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Scientific Committee on Emerging and Newly Identified Health Risks

SCENIHR

Health Effects of Smokeless Tobacco Products

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risks

The SCENIHR adopted this opinion at the 22nd plenary on 6 February 2008, after public consultation.

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

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Questions concerning emerging or newly-identified risks and on broad, complex or multi-disciplinary issues requiring a comprehensive assessment of risks to consumer safety or public health and related issues not covered by other Community risk- assessment bodies.

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Unfortunately Jagadeesan Nair died during the summer 2007. The whole working group appreciated his positive attitude during the many group meetings and recognises his great contribution to the opinion, in particular concerning the chemistry and role of nitrosamine adducts.

Mogens Thomsen, on behalf of the working group.

[1] Declared interest (see the minutes of the SCENIHR Plenary http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_mi_014.pdf)
[2] Declared interest (see the minutes of the SCENIHR Plenary http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_mi_011.pdf)
[3] Declared interest (see the minutes of the SCENIHR Plenary http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_mi_009.pdf)
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The views expressed in the SCENIHR opinion are not necessarily shared by all members of the working group.

ABSTRACT

The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has been asked to evaluate the health effects of smokeless tobacco products (STP) with particular attention to tobacco for oral use, moist snuff, which is called "snus" in Sweden. In addition to tobacco for oral use, STP include chewing tobacco, dry snuff and nasal snuff. The EC Tobacco Products Directive (2001/37/EC) defines tobacco for oral use as "…all products for oral use, except those intended to be smoked or chewed, made wholly or partly of tobacco, in powder or in particulate form or in any combination of those forms". Synonyms for "tobacco for oral use" are moist snuff (snus) and oral tobacco. Marketing of oral tobacco is banned in all EU-countries except Sweden while other STP are allowed in EU.

Adverse health effects of smokeless tobacco products

All STP contain nicotine, a potent addictive substance. They also contain carcinogenic tobacco-specific nitrosamines, albeit at differing levels. STP are carcinogenic to humans and the pancreas has been identified as a main target organ. All STP cause localised oral lesions and a high risk for development of oral cancer has been shown for various STP but the evidence for oral cancer in users of Swedish moist snuff (snus) is less clear. There is 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.

Addiction potential of smokeless tobacco products

Smokeless tobacco is addictive and withdrawal symptoms are broadly similar to those seen in smokers.

Use of STP as smoking cessation aid compared to pharmaceutical nicotine replacement products

Due to insufficient evidence it is not possible to draw conclusions as to the relative effectiveness of smokeless tobacco as an aid to smoking cessation in comparison with established therapies.

Impact of smokeless tobacco use on subsequent initiation of smoking

There is some evidence from the USA that smokeless tobacco use may lead to subsequent cigarette smoking. The Swedish data do not support the hypothesis that smokeless tobacco (i.e. Swedish snus) is a gateway to future smoking. Social, cultural and product differences between North America and Europe and within Europe suggest caution in translating findings across countries.

Extrapolation of the information on the patterns of smokeless tobacco use, smoking cessation and initiation from countries where oral tobacco is available to EU-countries where oral tobacco is not available.

It is not possible to extrapolate future patterns of tobacco use across countries. In particular, it is not possible to extrapolate the trends in prevalence of smoking and oral tobacco use if it were made available in an EU-country where it is now unavailable due to societal and cultural differences.

General conclusion

STP are addictive and their use is hazardous to health. Evidence on the effectiveness of STP as a smoking cessation aid is insufficient, and relative trends in progression from STP into and from smoking differ between countries. It is thus not possible to extrapolate the patterns of tobacco use from one country where oral tobacco is available to other countries.

Keywords: carcinogenic, health effects, moist snuff, nicotine, nitrosamines, oral tobacco, SCENIHR, smokeless tobacco, smoking, snus, STP

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EXECUTIVE SUMMARY

The SCENIHR has been asked to evaluate the health effects of smokeless tobacco products (STP) with particular attention to tobacco for oral use, moist snuff, which is called "snus" in Sweden. In addition to tobacco for oral use, STP include chewing tobacco, dry snuff and nasal snuff. The EC Tobacco Products Directive (2001/37/EC) defines tobacco for oral use as "…all products for oral use, except those intended to be smoked or chewed, made wholly or partly of tobacco, in powder or in particulate form or in any combination of those forms". Synonyms for "tobacco for oral use" are moist snuff (snus) and oral tobacco. Marketing of oral tobacco is banned in all EU countries except Sweden, while other STP are allowed in EU.

