockholm Diabetes Prevention Programme performed during 1992-1994 in four municipalities within Stockholm County (follow-up 10 years later). All odds ratios were	Overall Never-snus user: 1.0 (reference) Former snus use (n=6): 0.5 (0.2-1.2) Consistent snus use (n=16): 1.1 (0.6-2.0) Dose 1-5 boxes/week (n=7): 0.6 (0.2-1.4) >5 boxes/week (n=9): **3.3 (1.4-8.1)** Never-smoking snus (n=3): 2.3 (0.5-9.8)	Overall Never-smoker: 1.0 (reference) Former smoking (n=30): 0.9 (0.5-1.7) Consistent smoking (n=17): 1.5 (0.8-3.0) Dose 1-15 cigarettes/day (n=7): 0.8 (0.3-2.1) >15 cigarettes/day (n=10): 2.4 (1.0-5.8)

Study Information	Snus Use	Cigarette Use
adjusted for tobacco use, age, BMI, glucose tolerance at baseline, physical activity, alcohol consumption, socioeconomic position, and family history of diabetes. Type 2 diabetes (oral glucose tolerance test)		
Persson et al. 2000 Cross-sectional study Men living in 4 municipalities of Stockholm, Sweden 1992 – 1994 All risk estimates adjusted for age, sex (men only), BMI, family history of diabetes, physical activity, and alcohol consumption. Exclusive categories may include former smokers/snus users. Overall and dose estimates are unadjusted for tobacco use. Type 2 diabetes (oral glucose tolerance test)	Overall Never-snus user: 1.0 (reference) Former (n=5): 0.8 (0.3-2.0) Current (n=13): 1.5 (0.8-3.0) Dose ≤2 boxes/week (n=1): 0.2 (0.0-2.0) 3+ boxes/week (n=12): **2.7 (1.3-5.5)** Moist snuff only (n=4): **3.9 (1.1-14.3)**	Overall Never-cigarette user: 1.0 (reference) Former (n=21): 1.3 (0.7-2.7) Current (n=17): 1.3 (0.6-2.7) Dose 1-24 cigs/day (n=25): 1.1 (0.5-2.1) 25+ cigs/day (n=13): **2.6 (1.1-5.8)** Cigarettes only (n=15): 1.8 (0.7-4.5)
Wandell et al. 2008 Cross-sectional study Men living in Stockholm county, Sweden 1997-1999 Risk estimates adjusted for age (all 60), sex (men only), BMI, waist circumference, employment, educational level, living in an apartment, physical activity, alcohol intake and smoking and snuff duration. Newly diagnosed type 2 diabetes: fasting morning serum (fS) glucose levels ≥7.0 mmol/l in subjects with no known diagnosis of diabetes	Ex-snuffers: 3.10 (0.36-26.84) Current snuffers: 2.12 (0.25-17.71) Low consum. (<3 cans/w): 1.30 (0.49-3.40) High consum. (≥3 cans/w): 1.80 (0.67-4.85)	Ex-smokers: 1.41 (0.76-2.60) Current smokers: 1.40 (0.68-2.89) Smoking duration (<20y): 1.30 (0.64-2.66) Smoking duration (≥20y): 1.46 (0.79-2.68)

Studies not included due to insufficient control for tobacco use

Study Information	Snus Use	Cigarette Use
Hilding et al. 2005 (abstract) Cohort study Men living in Stockholm, Sweden Baseline: 1992-1994, followed for 10 years. Risk estimates adjusted for age, sex (men only), BMI, physical activity, and family history of diabetes (unclear if adjusted for tobacco use). Type 2 diabetes	Overall Never-snus user: 1.0 (reference) Current: 1.2 (0.7-2.1) Dose ≥ 4 boxes/week: 1.7 (0.8-3.4) ≥ 5 boxes/week: **2.3 (1.1-4.9)** ≥ 6 boxes/week: **3.6 (1.6-8.1)**	Overall Never-cigarette user: 1.0 (reference) Current: **2.0 (1.1-4.0)** Dose >10 cigs/day: **2.4 (1.1-5.0)**

Snus & Smoking Review Summary Estimates

Study Information	Summary Estimate
Cigarette Use	
Friedman et al. 1997 Kaiser Population 1979-1986, followed through 1987 Age-adjusted relative risk of death of current smokers compared to never-smokers.	Males Current smoker (n=1): 0.3 (0.0-2.7) Females Current smoker (n=3): 0.7 (0.2-2.6)
Willi et al. 2007 Smoking meta-analysis Relative risks adjusted for the most variables taken from each study.	Overall (active smokers): **1.44 (1.31-1.58)** Former smokers: **1.23 (1.14-1.33)** Heavy smokers (≥20 cigs/day): **1.61 (1.43-1.80)** Light smokers (<20 cigs/day): **1.29 (1.13-1.48)**
Snus Use	
None	

Summary of Study Quality & Comparability

Study design: Two cross-sectional studies, one cross-sectional study with follow up and two cohort studies.

Reference group comparability: One study used common reference groups for snus and smoking risk estimates; Eliasson et al. (2004): never-users of any tobacco. It is unclear which reference group(s) was used in another study (Wandell et al. 2008). The three other studies did not use common reference groups: never-user of snus and never-cigarette user (Ostenson et al. 2012; Persson et al. 2000; Hilding et al. 2005).

Confounding:

	Age	Sex	Weight/ BMI	Physical activity	Family history	Impaired glucose metabolism	Race	Diet	Alcohol	SES	Snus, Cigs
Eliasson et al. 2004	X	X	X								X
Hilding et al. 2005	X	X	X	X	X						
Ostenson et al. 2012	X	X	X	X	X	X			X	X	X
Persson et al. 2000	X	X	X	X	X				X		X
Wandell et al. 2008	X	X	X						X	X	X

Outcome comparability: Persson et al. (2000), Hilding et al. (2005) and Ostenson et al. (2012) tested for diabetes using an oral glucose tolerance test in men without known diabetes. Eliasson et al. (2004) identified individuals with known diabetes and Wandell et al. (2008) tested for diabetes by measuring fasting morning serum (fS) glucose levels ≥7.0 mmol/l in subjects with no known diagnosis of diabetes.

Exposure comparability (intra-study): Authors from all five studies used comparable exposure groups.

Dose-risk or duration-risk relationship:

Eliasson et al. (2004)
Smokers: no analysis.
Snus users: no analysis.

Hilding et al. (2005)
Smokers: no analysis.
Snus users: a dose-response was observed but no analysis for trend.

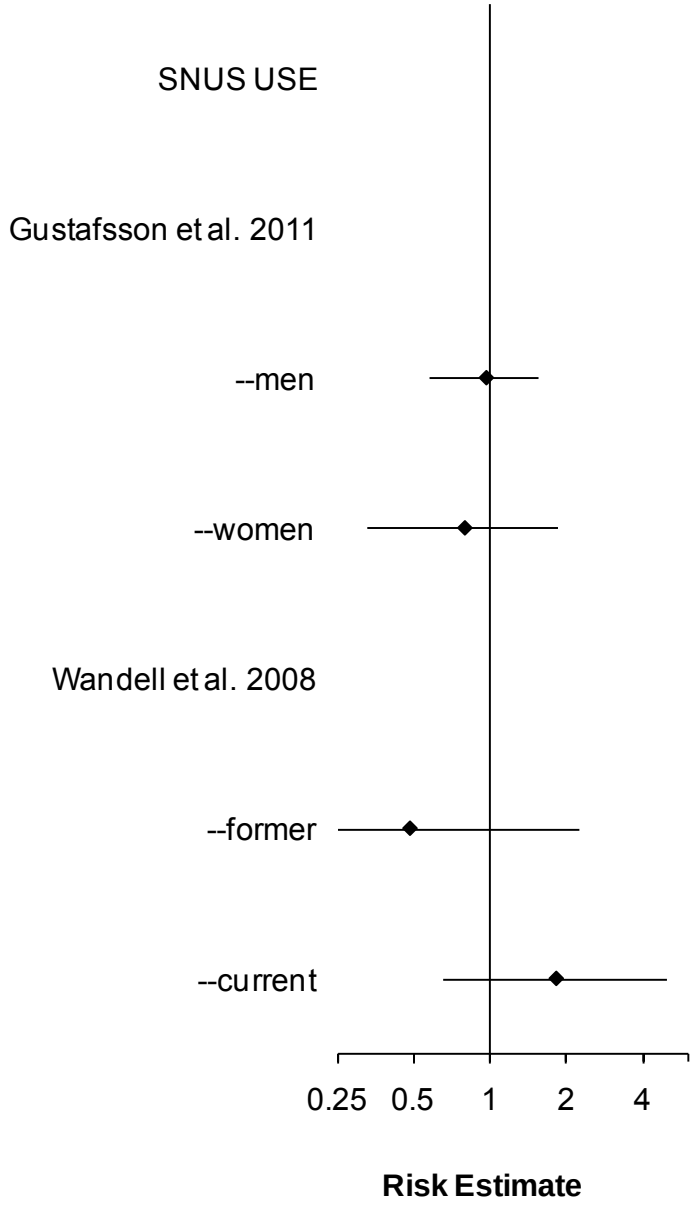
Ostenson et al. (2012)
Smokers: a dose-response was observed but no analysis for trend.
Snus users: a dose-response was observed but no analysis for trend.