Adverse health effects of smokeless tobacco products

Marketed STP vary considerably in form and content of toxicants, including nicotine, and thereby in associated health effects.

All STP contain nicotine, a potent addictive substance. The major group of carcinogens in STP includes non-volatile tobacco-specific nitrosamines (TSNA) and *N*-nitroamino acids. During the last two decades the levels of TSNA in snus have been considerably lowered. Some forms of STP contain polycyclic aromatic hydrocarbons depending on type of curing.

Aqueous and organic extracts of American and Swedish moist snuff and Indian chewing tobacco cause mutations and chromosomal damage in bacterial and mammalian cell cultures. Increased micronuclei formation in oral epithelial cells as evidence of chromosomal damage, has been associated with moist snuff use.

Use of American and Swedish moist snuff results in localised lesions in the oral epithelium, where the snuff is placed. These changes are reversible, whereas gingival retractions caused by moist snuff are not reversible. Moist snuff in portion-bag sachets gives less severe epithelial changes than snuff in loose form.

There is sufficient evidence that the use of a wide variety of STP causes cancer in humans. The pancreas has been identified as a main target organ in two Scandinavian cohort studies. Furthermore, several studies from the USA have provided additional support for a causal association between the use of smokeless tobacco and pancreatic cancer. There is inadequate evidence that STP cause lung cancer.

Risks of oral cancer have been found to be strongly associated with the use of American snuff in the USA. Studies in India, Pakistan and Sudan have reported large increases in the risk for oral cancers related to the use of various STP. In Sweden, the evidence for an increased risk of oral cancer in users of oral tobacco is less clear**.** In one study from Sweden among users of moist snuff, an increased risk of head and neck cancer has been found among never-smokers. A recent cohort study from Sweden reported a statistically significant three-fold increase of oral and pharyngeal cancer, adjusted for tobacco smoking and alcohol drinking.

There are suggestions that nasal snuff use increases the risk for certain cancers, including oral cancer.

It appears that the use of smokeless tobacco increases the risk of death after myocardial infarction, but that it does not increase the risk of myocardial infarction. Animal experiments and human studies indicate that oral tobacco use has short-term effects resulting in an increase of blood pressure and heart rate. Whether long-term use increases the risk of hypertension is uncertain. These data indicate a potential effect on the risk of cardiovascular disease.

The data on reproductive effects in relation to oral tobacco use during pregnancy are too sparse to allow conclusions. Nonetheless, studies in female Swedish users of moist snuff indicated an increased risk for prematurity and pre-eclampsia. Other studies indicate that

use of STP during pregnancy is associated with reduced birth weight and reduction in gestational age.

Various studies suggest that diabetes and other components of the metabolic syndrome might be associated with use of moist snuff.

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.

In conclusion, all STP contain nicotine, a potent addictive substance. They also contain carcinogenic tobacco-specific nitrosamines, albeit at differing levels. STP are carcinogenic to humans and the pancreas has been identified as a main target organ in American and Scandinavian studies. All STP cause localised 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.

Addiction potential of smokeless tobacco products

It is widely accepted that nicotine is the primary addictive constituent of tobacco, and nicotine demonstrates the properties of a drug of abuse. All commercially successful tobacco products deliver psychoactive levels of nicotine to users. Denicotinised tobacco products are typically not widely accepted by chronic tobacco users and are of marginal commercial importance.

Smokeless tobacco delivers quantities of nicotine comparable to those typically absorbed from cigarette smoking, although delivery of nicotine from STP lacks the high initial concentration and speed of delivery that results from inhalation of tobacco smoke, and may therefore have relatively less addiction potential than cigarettes. Nicotine levels obtained from STP are generally higher than those typically obtained from nicotine replacement therapy, which is considered to have a low addiction potential.

The time course and symptoms of withdrawal from smokeless tobacco are generally similar to those of cigarette smokers, although depressive symptoms and negative affect do not appear to be observed among abstinent STP users. It seems also that symptoms of withdrawal are stronger with some brands of smokeless tobacco delivering higher levels of nicotine compared to other brands with lower levels.

There is a lack of evidence relating to the effects of additives introduced to tobacco in the manufacturing process on the initiation of use of STP and subsequent dependence.

In conclusion, smokeless tobacco is addictive and withdrawal symptoms are broadly similar to those seen in smokers.

Use of smokeless tobacco as a smoking cessation aid compared to pharmaceutical nicotine replacement products

No randomized trial has been conducted on smokeless tobacco as an aid to smoking cessation and no randomized trial has compared smokeless tobacco to pharmaceutical nicotine replacement products in this respect. Some observational studies have looked at the use of smokeless tobacco, and in one study also nicotine replacement products, in relation to change in smoking habits but the results of these studies are inconsistent.

On the available evidence it is therefore not possible to draw conclusions as to the relative effectiveness of smokeless tobacco as an aid to smoking cessation in comparison with established therapies.

Impact of smokeless tobacco use on subsequent initiation of smoking

The association between smokeless tobacco use and cigarette smoking initiation is likely to be confounded by socio-demographic factors. In addition, across countries there are possible differences in risk for which the determinants are not fully understood. The associations observed may be due to an increased likelihood of all substance use (including STP and cigarettes) as part of a broader spectrum of risky and impulsive behaviours in adolescence.

There is some evidence from the USA that smokeless tobacco use may lead to subsequent cigarette smoking. The Swedish data do not support the hypothesis that smokeless tobacco (i.e. Swedish snus) is a gateway to future smoking. The marked social, cultural and product differences between North America and Europe suggest caution in translating findings across countries, also within Europe.

Extrapolation of the information on the patterns of smokeless tobacco use, smoking cessation and initiation from countries where oral tobacco is available to EU-countries where oral tobacco is not available

Presently, Sweden is the only EU-country in which it is legal to supply oral tobacco as defined by the EC (see above). All other smokeless tobacco products (chewing tobacco, nasal snuff) can be sold in all EU-countries. Aggregate data on smokeless tobacco product use and cigarette smoking show that particularly in Swedish men, there is a clear trend over recent decades for smoking prevalence to decrease and for use of oral tobacco (snus) to increase. The prevalence of smoking has also decreased markedly in Swedish women during this period, but to a lesser extent than in men, and in conjunction with a lesser increase in snus use. It has been suggested that the greater decline in smoking prevalence in men compared to women in Sweden is explained by the availability of snus, and this interpretation is supported by trends in longitudinal, within-person data from a population cohort in northern Sweden (report partly funded by the tobacco industry). However, these trends could also be due to successful non-smoking programs or other socio-cultural factors, and it is therefore not clear whether or by how much the availability of snus has influenced smoking prevalence. Overall smoking prevalence in Norway, as well as in young Norwegians, has decreased at the same rates in men and women during the last decade, whereas a marked increase in snus use during this time period has only occurred in young men. In California both the prevalence of smoking and smokeless tobacco use have decreased concurrently. These data imply that the association between patterns of smokeless tobacco use and smoking cessation differs between populations and is likely to be affected by cultural, societal and other factors.

In conclusion, it is not possible to extrapolate future patterns of tobacco use across countries. In particular, it is not possible to extrapolate the trends in prevalence of smoking and use of oral tobacco if it were made available in an EU country where it is now unavailable.

General conclusion

STP are addictive and their use is hazardous to health. STP contain various levels of toxic substances. Evidence on the effectiveness of STP as a smoking cessation aid is insufficient, and relative trends in progression from STP into and from smoking differ between countries. It is thus not possible to extrapolate the patterns of tobacco use from one country where oral tobacco is available to other countries due to societal, and cultural differences.

1. BACKGROUND

The prohibition on the marketing of tobacco for oral use (moist snuff, oral tobacco)[6] was introduced in 1992 (Directive 92/41/EEC[7]) and maintained in Article 8 of the recast Tobacco Products Directive (2001/37/EC[8]).

The rationale behind the ban was to protect public health by preventing people from starting to use a new tobacco product and to ensure proper functioning of the Internal Market since three Member States had already adopted such bans.

Sweden, where the use of oral tobacco called snus has been widespread, was granted derogation from the ban in its Act of Accession. Outside the EU, oral tobacco is used on a relatively wide scale in Norway, in the United States and in the Indian subcontinent. The Directive did not prohibit the marketing of other smokeless tobacco products - such as chewing tobacco and nasal snuff - which had a long tradition of use in the Community and were perceived as marginal products.