Persson et al. (2000)
Smokers: a dose-response was observed but no analysis for trend.
Snus users: a dose-response was observed but no analysis for trend.

Wandell et al. (2008)
Smokers: no significant dose-response observed.
Snus users: no significant dose-response observed.

Studies with no smoking comparison:
Janzon and Hedblad 2009

Figure A VI-9: Metabolic Syndrome



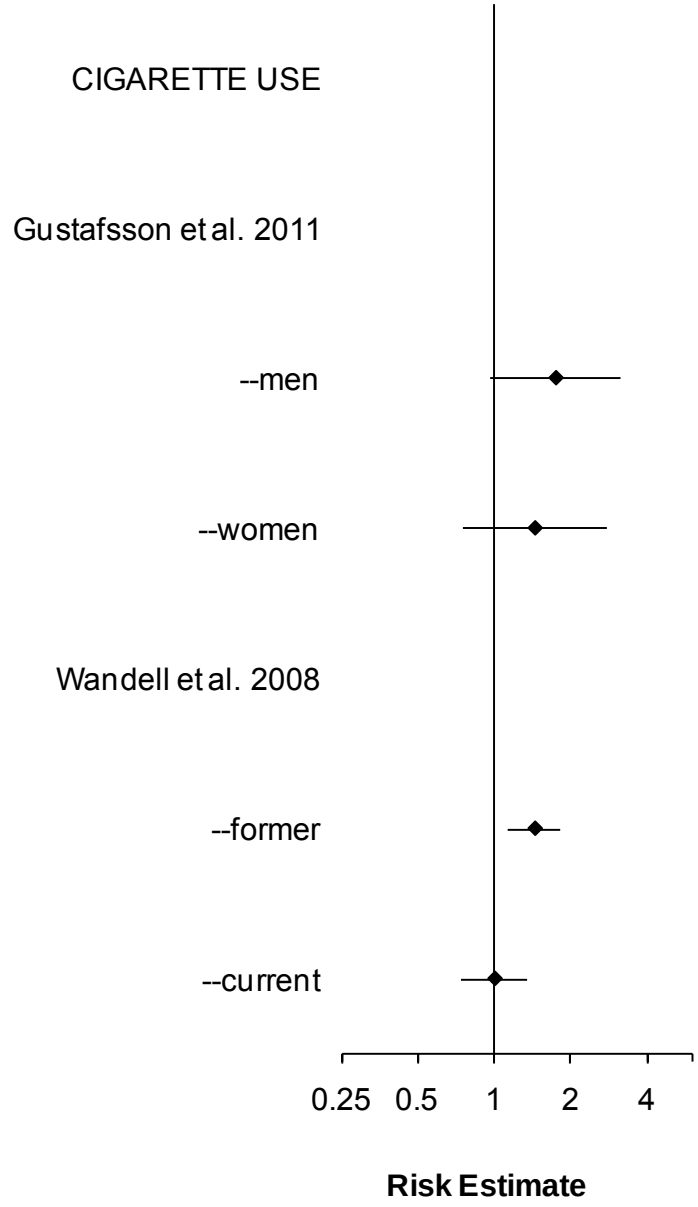


Table A VI-9: Metabolic Syndrome

Risk factors: The dominant underlying risk factors for this syndrome appear to be abdominal obesity and insulin resistance. Other conditions associated with the syndrome include physical inactivity, aging, hormonal imbalance and genetic predisposition (AHA 2014).

Head-to-Head Study Comparisons
Bold: statistically significant
Highlight: risk estimates used in forest plots
(n="# of exposed cases")

Study Information	Snus Use	Cigarette Use
Gustafsson et al. 2011 Cohort study (cross-sectional analysis of snus use) The Northern Swedish Cohort Followed from 1981 and followed up in 1983, 1986, 1995, and 2008. All risk estimates adjusted for SES, BMI, SBP, DBP, daily smoking, daily snuff use, alcohol consumption, and physical inactivity. Metabolic syndrome was diagnosed at a health examination according to the definition by the International Diabetes Federation (IDF).	Current Women: 0.79 (0.33-1.86) Men: 0.96 (0.58-1.56)	Current Women: 1.44 (0.76-2.76) Men: 1.74 (0.97-3.14)
Wandell et al. 2008 Cross-sectional study Men living in Stockholm county, Sweden 1997-1999 Risk estimates adjusted for age (all 60), sex (men only), BMI, waist circumference, employment, educational level, living in an apartment, physical activity, alcohol intake. Risk estimates presented by metabolic syndrome definition: National Cholesterol Education Program Adult Treatment Panel III (ATP III), from the	Ex-snuffers ATP III (n=2): 0.69 (0.14-3.28) EGIR (n=2): 0.97 (0.20-4.67) IDF (n=2): 0.48 (0.10-2.26) Current snuffers ATP III (n=5): 1.55 (0.52-4.62) EGIR (n=2): 0.71 (0.16-3.24) IDF (n=7): 1.81 (0.65-5.02) Ex-smokers, current snuffers ATP III (n=32): 1.14 (0.71-1.82) EGIR (n=26): 1.29 (0.78-2.14) IDF (n=42): 1.18 (0.76-1.83)	Ex-smokers ATP III (n=233): **1.49 (1.15-1.92)** EGIR (n=183): **1.55 (1.17-2.06)** IDF (n=295): **1.44 (1.14-1.83)** Current smokers ATP III (n=108): 1.18 (0.86-1.62) EGIR (n=72): 0.95 (0.66-1.37) IDF (n=124): 1.00 (0.74-1.35)

Study Information	Snus Use	Cigarette Use
European Group for the Study of Insulin Resistance (EGIR), and from the International Diabetes Federation (IDF).		

Studies not included due to insufficient control for tobacco use

Study Information	Snus Use	Cigarette Use
Norberg et al. 2006 Cohort study Vasterbotten Intervention Programmme, Vasterbotten, Sweden 1990 – 1994, followed up 10 years later All risk estimates adjusted for age, sex, and family history of CVD and/or diabetes. Metabolic syndrome was identified at follow-up according to the new definition from the International Diabetes Federation (IDF).	No-use: 1.0 (reference) ≤4 cans/week (n=174): 1.0 (0.85-1.22) >4 cans/week (n=74): **1.6 (1.26-2.15)**	Non-smoking: 1.0 (reference) Ex-smoker (n=416): **1.2 (1.06-1.38)** Daily smoking (n=402): 1.0 (0.89-1.16)

Snus & Smoking Review Summary Estimates

Study Information	Summary Estimate
Cigarette Use	
None	
Snus Use	
None	

Summary of Study Quality & Comparability

Study design: One cross-sectional study and two cohort studies.

Reference group comparability: One study did not use common reference groups for snus and smoking risk estimates; Norberg et al. (2006): no snus vs. non-smoking. It is unclear which reference group(s) was used in the other two studies (Gustafsson et al. 2011; Wandell et al. 2008).

Confounding:

	Age	Sex	Weight / BMI	Physical activity	Family history	Impaired glucose metabolism	Race	Alcohol	BP	SES	Snus, Cigs
Gustafsson et al. 2011	X	X	X	X				X	X	X	X
Norberg et al. 2006	X	X			X						
Wandell et al. 2008	X	X	X					X		X	X

Outcome comparability: All three studies reported risk estimates using the IDF definition of metabolic syndrome.

Exposure comparability (intra-study): Wandell et al. (2008) and Gustafsson et al. (2011) used comparable exposure groups. Norberg et al. (2006) reported dose groups for snus use and only reported risk estimates for daily smoking and ex-smoking for the smoking group.

Dose-risk or duration-risk relationship:

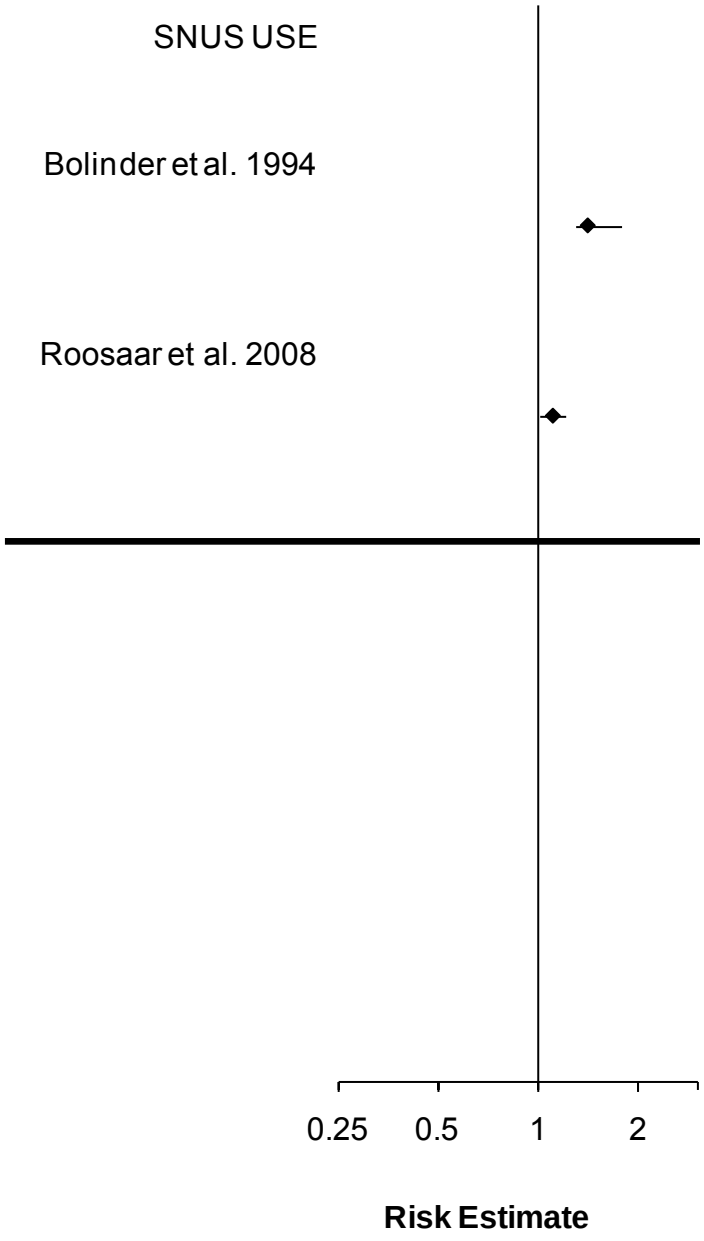
Gustafsson et al. (2011)
Smokers: no analysis.
Snus users: no analysis.

Norberg et al. (2006)
Smokers: no analysis.
Snus users: a dose-response was observed but no analysis for trend.

Wandell et al. (2008)
Smokers: no analysis.
Snus users: no analysis.

Studies with no smoking comparison:
None

Figure A VI-10: All-Cause Mortality



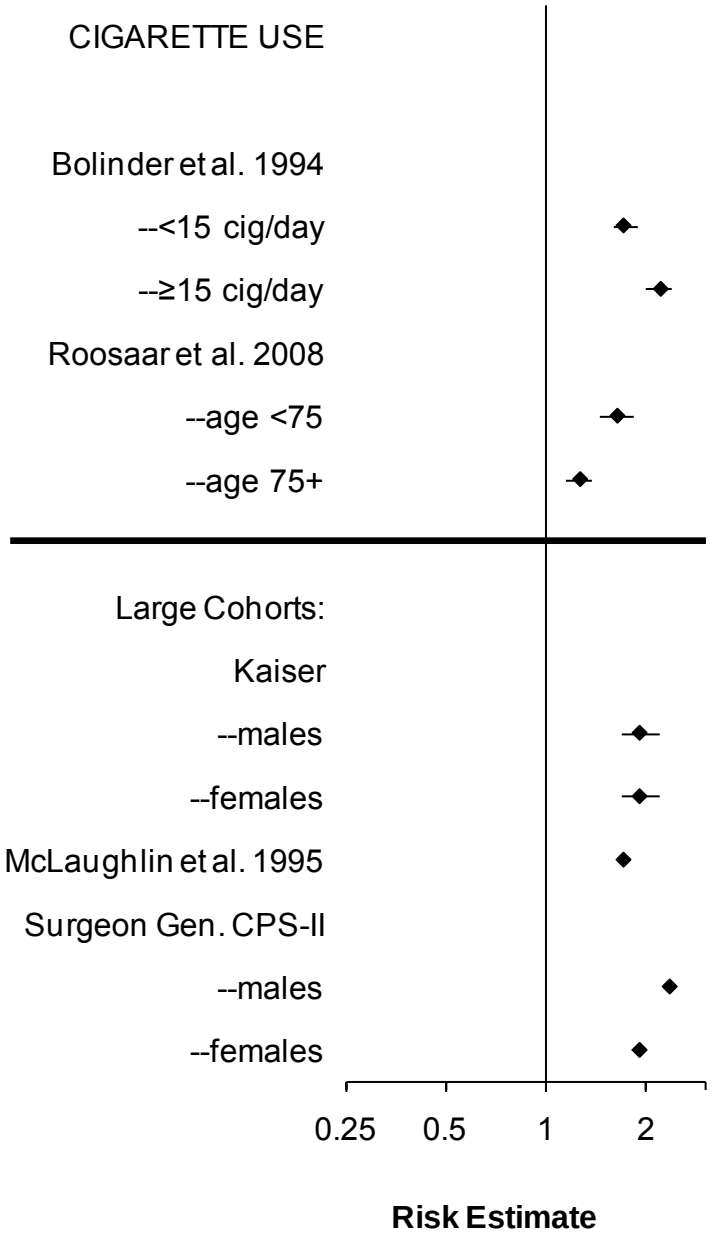


Table A VI-10: All-Cause Mortality

Head-to-Head Study Comparisons
Bold: statistically significant
Highlight: risk estimates used in forest plots
(n="# of exposed cases")

Study Information	Snus Use	Cigarette Use
Bolinder et al. 1994 Cohort study Swedish Construction Worker cohort 1971 – 1974, followed through 1985 Risk estimates adjusted for age, sex (men only) and region of origin. Tobacco use categories were exclusive. All-causes of death	All-Cause Mortality Never-users of tobacco: 1.0 (reference) Snuff users (n=440): **1.4 (1.3-1.8)** All-Cause Mortality Ages 35-54 Never-users of tobacco: 1.0 (reference) Snuff users (n=105): **1.9 (1.6-2.4)** All-Cause Mortality Ages 55-65 Never-users of tobacco: 1.0 (reference) Snuff users (n=301): 1.2 (1.0-1.3)	All-Cause Mortality Never-users of tobacco: 1.0 (reference) <15 cig/day (n=900): **1.7 (1.6-1.9)** ≥15 cig/day (n=923): **2.2 (2.0-2.4)** Ex-smokers, 1-5 years (n=350): **1.3 (1.2-1.5)** Ex-smokers, >5 years (n=776): 1.1 (1.0-1.2) All-Cause Mortality Ages 35-54 Never-users of tobacco: 1.0 (reference) <15 cig/day (n=317): **2.0 (1.7-2.3)** ≥15 cig/day (n=437): **2.6 (2.3-3.0)** Ex-smokers, 1-5 years (n=114): **1.3 (1.1-1.6)** Ex-smokers, >5 years (n=189): 1.0 (0.9-1.2) All-Cause Mortality Ages 55-65 Never-users of tobacco: 1.0 (reference) <15 cig/day (n=496): **1.6 (1.5-1.8)** ≥15 cig/day (n=377): **2.0 (1.8-2.2)** Ex-smokers, 1-5 years (n=212): **1.4 (1.2-1.6)** Ex-smokers, >5 years (n=576): 1.1 (1.0-1.2)
Roosaar et al. 2008 Cohort study Uppsala County, central Sweden Exposure information collected 1973-1974 and followed through 2002 All risk estimates adjusted for age, sex (men only), calendar period (attained), area of residence, alcohol consumption and smoking or snus use. All-cause mortality	Smoking adjusted Never snus user: 1.0 (reference) Snus use (n=641): **1.10 (1.01-1.21)** Among never-smokers Never snus user: 1.0 (reference) Snus use: **1.23 (1.09-1.40)**	Snus adjusted Never-smoker: 1.0 (reference) Smoking ever age <75: **1.63 (1.45-1.83)** Smoking ever age 75+: **1.26 (1.15-1.38)**

Snus & Smoking Review Summary Estimates

Study Information	Summary Estimate
Cigarette Use	
Friedman et al. 1997 Kaiser Population 1979-1986, followed through 1987 Age-adjusted relative risk of death of current smokers compared to never-smokers.	Males Current smoker (n=308): **1.9 (1.7-2.2)** Females Current smoker (n=308): **1.9 (1.7-2.2)**
McLaughlin et al. 1995 Cohort study US veterans who held government life insurance policies active at the end of 1953. Followed through 1980. Mortality	Current smoker: **1.7 (1.67-1.72)** Former smoker: **1.2 (1.18-1.22)**
US Surgeon General 1989 CPS II Population 1982 – 1986 Relative risk of death among adults aged 35 and older.	Males Current smoker: **2.34 (2.26-2.43)** Former smokers: **1.58 (1.53-1.64)** Females Current smoker: **1.90 (1.82-1.98)** Former smokers: **1.32 (1.27-1.37)**
Snus Use	
None	

Summary of Study Quality & Comparability

Study design: Two cohort studies.

Reference group comparability: One study used common reference groups for snus and smoking risk estimates; Bolinder et al. (1994): never-users of any tobacco. Roosaar et al. (2008) did not use common reference groups. The authors used never snus users and never smokers as the reference groups for snus users and smokers respectively.

Confounding:

	Age	Sex	Area of residence/region of origin	Alcohol	Tobacco Use
Bolinder et al. 1994	X	X	X		X
Roosaar et al. 2008	X	X	X	X	X

Outcome comparability: Both studies identified deaths from any cause.

Exposure comparability (intra-study): Both studies compared different exposure groups. Bolinder et al. (1994) compared smokeless tobacco use with smoking dose, while Roosaar et al. (2008) compared snus users with smokers stratified by age (<75 years and 75+).