The literature suggests that smokeless tobacco, including all of the above-mentioned tobacco products, is not harmless and the harm posed could vary from one product to another, depending on the production techniques and the levels of addictive, carcinogenic and other toxic substances a product contains.

Given recent developments with regard to the composition of some smokeless tobacco products and the claims that the use of smokeless tobacco could reduce harm related to other tobacco products, DG SANCO wishes to review the scientific basis for the current regulatory framework.

2. TERMS OF REFERENCE

In the light of most recent scientific information, the Scientific Committee is requested to answer the following questions:

1. What are the adverse health effects of smokeless tobacco products?

2. What is the addiction potential of smokeless tobacco products?

3. Does the available data support the claim that smokeless tobacco may constitute a smoking cessation aid comparable to pharmaceutical nicotine replacement products?

4. What is the impact of smokeless tobacco use on subsequent initiation of smoking?

5. Is it possible to extrapolate the information on the patterns of smokeless tobacco use, smoking cessation and initiation from countries where oral tobacco is available to EU-countries where oral tobacco is not available?

[6] 'tobacco for oral use' means all products for oral use, except those intended to be smoked or chewed, made wholly or partly of tobacco, in powder or in particulate form or in any combination of those forms, particularly those presented in sachet portions or porous sachets, or in a form resembling a food product (as defined in the Tobacco Products Directive (2001/37/EC))

[7] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31992L0041:EN:HTML

[8] http://eur-lex.europa.eu/pri/en/oj/dat/2001/l_194/l_19420010718en00260034.pdf

3. SCIENTIFIC RATIONALE

3.1. Introduction

Every year, the use of tobacco products causes a heavy toll of deaths and severe human disease worldwide. The number of deaths per year due to tobacco related diseases is about 5 million and if current smoking patterns continue, about 10 million deaths are expected to occur each year due to tobacco smoking by the year 2020 (WHO 2007). The same source estimates that about half of the people that smoke today (about 650 million people) will be killed by their tobacco use, unless they quit smoking. Smokeless tobacco products (STP) are used without combustion and this eliminates the danger of direct exposure of toxic combustion compounds to the lung and other tissues of the user and of the people around. But the use of STP may result in other health hazards, local or systemic according to the way of administration and to the content of various toxic products, including nicotine and tobacco-specific nitrosamines. STP can be divided into three kinds: nasal snuff which is relatively rarely used in Europe, chewing tobacco that in some communities is mixed with other products as areca nut, catechu, and lime (see section 3.3), and finally snuff, especially moist snuff - a product that has been developed in Sweden under the name of snus.

The marketing of moist snuff was prohibited in the EU in 1992. Sweden was granted derogation from the ban on its entry to the EU in 1995 due to a long tradition of the use of snus in this country; currently 24% of the men are using it. Finland entered the EU at the same time as Sweden, but did not ask for derogation. In another neighbouring country, Norway, which is not member of the EU but member of the European Economic Area, the marketing of moist snuff is allowed, and about 11% of males use moist snuff daily. The marketing of other STP (chewing tobacco, dry snuff and nasal snuff) is not banned in EU countries.

In recent decades the use of snus in Sweden has increased while the number of smokers in this country has decreased. This is in particular the case for males. There is general agreement that the use of moist snuff is less dangerous than tobacco smoking, but the level of risk for developing cardiovascular diseases and cancer in STP users compared to the population that is not using tobacco is still debated in the scientific literature. The addiction to nicotine and possibly other substances in tobacco is another important issue.

The tobacco industry claims that improved production methods have reduced the contents of toxic products in STP, in particular the substances suspected of causing cancer. It is undeniable that for an individual substitution of tobacco smoking by the use of moist snuff would decrease the incidence of tobacco related diseases. It has also been proposed that the use of moist snuff could be a way of quitting totally the use of tobacco. On the other hand, the use of moist snuff might also initiate individuals, especially young people, to habits of tobacco consumption and maybe even to smoking. In the scientific literature both viewpoints have been advocated and a public debate is currently going on in Sweden and elsewhere concerning the health risks of moist snuff and the possible harm reduction potential of moist snuff use compared to other smoke cessation measures.