Dose-risk or duration-risk relationship:

Bolinder et al. (1994)
Smokers: a dose-response was observed but no analysis for trend.
Snus users: no analysis.

Roosaar et al. (2008)
Smokers: no analysis.
Snus users: no analysis.

Studies with no smoking comparison:
None

Appendix VII

Comparison of Risks from Dual Use, Switching, and Quitting

Appendix (VII) to Chapter 5: Human Health Effects of Snus: Comparison of Risks from Dual Use, Switching, and Quitting

Introduction

Dual tobacco use is an important issue in trying to understand the role of the various tobacco products in tobacco use initiation and cessation. It is therefore important to understand differences in health risks, and changes in health risks, for individuals who transition from one kind of tobacco use to another (if at all). In Scandinavia, particularly in Sweden, people who have ever used snus are more likely to have ever smoked than people who never used snus. It is less clear from the literature that people who are current snus users are also more likely to smoke simultaneously, and if yes, the frequency and duration of use of both tobacco products, as the study designs used often do not allow for an understanding of temporality necessary to discern patterns of use. Another challenge is determining the amount of cigarettes and smokeless tobacco used. There is evidence that smokers who use snus smoke fewer cigarettes per day or per other specified period than smokers who are not dual users. However, it is not often possible to understand the temporal sequence of product initiation, since few of these studies measured frequency and intensity of tobacco use.

This appendix provides additional information on the potential health risks of Swedish snus, using a subset of studies that were reviewed in Chapter 5: Health Effects of Snus. The evidence from several different cohorts suggests that dual users do not face a higher disease risk than exclusive smokers, and that generally, the health risks among dual users appear to be similar to those observed among exclusive smokers. The health risks among those who switch to snus from cigarettes were clearly lower than those observed among individuals who continued to smoke cigarettes, and were generally comparable to, or had lower point estimates than the risks estimates observed among those who quit tobacco entirely.

The studies selected for inclusion in this Appendix were all studies that provided relative risk estimates for snus users who were also former smokers (switchers), and studies that provided relative risk estimates for any other varying categories of snus users in combination with smoking, such as dual users of snus and cigarettes. These studies allow the comparison of available risk estimates to examine potential differences in risks among switchers and dual users compared to nontobacco users, individuals who quit tobacco entirely, and individuals that continue smoking cigarettes.

Methods

The available epidemiology studies summarized as part of the snus health effects review were reviewed to examine for evidence of health effects among switchers and dual users; that is, studies that provided relative risk estimates for snus users who were former smokers, and concurrent users of snus and cigarettes, respectively. Relative risk estimates for smokers who quit tobacco entirely, or continue smoking, were also extracted from these studies using methodology similar to that described in Appendix VI, notably, where relative risks were presented for the various types of tobacco users within the same study population using a common referent group (ideally, nontobacco users). The health outcomes considered in this Appendix include the same smoking-related outcomes as those included in Appendix VI. Among the available studies of switchers, the outcomes evaluated included oral cancer,

metabolic syndrome, diabetes, and various cardiovascular outcomes including overall cardiovascular disease, myocardial infarction (MI), ischemic heart disease, sudden cardiac death, coronary heart disease and stroke. These same outcomes were available for dual users, plus for several additional cancers: pancreatic, lung, stomach, and esophageal.

In addition to the smoking-related outcomes included in the summary table, below, we discuss several additional health outcomes that were studied among smokers, snus users, and dual users or switchers in order to ascertain whether combined use might present unique health risks for disease other than those considered smoking-related. These include several additional cancer types, neurologic diseases, gastric conditions, and potential effects on body weight.

Results

The health risks of quitting smoking without a substitute, switching to snus from cigarettes, using snus and cigarettes concurrently, and continued smoking are presented in Table A VII-1 and described below. In this table, results for dual users are bolded; results for switchers (former smokers who switched to snus) are italicized. Results by health outcome are discussed following the table.

Table A VII-1: Health Risks of Dual Use, Switching to Snus from Cigarettes vs. Quitting Tobacco, Continued Smoking, and Non-use of Tobacco

Study Info	Outcome	Results	Definition of Former Smoking Status & Dual Use Details
Boffetta et al. (2005) Cohort study Two sources: General Norwegian population (1960 census) and relatives of Norwegian migrants to the US (1964-1967 questionnaire). All risk estimates adjusted for age, sex and smoking (smoking estimates are among snuff users).	<u>Lung Cancer</u> Never-smokers/ever snus users **Current smokers/ever snus users** Never-users Ever snus user Current snus users <u>Pancreatic Cancer</u> Never-smokers/ever snus users **Current smokers/ever snus users** Never-users Ever snus user Current snus users	<u>Relative Risk (95% CI)</u> 1.00 (reference) **0.68 (0.51-0.90)**** 1.00 (reference) 0.80 (0.61-1.05) 0.80 (0.58-1.11) 1.00 (reference) **1.86 (1.13-3.05)*** 1.00 (reference) 1.67 (1.12-2.50)* 1.60 (1.00-2.55)	Use of cigarettes and snus may not have been concurrent.
Bertuccio et al. (2011) Pooled-analysis of 11 case-control studies (international, non-snus) Adjusted for center, race, sex, age, education, history of diabetes, body mass index and total alcohol consumption.	<u>Pancreatic Cancer</u> Never tobacco users Ever smokeless tobacco user Exclusive smokeless tobacco user **Smokeless tobacco users and cigarette** Cigarette-only smokers	<u>Odds Ratio (95% CI)</u> 1.00 (reference) 0.98 (0.75-1.27) 0.62 (0.37-1.04) **1.36 (0.94-1.96)** 1.50 (1.39-1.62)*	Use of cigarettes and smokeless tobacco may not have been concurrent.

Table A VII-1: Health Risks of Dual Use, Switching to Snus from Cigarettes vs. Quitting Tobacco, Continued Smoking, and Non-use of Tobacco

Study Info	Outcome	Results	Definition of Former Smoking Status & Dual Use Details
Haglund et al. (2007) Cohort study Swedish population 1988 – 1989 through 2003 All risk estimates adjusted for age, sex (men only), socioeconomic status, residential area, self-reported health, number of longstanding illnesses, and physical activity. Tobacco use categories were exclusive (but may include former smokers/snuff users). ICD9: 410-414; ICD10: I20-I25 (IHD)	<u>IHD (incidence)</u> No tobacco Smoke Snuff **Smoke and snuff** <u>IHD (mortality)</u> No tobacco Smoke Snuff **Smoke and snuff** <u>Stroke (incidence)</u> No tobacco Smoke Snuff **Smoke and snuff** <u>Stroke (mortality)</u> No tobacco Smoke Snuff **Smoke and snuff**	<u>IRR or MRR (95% CI)</u> 1.00 (reference) 1.74 (1.41–2.14)* 0.77 (0.51–1.15) **1.64 (0.96–2.79)** 1.00 (reference) 1.98 (1.35–2.91)* 1.15 (0.54–2.41) **1.69 (0.52–5.46)** 1.00 (reference) 1.40 (1.03–1.91)* 1.07 (0.65–1.77) **1.98 (1.00–3.95)** 1.00 (reference) 1.02 (0.50–2.05) 1.01 (0.35–2.92) **4.30 (1.22–15.1)***	Dual users were concurrent users of both cigarettes and snus.
Hansson et al. (2009) Cohort Study Swedish Twin Registry All risk estimates adjusted for age, sex, diabetes, high blood pressure, and high cholesterol. All CVD; ICD10: I20-I21, I24-I25 [excluding I25.2] IHD: MI or coronary revascularization procedures Stroke: ICD10: I60-I61, I63-I64, G45; ICD9: 430-431, 433-436	<u>Ischemic Heart Disease</u> Never smoking/never snus Former smoking/never snus *Former smoking/current snus* Current smoking/never snus Never smoking/current snus **Current smoking/current snus** <u>All CVD</u> Never smoking/never snus Former smoking/never snus *Former smoking/current snus* Current smoking/never snus Never smoking/current snus **Current smoking/current snus** <u>Stroke</u> Never smoking/never snus Former smoking/never snus *Former smoking/current snus* Current smoking/never snus Never smoking/current snus **Current smoking/current snus**	<u>Relative Risk (95% CI)</u> 1.00 (reference) 1.34 (1.10-1.64)* *1.22 (0.82-1.74)* 1.99 (1.59-2.50)* 0.85 (0.51-1.41) **1.50 (0.73-3.08)** 1.00 (reference) 1.17 (1.00-1.38) *1.04 (0.78-1.39)* 1.86 (1.56-2.22)* 1.00 (0.69-1.46) **1.51 (0.86-2.65)** 1.00 (reference) 1.01 (0.78-1.30) *0.77 (0.46-1.29)* 1.61 (1.22-2.13)* 1.18 (0.67-2.08) **1.45 (0.58-3.62)**	Information on tobacco use was ascertained through the question 'Have you ever smoked or used snus?'. Subjects stated whether they were never, former or current snus users and/or smokers, including regular and occasional use, such as 'now and then' or 'at parties'. Dual users were concurrent users of both cigarettes and snus.
Hergens et al. (2005) Case-control Study	<u>All Cases of MI</u> Never snuff/never smoking Never snuff/ former smoking *Current snuff/former*	<u>Odds Ratio (95% CI)</u> 1.00 (reference) 1.30 (1.10-1.60)* *1.60 (1.10-2.20)**	Subjects who at enrollment had been using snuff within the last 2 years were classified as current

Table A VII-1: Health Risks of Dual Use, Switching to Snus from Cigarettes vs. Quitting Tobacco, Continued Smoking, and Non-use of Tobacco			
Study Info	**Outcome**	**Results**	**Definition of Former Smoking Status & Dual Use Details**
Residents of Stockholm County All risk estimates adjusted for age, sex, hospital catchment area, diabetes, hyperlipidemia, hypertension, overweight, physical inactivity, and job strain. Myocardial Infarction (MI)	*smoking* Never snuff/current smoking Current snuff/never smoking **Current snuff/current smoking** <u>Nonfatal MI</u> Never snuff/never smoking Never snuff/ former smoking *Current snuff/former smoking* Never snuff/current smoking Current snuff/never smoking **Current snuff/current smoking** <u>Fatal MI within 28 days</u> Never snuff/never smoking Never snuff/ former smoking *Current snuff/former smoking* Never snuff/current smoking Current snuff/never smoking **Current snuff/current smoking**	 2.80 (2.30-3.40)* 0.73 (0.35–1.5) **2.30 (1.6–3.4)*** 1.00 (reference) 1.20 (0.98-1.50) *1.60 (1.10-2.20)** 2.70 (2.20-3.30)* 0.59 (0.25-1.4) **2.10 (1.4-3.1)*** 1.00 (reference) 1.70 (1.60-2.60)* *1.50 (0.69-3.20)* 3.60 (2.40-5.20)* 1.70 (0.48-5.5) **3.80 (1.9-7.5)***	snuff users. Subjects who had stopped smoking more than 1 year before were classified as former smokers and those who had smoked within the past year were classified as current smokers. Dual users were concurrent users of both cigarettes and snus. Among controls, dual users smoked slightly fewer cigarettes than those who exclusively smoked (16.4 vs. 18.6 cigs/day). Similar for former smokers. This was also true for the former smokers (18.4 cigarettes per day with snuff use and 20.6 cigarettes per day without snuff).
Huhtasaari et al. (1999) Case-control study Northern Sweden MONICA project: Norrbotten and Vasterbotten provinces. 1991 – 1993 Multivariate estimates adjusted for age (matched) and sex (men only), hypertension, diabetes, high cholesterol, family history of early cardiac death, low education level, and marital status. Tobacco use categories were exclusive (but may include former smokers/snuff users). ICD:410-414 (MI)	<u>MI</u> Never users of tobacco Current snuff/no smoking Current smoking/no snuff Former smoker/never snuff **Current concomitant user**	<u>Odds Ratio (95% CI)</u> 1.00 (reference) 0.96 (0.65–1.41) 3.65 (2.67–4.99)* 1.05 (0.77–1.43) **2.66 (1.24–5.71)***	Dual users were daily, concurrent users of both cigarettes and snus.
Johansson et al. (2005) Cohort study	<u>Coronary Heart Disease</u> Never-smoker Former smoker *Daily snuffer/former smoker*	<u>Hazard Ratio (95% CI)</u> 1.00 (reference) 1.47 (1.07-2.03)* *1.18 (0.67-2.06)*	No definition of former smokers given. Dual users were daily,

Table A VII-1: Health Risks of Dual Use, Switching to Snus from Cigarettes vs. Quitting Tobacco, Continued Smoking, and Non-use of Tobacco			
Study Info	**Outcome**	**Results**	**Definition of Former Smoking Status & Dual Use Details**
Random sample from Swedish population: SALLS survey All risk estimates adjusted for age, sex (men only), BMI, physical activity, diabetes, and hypertension. Risk estimates did not change much when socioeconomic status was considered. ICD9: 410-414; ICD10: I20-I25 (CHD event)	Daily smoker Daily snuffer/never-smoker **Daily snuffer and smoker**	2.30 (1.66-3.19)* 1.41 (0.61–3.28) **2.73 (1.35–5.53)***	concurrent users of both cigarettes and snus.
Schildt et al. (1998) Case-control study 4 Northernmost counties of Sweden Matched for gender, age and county.	<u>Oral Cancer</u> Never snuff/never smoker Never snuff/ex-smoker *Active snuff/ex-smoker* Never snuff/active smoker Active snuff/never-smoker **Active snuff/active smoker**	<u>Odds Ratio (95% CI)</u> 1.0 (reference) 0.9 (0.6-1.4) *0.6 (0.3-1.3)* 1.7 (1.1-2.6)* 0.7 (0.4–1.2) **1.2 (0.6–2.4)**	An ex-smoker or ex-snuff user was defined as a person who had quit the habit at least 1 year before the diagnosis; for controls, the corresponding year was the year of diagnosis for the respective case. Subjects who had stopped smoking or stopped using moist snuff within the year before diagnosis were coded as current users of tobacco. Dual users were concurrent users of both cigarettes and snus.
Wandell et al. (2008) Cross-sectional study Men living in Stockholm county, Sweden Risk estimates adjusted for age (all 60), sex (men only), BMI, waist circumference, employment, educational level, living in an apartment, physical activity, alcohol intake. Metabolic syndrome definition: International Diabetes Federation (IDF).	<u>Diabetes</u> Reference not provided Never snuff/ex-smoker *Current snuff/ex-smoker* Never snuff/current smoker Never smoker/current snuff **Current smokers and snuffers** <u>Metabolic Syndrome</u> Reference not provided Never snuff/ex-smoker *Current snuff/ex-smoker* Never snuff/current smoker Never smoker/current snuff **Current smokers and snuffers**	<u>Odds Ratio (95% CI)</u> ---- 1.41 (0.76-2.60) *1.71 (0.67-4.35)* 1.40 (0.68-2.89) 2.12 (0.25-17.71) **2.48 (0.52-11.82)** ---- 1.44 (1.14-1.83)* *1.18 (0.76-1.83)* 1.00 (0.74-1.35) 1.81 (0.65-5.02) **0.85 (0.36-2.02)**	No definition of former smokers given. Dual users were daily, concurrent users of both cigarettes and snus.

Table A VII-1: Health Risks of Dual Use, Switching to Snus from Cigarettes vs. Quitting Tobacco, Continued Smoking, and Non-use of Tobacco			
Study Info	**Outcome**	**Results**	**Definition of Former Smoking Status & Dual Use Details**
Wennberg et al. (2007) Prospective incident case-referent study (Nested case-control study) Nested in northern Sweden MONICA cohort: Norrbotten and Vasterbotten provinces. All risk estimates adjusted for age, sex, BMI, leisure time physical activity, educational level and cholesterol level. Tobacco use categories were exclusive, but current smoking category may have included some past snuff users. ICD9: 410-414, 429.2; ICD10: I20-I25 (MI, fatal MI, Sudden cardiac death (SCD))	<u>MI</u> Never used tobacco Former smoker/never snuff *Former smoker/current snuff* Current smoker/no current snuff Never smoked/current snuff **Current smoker/current snuff** <u>Fatal MI within 28 days</u> Never used tobacco Former smoker/never snuff *Former smoker/current snuff* Current smoker/no current snuff Never smoked/current snuff **Current smoker/current snuff** <u>SCD with survival <24 h</u> Never used tobacco Former smoker/never snuff *Former smoker/current snuff* Current smoker/no current snuff Never smoked/current snuff **Current smoker/current snuff** <u>SCD with survival <1 h</u> Never used tobacco Former smoker/never snuff *Former smoker/current snuff* Current smoker/no current snuff Never smoked/current snuff **Current smoker/current snuff**	<u>Odds Ratio (95% CI)</u> 1.00 (reference) 1.18 (0.82-1.70) *1.25 (0.80-1.96)* 2.60 (1.91-3.54)* 0.82 (0.46–1.43) **2.14 (1.28–3.60)*** 1.00 (reference) 1.02 (0.45-2.31) *1.24 (0.44-3.53)* 3.53 (1.83-6.84)* 1.12 (0.38-3.29) **1.11 (0.34-3.69)** 1.00 (reference) 0.74 (0.28-1.97) *1.39 (0.44-4.42)* 3.12 (1.53-6.33)* 1.18 (0.38-3.70) **0.75 (0.17-3.28)** 1.00 (reference) 0.35 (0.07-1.78) *2.67 (0.52-13.80)* 4.54 (1.55-13.25)* 0.38 (0.08-1.89) **0.13 (0.01-2.10)**	No definition of former smokers given. Dual users were daily, concurrent users of both cigarettes and snus.
Ye et al. (1999) Case-control (population-based) Swedish population (5 counties) 1989-1995 Risk estimates for snuff use were adjusted for age, residence area, BMI, socio-economic status, and smoking. Odds ratios among smokers and exclusive tobacco groups were	<u>Stomach Cancer</u> Never-smokers/never-users Never-smokers/ever-users Ex-smokers/never-users Ex-smokers/ever-users Current smokers/never-users **Current smokers/ever-users**	<u>Odds Ratio (95% CI)</u> 1.0 (reference) 0.5 (0.2-1.2) 1.2 (0.9-1.8) 1.2 (0.8-1.9) 2.0 (1.3-2.9)* **1.0 (0.5-1.8)**	Use of cigarettes and snus may not have been concurrent. Dual users smoked less and for a shorter duration than smokers who did not (ever) use snuff.