Article 11 of the directive 2001/37/EC concerning the manufacture, presentation and sale of tobacco products in EU Member States stipulates that the commission shall report regularly on the application of the directive. The first report was published in July 2005 (COM (2005) 339 final), and was based on questionnaires sent to the Member States. It was concluded that positive effects on the regulation of tobacco products are emerging at EU level. However, the report did not treat separately the question on STP because of lack of new information from the Member States. It was also considered that there was not enough new scientific information on ingredients that encourage addiction or on products that may have the potential to reduce harm.

It is the purpose of the present opinion to evaluate the most recent scientific information in order to respond to the questions formulated by the Commission. The procedures for inclusion of information are described in detail in section 3.2. In this opinion we will consider

STP that are commonly used in the EU. We will pay special attention to the Swedish STP "snus" because the marketing of this product is banned in all countries of the EU except Sweden while many other STP are widely available in EU Member States.

3.2. Methodology

The sections of the opinion that deal with cancer are mainly based on the extensive review on the health effects of STP provided previously by an expert group from the International Agency for Research on Cancer (IARC). The references from the IARC monograph (IARC 2007) have been supplemented with scientific work published after the editing of the report. For other sections of the opinion not relating to cancer, also earlier studies and reports have been considered. In order not to omit essential scientific information, a public call for information has been sent out in 2006, giving the principal stakeholders the opportunity to submit relevant scientific information concerning STP. The information received has been scrutinised carefully according to the principles described below. In general, only scientific reports that are published in English peer-reviewed scientific journals are considered. This does not imply that all published articles are considered to be equally valid and relevant for health risk assessment. On the contrary, a main task is to evaluate and assess the articles and the scientific weight that is to be given to each of them. Only studies that are considered relevant for the task are commented upon in the opinion.

Relevant research for assessment of health risks of STP can be divided into broad sectors such as epidemiologic studies, experimental studies in humans, experimental studies in animals, and cell culture studies. A health risk assessment evaluates the evidence within each of these sectors and then weighs together the evidence across the sectors to a combined assessment. This combined assessment should address the question of whether or not a hazard exists i.e., if there exists a causal relationship between exposure and some adverse health effect. The answer to this question is not necessarily a definitive yes or no, but may express the weight of the evidence for the existence of a hazard. If such a hazard is judged to be present, the risk assessment should also address the magnitude of the effect and the shape of the dose-response function, used for characterising the magnitude of the risk for various exposure levels and exposure patterns.

A full risk assessment also includes exposure assessment in the population and estimates of the impact of exposure on burden of disease. Epidemiological and experimental studies are subject to similar treatment in the evaluation process. It is of equal importance to evaluate positive and negative studies, i.e., studies indicating that STP have an effect and studies not indicating the existence of such an effect. In the case of positive studies the evaluation focuses on alternatives to causation as explanation of the positive result: What is the degree of certainty for ruling out the possibility that the observed positive result is produced by bias, e.g. confounding or selection bias, or chance. In the case of negative studies one assesses the certainty with which it can be ruled out that the lack of an observed effect is the result of (masking) bias, e.g. because of too small exposure contrasts or too crude exposure measurements; one also has to evaluate the possibility that the lack of an observed effect is the result of chance, a possibility that is a particular problem in small studies with low statistical power.

Obviously, the presence or absence of statistical significance is only one factor in this evaluation. In addition, the evaluation considers a number of other characteristics of the study. Some of these characteristics are rather general, such as study size, assessment of participation rate, level of exposure, and quality of exposure assessment. Particularly important aspects are the observed strength of association and the internal consistency of the results including aspects such as dose-response relation. Regarding experimental studies, additional important characteristics that are taken into consideration are the types of controls that have been used and to what degree replication studies have been performed. It is worth noting that the result of this process is not an assessment that a

specific study is unequivocally negative or positive or whether it is accepted or rejected. Rather, the assessment will result in a weight that is given to the findings of a study.

In the final overall evaluation phase, the available evidence is integrated over various sectors of research. This phase combines the existing relevant pieces of evidence on a particular endpoint from studies in humans, from animal models, *in vitro* studies, and from other relevant areas. The integration of the separate lines of evidence should take place as the last stage, after the critical assessment of all (relevant) available studies for particular endpoints. In the first phase, epidemiological studies should be critically evaluated for quality irrespective of the putative mechanisms of biological action of a given exposure. In the final integrative stage of evaluation, however, the plausibility of the observed or hypothetical mechanism(s) of action and the evidence for the mechanism(s) is a factor to be considered. The overall result of the integrative phase of evaluation, combining the degree of evidence across epidemiology, animal studies, *in vitro* and other data depends on how much weight is given to each line of evidence from different categories.