Table A VII-1: Health Risks of Dual Use, Switching to Snus from Cigarettes vs. Quitting Tobacco, Continued Smoking, and Non-use of Tobacco			
Study Info	**Outcome**	**Results**	**Definition of Former Smoking Status & Dual Use Details**
adjusted for age, gender, residence area, BMI, SES, use of smokeless tobacco, and use of beer, wine and liquor. Gastric cancer			
Zendehdel et al. (2008) Cohort study Swedish Construction Worker cohort 1971 – 1993 and followed through 2004 All risk estimates adjusted for attained age and BMI.	<u>Esophageal Adenocarcinoma</u> Ever-smokers/non snus users **Ever-smokers/snus use** Never-users of any tobacco User of snus only Smoker only <u>Esophageal squamous cell carcinoma</u> Ever-smokers/non snus users **Ever-smokers/snus use** Never-users of any tobacco User of snus only Smoker only <u>Stomach cancer-cardia</u> Ever-smokers/non snus users **Ever-smokers/snus use** Never-users of any tobacco User of snus only Smoker only <u>Stomach cancer-noncardia</u> Ever-smokers/non snus users **Ever-smokers/snus use** Never-users of any tobacco User of snus only Smoker only	<u>Relative Risk (95% CI)</u> 1.0 (reference) **1.0 (0.6-1.5)** 1.0 (reference) 0.2 (0.0-1.9) 2.9 (1.8-4.8)* 1.0 (reference) **0.8 (0.6-1.2)** 1.0 (reference) 3.5 (1.6-7.6)* 7.6 (4.5-12.7)* 1.0 (reference) **0.9 (0.7-1.3)** 1.0 (reference) 0.9 (0.4-2.0) 2.3 (1.6-3.3)* 1.0 (reference) **1.0 (0.9-1.2)** 1.0 (reference) 1.4 (1.1-1.9)* 1.4 (1.2-1.6)*	Use of cigarettes and snus may not have been concurrent.
* denotes statistically significant increase in risk ** denotes statistically significant decrease in risk **Bold:** dual use category *Italics:* switching category			

Switching

Oral Cancer

One case-control study reported risk estimates of oral cancer among former smoking snus users along with risk estimates among former smokers who quit tobacco entirely and those who were current smokers (Schildt et al. 1998). The risk of oral cancer among ex-smokers was not increased, including ex-smokers who were active snus users. The risk among active smokers who had never used snus was significantly increased. This study observed that among those who switched from cigarettes to snus, no increased risk of oral cancer was observed compared to those who were active smokers.

Diabetes and Metabolic Syndrome

One cross-sectional study reported risk estimates of diabetes and metabolic syndrome among former smoking snus users, former smokers who quit tobacco entirely, and those who were current smokers (Wandell et al. 2008). None of the risk estimates were significantly elevated for either outcome except for a significantly increased risk of metabolic syndrome among ex-smokers who quit tobacco entirely (i.e., did not switch to snus).

Stroke

One cohort study reported risk estimates of stroke among former smoking snus users, former smokers who quit tobacco entirely, and those who are current smokers (Hansson et al. 2009). The risk of stroke among former smokers, whether they had quit tobacco use altogether or had switched to snus are equivalent, and show no increased risk. The risk of stroke among active smokers who had never used snus was significantly increased.

Cardiovascular Disease

Two cohort (Hansson et al. 2009; Johansson et al. 2005) and two case-control (Hergens et al. 2005; Wennberg et al. 2007) studies reported risk estimates of all cardiovascular disease, MI, ischemic heart disease, sudden cardiac death, or coronary heart disease among former smoking snus users, former smokers who quit tobacco entirely, and those who are current smokers. Three of the four studies did not find a significantly increased risk for the various CVD-related outcomes examined, including ischemic heart disease, all cardiovascular disease, coronary heart disease, MI (overall or fatal within 28 days) or sudden cardiac death (<24 hours and <1 hour) among former smoking snus users. In the fourth study, which examined MI (all, fatal, nonfatal), Hergens and colleagues (2005) did report significantly increased risks of any MI, and non-fatal MI among former smoking snus users, but no significantly increased risk of fatal MI. Additionally, these risks were either lower than or not significantly different from those observed among smokers, where the risks of the various CVD outcomes were consistently significantly increased among current smokers in all of the studies.

Dual Use

Diabetes and Metabolic Syndrome

One cross-sectional study reported risk estimates of diabetes and metabolic syndrome among concurrent users of snus and cigarettes (Wandell et al. 2008). None of the risk estimates were significantly elevated among participants who were current smokers and current snuff users for

either outcome; a significantly increased risk of metabolic syndrome was observed among ex-smokers.

Esophageal Cancer

One cohort study investigated the potential effects of dual use on esophageal cancer among snus users who were ever users of cigarettes (Zendehdel et al. 2008), though the use of snus and cigarettes may not have been concurrent among the study participants, and no information was provided on the amount of tobacco consumed by type. Among these dual users, the risks of esophageal adenocarcinoma and squamous cell carcinoma were not increased compared to ever smokers/non snus users, while the risks of both cancer subtypes were significantly elevated among exclusive smokers when compared to never tobacco users.

Lung Cancer

One cohort study investigated the potential risk of dual use on lung cancer among ever snus users who were current smokers (Boffetta et al. 2005). Though the use of snus and cigarettes may not have been concurrent among the study participants, and no information was provided on the amount of tobacco consumed by type, the risk of lung cancer was significantly lower among dual users. A risk estimate for exclusive smokers was not available for comparison with that of dual users.

Oral Cancer

One case-control study investigated the potential effects of dual use on oral cancer among concurrent users of snus and cigarettes (Schildt et al. 1998). Though no information is given on the amount of snus or cigarettes consumed by dual users, the risk of oral cancer among dual users was not significantly increased, while the risk among current smokers was significantly increased. The risk among snus users was near unity, suggesting no increased risk of snuff use.

Pancreatic Cancer

One cohort study of Swedish snus users investigated the potential effects of dual use on pancreatic cancer among ever snus users who were current smokers (Boffetta et al. 2005). The risk of pancreatic cancer was significantly increased among dual users, though the use of snus and cigarettes may not have been concurrent among the study participants, and no information was provided on the amount of tobacco consumed by type. A risk estimate for exclusive smokers was not available for comparison with dual users.

Though there is limited snus-specific data, additional evidence, though not specific to snus, was provided by a recent pooled-analysis of 11 studies of cigarette and Western population smokeless tobacco users (Bertuccio et al. 2011). In this study, dual users and exclusive smokeless tobacco users did not face a significantly increased risk of pancreatic cancer, whereas the risk of pancreatic cancer was significantly increased among smokers. Given that the smokeless tobacco used by participants in these studies likely contained higher levels of TSNAs compared to Swedish snus, the principal component of tobacco thought to be associated with the development of pancreatic cancer (Boffetta et al. 2008), it is unlikely that Swedish snus poses a risk for pancreatic cancer.

Stomach Cancer

One cohort study (Zendehdel et al. 2008) and one case-control study (Ye et al. 1999) reported risk estimates of stomach cancer among dual users of snus and cigarettes. The cohort study investigated the potential effects of dual use on stomach cancer among snus users who were ever users of cigarettes. Though the use of snus and cigarettes may not have been concurrent among the study participants, and no information was provided on the amount of tobacco consumed by type, the risks of cardia and non-cardia stomach cancer were not increased among dual users, while the risks of both cancer subtypes were significantly elevated among exclusive smokers (Zendehdel et al. 2008).

The case-control study investigated the potential effects of dual use on stomach cancer among smokers who were ever users of snus (Ye et al. 1999). Though the use of snus and cigarettes may not have been concurrent among the study participants, the risk of stomach cancer was not increased among dual users, while the risk of stomach cancer was significantly elevated among exclusive smokers. The authors reported that dual users smoked less and for a shorter duration than smokers who did not (ever) use snuff.

Stroke

Two cohort studies reported risk estimates for stroke among concurrent users of snus and cigarettes (Haglund et al. 2007; Hansson et al. 2009). Hansson and colleagues (2009) found that dual users did not face a significantly increased risk of stroke, while the risk of stroke was significantly increased among current exclusive smokers. Haglund and colleagues (2007) found that the risk of incident stroke was elevated and of borderline significance among dual users, and that fatal stroke was also elevated, and statistically significant, but based on three cases available for analysis. The risk of fatal stroke was not significantly elevated among cigarette smokers. Neither study provided information on the amount of tobacco consumed by type.