3.3. Smokeless Tobacco Products - Types, Use and Exposure

3.3.1. Types and mode of consumption

There are different types of STP in use around the world and the health risks related to their use vary considerably. Smokeless tobacco comes in two main forms: snuff (finely ground or cut tobacco leaves that can be dry or moist, loose or portion packed in sachets, and administered to the mouth, or the dry products to the nose or mouth) and chewing tobacco (loose leaf, in pouches of tobacco leaves, "plug" or "twist" form). When administered orally, the tobacco can also be mixed with other psychoactive ingredients. The Swedish moist snuff "snus" is sold in loose weight in boxes or in small "tea-bag"-like sachets.

In India, use of domestic types of chewing tobacco is a major cause of oral cancer and is also harmful in pregnancy (see chapter 3.6.4. and 3.6.5.2.). As these types of STP are allowed in Europe, this is also a cause of concern here.

An attempt to list the wide range of oral and nasal tobacco products used is presented below. This list is by no means exhaustive as there almost certainly exist as yet undescribed varieties in the world. With the present rate of immigration many of these products may find their way into EU countries, and their use is typically clustered in local communities. A similar clustering of use may be seen with now increasingly rarer traditional European products such as nasal snuff. Products of established and significant use in EU countries are underlined in table 1.

Table 1. Smokeless Tobacco Products used, by region.

Common name	Where used, Brand names	Contents	How used	Who uses	Processing
Europe					
Moist snuff, Snus (Other forms of smokeless tobacco: chewing tobacco or dry snuff are very rarely used in the Nordic countries)	Sweden, Norway, Finland *Catch; General; Ettan, Grovsnus; Göteborgs Rapé; Göteborgs Prima fint; Rallarsnus; Probe; Röda Lacket (Swedish Match); Gustavus (Gallaher); Skruf (Skruf); Gellivare; Landströms (Gellivare Snusfabrik) Metropol, Granit, Mocca (Fiedler & Lundgren); Lucky Strike (BAT), Prince (House of Prince); Roots (Snusab)*	Tobacco; water; sodium carbonate; sodium chloride; moisturizer; flavouring; nicotine	A pinch (called a dip) is usually placed in the upper gingivolabial sulcus. The average user keeps snus in the oral cavity for 11 to 14 hours per day.	24% of Swedish men and 3% of Swedish women use snus daily (Statistics Sweden 2007) Snus is used by 5% of Norwegian males, very little by females. Although banned, there is an increasing use in Finland, (see chapter 3.3.3.3).	Finely ground dry tobacco is mixed with aromatic substances, salts, water, and humidifying agents. The product is pasteurised by heating and kept cool to avoid ageing.
Dry snuff	Germany, UK, Republic of Georgia *European brand names: Bernards, Lotzbeck, Pöschl (Germany). Fribourg & Treyer, Gawith Hoggarth, Hedges, McChrystal's, Wilsons of Sharrow (UK). Burnuthi (Georgia)*	Tobacco + flavouring	Inhaled up the nostril	No data Annual production low	Tobacco is fire-cured and air-cured, then fermented or simply mixed with other ingridients and processed into a dry, powdered form. The moisture content of the finished product is less than 10%. It is packaged and sold in small metal or glass containers.
Tobacco gum (non-pharmaceutical)	Sweden, Denmark (introduced 2006) *Firebreak*	Tobacco, chewing-gum base, xylitol	Gum to be chewed	No data	Finely ground tobacco (3%) embedded in chewing gum
Gutkha	Some products are available in Europe	Tobacco, areca nut	See below	No data	See below