Cardiovascular Disease

Three cohort (Haglund et al. 2007; Hansson et al. 2009; Johansson et al. 2005) and three case-control (Hergens et al. 2005; Huhtasaari et al. 1999; Wennberg et al. 2007) studies reported risk estimates of all cardiovascular disease, MI, ischemic heart disease, sudden cardiac death, or coronary heart disease among concurrent users of snus and cigarettes. Haglund and colleagues (2007) reported no significantly increased risk of IHD incidence or mortality among dual users, while the risk among smokers was significantly elevated for both. Hansson and colleagues (2009) also reported that the risk of IHD was not significantly increased along with all cases of CVD among dual users, while the risks among smokers for both of these outcomes were significantly elevated. Johansson and colleagues (2005) reported a significantly increased risk of coronary heart disease among dual users, which was lower than the risk observed among exclusive smokers in this study. Hergens and colleagues (2005) reported a significantly increased risk of all cases of MI, nonfatal MI, and fatal MI within 28 days among dual users. These risks were generally comparable to those observed among smokers in this study. Huhtasaari and colleagues (1999) reported a significantly increased risk of MI among dual users, though this risk was lower compared to that observed among current exclusive smokers. Wennberg and colleagues (2007) reported a significantly increased risk of MI among dual users, but increased risks were not observed for fatal MI within 28 days, sudden cardiac death (SCD)

with survival <24 hours, and SCD with survival <1 hour, while the risk among smokers for all of these outcomes were significantly elevated.

None of the studies of CVD provided information on the amount of tobacco consumed with the exception of Hergens et al. (2005). Hergens and colleagues (2005) reported that among controls, dual users smoked slightly fewer cigarettes than those who exclusively smoked cigarettes (16.4 vs. 18.6 cigs/day). The authors reported that this was also true for the former smokers (18.4 cigs/day with snuff use and 20.6 cigs/day without snuff). Overall, the risks of the various cardiovascular outcomes among dual users were either not increased, lower than that observed among smokers, or comparable to the risk observed among smokers; in no instance was the risk of CVD outcomes higher than that observed among smokers who did not use snus.

Other Outcomes

The results from studies of other outcomes besides those presented in Table A VII-1 of dual snus/cigarette users were also investigated in order to ascertain whether combined use might present unique health risks for disease other than those considered smoking-related. Similar to the results provided in this appendix, dual users either did not face any risk or faced a risk not significantly different from exclusive smokers for outcomes that included various types of skin and blood cancers, ALS, multiple sclerosis, sarcoidosis, rheumatoid arthritis, and rectal, and anal cancers (Carlens et al. 2010; Fang et al. 2006; Fernberg et al. 2006; Fernberg et al. 2007; Nordenvall et al. 2010; Odenbro et al. 2005; Odenbro et al. 2007).

There were a few exceptions where dual users faced a significantly increased risk where exclusive smokers did not, which included one cancer study that reported a significantly increased risk of colon cancer for dual users but not among pure smokers (Nordenvall et al. 2010). The confidence intervals overlapped, however, and pure snus use was not associated with this outcome. Details regarding cigarette and snus consumption, and potential lifestyle differences among different tobacco user groups were also not provided. Similar results were observed in one study of ulcerative colitis and Crohn's disease (Persson et al. 1993), where the risks of these outcomes were significantly increased among dual users, but not among smokers. However, another study presented risk estimates that were similar, and significantly increased among both smokers and dual users for these conditions (Carlens et al. 2010). Not all of the participants in the Carlens et al. (2010) and Persson et al. (1993) studies may have actually used snus and cigarettes concurrently. Though the confidence intervals overlapped, Aro and colleagues (2010) reported risk estimates that were significantly increased among dual users but not among current smokers for some, but not all of the gastric conditions investigated in that study. Dual users in this study were the highest consumers of alcohol, while, potential unhealthy lifestyle habits were not investigated in the other studies that observed significantly increased risks of gastric conditions among dual users.

Among studies that investigated the potential effects of concurrent dual use on BMI, body weight or incident weight gain, some found that dual use conferred a significantly increased risk for some of these outcomes, or a similar risk to exclusive smokers. Aro et al. (2010) observed that the mean BMI of dual users was similar to never-users of tobacco, while the mean BMI among current smokers was significantly greater than never-users of tobacco. Engstrom and colleagues (2010) did not find an increased prevalence of being underweight, but did report a

significantly increased prevalence of being overweight or obese among dual using men, whereas the risks were not significantly increased among exclusive smokers (though the confidence intervals did overlap). A significantly increased prevalence of overweight or obesity was not observed among women who were dual users. Hansson and colleagues (2011) and Rodu and colleagues (2004) reported a significantly increased risk of incident weight gain and becoming overweight, respectively, among dual users, while the risk was not significantly elevated among smokers. Hansson et al. (2011) did not report a significantly increased risk of becoming obese among dual users.

Discussion

The relative risk estimates of specific smoking-related health outcomes were examined among switchers (former smokers who were current snus users) and dual users, who use both snus and smoke cigarettes. Among switchers, risks of the health outcomes examined (oral cancer, metabolic syndrome, diabetes, stroke and various cardiovascular outcomes) were either not statistically significantly increased, or were lower than those observed among current smokers, with the exception of an increased risk of MI and non-fatal MI in one case-control study (Hergens et al. 2005). The risk of non-fatal MI among switchers was not significantly different from, and the risk of all cases of MI in this study was lower than, that observed among current smokers. The risks of MI, CHD, IHD, overall CVD or SCD were not significantly increased among switchers in two cohort studies (Hansson et al. 2009; Johansson et al. 2005) and one other case-control study (Wennberg et al. 2007). The relative risk estimates for all outcomes among switchers were either similar to or had lower point estimates than that of former smokers who quit tobacco entirely, with the exception of non-fatal MI in the Hergens et al. (2005) study.

These conclusions for Swedish snus differ from those reported by Henley and colleagues (2007) who investigated the potential health effects of switching from cigarettes to smokeless tobacco in the US American Cancer Society Cancer Prevention Study II cohort. The authors reported that men who switched from smoking cigarettes to using smokeless tobacco (using data that was collected at baseline only) had a higher rate of death from all causes, lung cancer, coronary heart disease, and stroke than those who had never used tobacco or those who were former cigarette smokers and quit using tobacco entirely following adjustment for several relevant potential confounders. The authors noted that switchers, compared to those who quit tobacco entirely, were less educated, more often employed in blue-collar occupations, and had a less healthy diet. Because information on tobacco use was collected only at baseline and not updated during follow-up, it is possible that men who quit smoking before enrollment, but resumed during the follow-up period, and those who initiated or discontinued using spit tobacco after enrolment, could have been misclassified, in fact, a subset of the cohort whose smoking status was updated after 10 years, had low overall rate of recidivism, but was statistically significantly higher among switchers (3.0%) than among those who quit using tobacco entirely (1.4%). Additional limitations of the study include lack of information on intensity of smoking, and the possibility that addiction may have influenced both smoking behavior and use of smokeless tobacco. Former smokers who switched may have been more addicted on average and may have smoked differently than those who quit tobacco entirely.

For dual users of snus and cigarettes, most of the relative risk estimates reported in the available studies were not significantly increased or were similar to those observed among

exclusive smokers. The health outcomes for which none of the relative risk estimates were significantly increased for dual users included esophageal cancer, lung cancer, oral cancer, stomach cancer, diabetes, and metabolic syndrome. Among the studies that reported significantly increased health risks among dual users, all of these risks were similar to, or had lower point estimates than, those observed among exclusive smokers, with the exception of one stroke subanalysis of fatal stroke (Haglund et al. 2007). In that study, though the point estimate of the relative risk was higher, the confidence intervals overlapped with the relative risk among exclusive smokers. In this study, details regarding cigarette and snus consumption were not reported, and there were only three cases. With the exception of fatal stroke, the relative risk estimates for dual users among the studies of the other outcomes, which included pancreatic cancer, and the various cardiovascular outcomes, were either not significantly increased, or were comparable to the risk observed among smokers.

A limitation of these studies is that most of the studies of dual users did not provide qualitative or quantitative information on consumption of individual tobacco types among dual users with the exception of two of the twelve studies (Hergens et al. 2005; Ye et al. 1999). In both of these studies, the authors reported that dual users smoked slightly less compared to exclusive smokers, and in the Ye et al. (1999) study, smoked for a shorter duration. Though dual users smoked less in these two studies, the authors of at least one US study have reported that dual users smoked more than exclusive smokers in that particular study population (Accortt et al. 2002). Among the studies where the amount of tobacco consumption by type is not provided, it is not known how smoking intensity may affect the interpretation of the reported risk estimates.

Additionally, though most of the studies reported relative risk estimates among concurrent users of snus and cigarettes (those who used both tobacco types at the same time, typically daily), four of the twelve studies reported relative risk estimates among dual users who were either ever users of snus, cigarettes, or both (Bertuccio et al. 2011; Boffetta et al. 2005; Ye et al. 1999; Zendehdel et al. 2008). Thus, it is likely that not all of the participants were concurrent users of both tobacco types when they developed a disease.

It is also possible that the lifestyles, especially unhealthy habits know to affect disease risk, may differ significantly among the various tobacco groups, and may not be accounted for in the studies. Several individual studies have found that unhealthy lifestyle habits are be more prevalent among dual users of tobacco compared to exclusive tobacco user groups, and nontobacco users. Engstrom and colleagues (2010) reported that unhealthy lifestyle was strongly associated with dual use among Swedish men and women. This included risky alcohol consumption, binge drinking, low fruit and vegetable consumption, and a sedentary lifestyle. Bombard and colleagues (2009) reported that lifetime polytobacco users in Canada were more likely to use drugs and alcohol. Klesges and colleagues (2011) reported that US Air Force recruits, who were dual users, had a higher prevalence of heavier alcohol consumption, more risk-taking behaviors, and were more likely to be surrounded by smokers. Johansson and colleagues (2005) reported that the highest percentage of “no physical activity” was observed among daily smokers and dual users in a Swedish population. The highest percentage of overweight and obesity was also found among dual users in this study. Aro and colleagues (2010) found that the high alcohol consumption (>100 g/week) was highest among dual users in a Northern Swedish study population.

Dual use of cigarettes and nicotine replacement therapy (NRT) products has also been reported. Hughes and colleagues (2005) investigated the potential off-label use of a nicotine inhaler that had recently been prescribed to US smokers in a prospective study. Off-label use included using the inhaler and cigarettes concurrently or using the inhaler for non-cessation reasons. The authors reported that many smokers used the inhaler and cigarettes concurrently on the same day (43-55%) at some time during the six month follow-up period but found that this behavior did not persist in most individuals. Repeated concurrent use (weekly concurrent use for at least a month) was reported by only 7-12% of participants. The participants did not appear to become dependent on the inhaler (only 1.4% self-reported the DSM-IV or ICD-10 criteria for dependence, but a clinician who interviewed them did not believe any were dependent). The authors concluded that although concurrent use of NRT and cigarettes occurs in some users, harm from and dependence on NRT is rare.

Despite the potential limitations of the studies of dual users of Swedish snus and cigarettes, the evidence from several different cohorts suggests that dual users do not face a higher disease risk than exclusive smokers, and that generally, the health risks among dual users appear to be similar to those observed among exclusive smokers. A number of smoking-related diseases were examined, including various cardiovascular outcomes, smoking-related cancers and other non-smoking-related diseases. Thus, no unique or multiplicative health risks were identified among dual users of tobacco. These conclusions are consistent with that reached by Frost-Pineda and colleagues (2010), who reviewed the available literature on the health effects of dual use from US and European epidemiology studies. Those authors concluded that "the evidence is sufficient and clear that there are no unique health risks (either qualitative or quantitative) associated with dual use of cigarettes and smokeless tobacco products, which are not anticipated or observed from single use of these products for the major health effects associated with smoking and smokeless tobacco. Some data indicate that the risks of dual use are lower than those of exclusive smoking." In this current review, the health risks among those who switch to snus from cigarettes were clearly lower than those observed among individuals who continued to smoke cigarettes, and were generally comparable to, or had lower point estimates than the risks estimates observed among those who quit tobacco entirely. These conclusions are also consistent with those reached by Lee (2013), who reviewed the health effects of switching among the same studies of smoking-related outcomes included in this analysis. Lee (2013) compared risk estimates of switchers with quitters and continuing smokers quantitatively, and where appropriate, provided combined summary estimates of switching vs. continued smoking (0.55; 95% CI: 0.45-0.68) and quitting (1.02; 95% CI: 0.83-1.26). Lee (2013) concluded that "the findings consistently demonstrate that switching from cigarettes to snus is associated with a clearly lower risk of CVD and cancer than is continuing to smoke. The risk in switchers is no different than that in smokers who quit smoking."

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Appendix VIII

Smokeless Tobacco Reviews and Meta-analyses

Appendix (VIII) to Chapter 5: Smokeless Tobacco Reviews and Meta-analyses

A summary of statements or conclusions by researchers or public health organizations related to smokeless tobacco (often in comparison to health risks from smoking)

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.

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.
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).

Reference	Objective	Author(s) Conclusion
		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.
Frost-Pineda K, Appleton S, Fisher M, Fox K, and Gaworski CL. (2010). Does dual use jeopardize the potential role of smokeless tobacco in harm reduction? Nicotine Tob Res 12:1055-1067.	To review the health effects of the use among dual users from a variety of US and European epidemiological studies.	The evidence is sufficient and clear that there are no unique health risks (either qualitative or quantitative) associated with dual use of cigarettes and smokeless tobacco products, which are not anticipated or observed from single use of these products for the major health effects associated with smoking and smokeless tobacco. The current evidence suggests that smokeless tobacco use can contribute to reducing smoking-related harm and that the potential for dual use of both products should not be a barrier to using smokeless tobacco in harm-reduction strategies. Dual users are more likely to reduce smoking intensity or to cease smoking cigarettes than exclusive smokers. This is despite the fact that, at least for the U.S. cohorts assessed herein, dual users as a group have higher prevalence of demographic variables that are typically associated with lower rates of smoking cessation, such as younger age, lower educational attainment (a strong correlate with poverty), and unmarried status.
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.

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.
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

Reference	Objective	Author(s) Conclusion
		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.
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. (2009a). The relation between smokeless tobacco and cancer in Northern Europe and North America. A commentary on differences between the conclusions reached by two recent reviews. BMC Cancer 9:256.	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. (2009b). Systematic review of the relation between smokeless tobacco and cancer in Europe and North America. BMC	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	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

Reference	Objective	Author(s) Conclusion
Med 7:36.	and smoking (attributable risk).	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. Of 142,205 smoking-related male US cancer deaths in 2005, 104,737 are smoking attributable. Smokeless tobacco-attributable deaths would be 1,102 (1.1%) if as many used smokeless tobacco as had smoked, and 2,081 (2.0%) if everyone used smokeless tobacco.
Lee PN. (2011). Summary of the epidemiological evidence relating snus to health. Regul Toxicol Pharmacol 59:197-214.	To conduct a meta-analysis and systematic review of the health effects of Swedish snus.	It seems clear that any risks from snus are overall much lower than from smoking. For cancer, Lee and Hamling (2009a) estimated that tobacco-attributable deaths would reduce by about 99% if all smokers switched to smokeless tobacco (as used in North America or Europe) and had the excess risks of smokeless tobacco users. As the association with cancer seems no greater for snus than smokeless tobacco (Lee and Hamling, 2009a), it can be concluded that snus-related cancer deaths (if they exist) are much lower than smoking-related deaths. For CID, one can compare meta-analysis RR estimates of 1.01 (0.91–1.12) for IHD/AMI and 1.05 (0.95–1.15) for stroke with estimates for smoking of 2.95 (2.77–3.14) for AMI from a 52 country study (Teo et al., 2006) and a similar estimate for stroke from a review (Hankey, 1999). Again any excess risk from snus seems two orders of magnitude less. Respiratory disease, particularly COPD, is another major cause of smoking-related death. Though evidence is lacking for snus, it seems unlikely that any major effect exists, partly as one might have been reported had it existed, and partly as snus does not produce smoke.

Reference	Objective	Author(s) Conclusion
Lee PN. (2013). The effect on health of switching from cigarettes to snus - A review. Regul Toxicol Pharmacol 66:1-5.	To evaluate health effects associated specifically with switching from smoking to Swedish snus by comparing switchers with those who continue to smoke or who quit smoking rather than switch.	The findings consistently demonstrate that switching from cigarettes to snus is associated with a clearly lower risk of CVD and cancer than is continuing to smoke. The risk in switchers is no different than that in smokers who quit smoking.
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.
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. (2006b). 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%.

Reference	Objective	Author(s) Conclusion
Phillips CV, Guenzel B, and Bergen P. (2006a). 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.
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.
Piano MR, Benowitz NL, Fitzgerald GA, Corbridge S, Heath J, Hahn E, Pechacek TF, and Howard G. (2010). Impact of smokeless tobacco products on cardiovascular disease: implications	To review the epidemiology evidence on the relationship between smokeless tobacco and cardiovascular disease, and comment on the implications for policy.	As a national nonprofit health organization committed to promoting tobacco control research and policy efforts, the American Heart Association does not recommend the use of ST as an alternative to cigarette smoking or as a smoking cessation product. Although the evidence is consistent with the suggestion

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for policy, prevention, and treatment: a policy statement from the American Heart Association. Circulation 122:1520-1544.		that the CV risks are lower with ST products, ST products are not without harm.
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.
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	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.

Reference	Objective	Author(s) Conclusion
	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.	
Rodu B. (2011). The scientific foundation for tobacco harm reduction, 2006-2011. Harm Reduct J 8:19.	To review recent epidemiology and behavioral evidence for tobacco harm reduction.	Pregnant women who use snus are at risk for slightly smaller babies, and they also have modestly elevated risks for premature delivery, stillbirth and possibly preeclampsia. Although any form of nicotine should be avoided during pregnancy, the highest risks for the developing baby are associated with smoking. There is extensive research evidence that ST use has been a key factor in the declining rates of smoking and of smoking-related diseases in Sweden. While it cannot be proven that the availability of ST would reduce smoking prevalence in other countries, the potential population health benefits of ST are far greater than the potential risks.
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).
Royal College of Physicians. (2007). *Harm reduction in nicotine addiction. Helping people who can't quit. A report*	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

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by the Tobacco Advisory Group of the Royal College of Physicians. http://www.tobaccoprogram.org/pdf/4fc74817-64c5-4105-951e-38239b09c5db.pdf		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.

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