Common name	Where used, Brand names	Contents	How used	Who uses	Processing
Chewing tobacco	*Oliver Twist* (Nordic countries) Other products are available in Europe	Tobacco, water, flavouring	Chewed or smoked in pipes	No data	Pieces of twisted tobacco used orally. Handmade from unfermented tobacco (Oliver Twist).
North America					
Dry snuff Same/similar to European	USA *Bruton, Garrett, Honest Scotch, Railroad Mills and Red Seal.*	Tobacco + aromatic oils, spices	Put in oral cavity	Mainly women	Tobacco is fire-cured, then fermented and processed into a dry, powdered form. The moisture content of the finished product is less than 10%. It is packaged and sold in small metal or glass containers
Loose leaf chew	USA *Red Man, Red Man Golden Blend, Red Man Select, Granger, Work Horse (Swedish Match products); Scotten, Dillon, Levi Garrett, HB Scott, Taylors Pride, Red Fox (Conwood products); Beech-Nut Regular, Beech-Nut Wintergreen, Beech-Nut Spearmint (National products); Chattanooga Chew (Swisher product)*	Leaf tobacco; sweetener and/or liquorice	A piece of tobacco 0.75 to 1 inch in diameter is tucked between the gum and jaw, typically toward the back of the mouth. It is either chewed or held in place.1 Saliva spit or swallowed.	Predominantly southern white, blue collar males	Commercially manufactured. Loose cigar tobacco leaves are air-cured, then stemmed, cut or granulated and loosely packed to form small strips of shredded tobacco. Most brands are sweetened and flavoured with liquorice. Typically sold in pouches weighing about 3 ounces. Loose-leaf tobacco has a high average sugar content (approximately 35%).
Moist plug Chewing tobacco, spit tobacco	USA *Red Man Moist Plug, Totems, RJ Gold (Swedish Match products); Levi Garrett Plus, Taylors Pride (Conwood products)*	Enriched tobacco leaves; fine tobacco; sweetener and/or liquorice	Chewed or held between the cheek and lower lip. Saliva may be spit or swallowed.	Predominantly southern white, blue collar males	Commercially manufactured. Enriched tobacco leaves (Burley and bright tobacco or cigar tobacco) or fragments are wrapped in fine tobacco and pressed into bricks. Moist plug tobacco has at least 15% moisture. Most plug tobacco is flavoured and sweetened with liquorice. Plus tobacco is packaged as a compressed brick or flat block wrapped inside natural tobacco leaves. Typically weighs 7 to 13 ounces. Sugar content is approximately 24%

Common name	Where used, Brand names	Contents	How used	Who uses	Processing
Moist snuff	USA *Copenhagen, Cougar, Grizzly, Kayak, Kodiak, Red Seal, Red Wood, Rooster, Silver Creek, SkoalL, Timber Wolf*	Tobacco	A pinch "dip" or held between the cheek/gum. Saliva may be swallowed.	Used more and more by "non-traditional" users. Increasing market share.	Tobacco is either air- or fire-cured, then processed into fine particles ("fine cut") or strips ("long cut"). Tobacco stems & seeds not removed. Moisture content up to 50%. Sold loose (Skoal, Copenhagen and Kodiak) or in sachets (Skoal Bandits). Nicotine released more rapidly from fine cut due to the greater surface area.
Plug chew Chewing tobacco	USA *Days Work (Swedish Match product); Conwood (Conwood product); Brown & Williamson (Brown & Williamson product)*	Enriched tobacco leaves; fine tobacco; sweetener and/or liquorice	Chewed or held between the cheek and lower lip.1 Saliva may be spit or swallowed.	Predominantly southern white, blue collar males	Enriched tobacco leaves (Burley and bright tobacco and cigar tobacco) are wrapped in fine tobacco and pressed into bricks with less than 15% moisture. Most plug tobacco is flavoured and sweetened with liquorice. Plus tobacco is packaged as a compressed brick or flat block wrapped inside natural tobacco leaves. Package typically weighs 7 to 13 ounces
Twist roll (chew) Chewing tobacco	USA *Conwood (Conwood product), R.C. Owen (R.C. Owen product), R.J. Reynolds (R.J. Reynolds product)*	Tobacco; tobacco leaf Extract	Chewed or held between the Cheek and lower lip. Saliva may be spit or swallowed.	Predominantly southern white, blue collar males	Handmade by commercial manufacturers. Dark, aircured leaf tobacco is treated with a tar-like tobacco leaf extract and twisted into rope-like strands that are dried. Typically, no flavouring or sweetener is added. The final product is a pliable but dry rope. The product is sold by the piece is small (1 to 2 ounce) or larger sizes based on the number of leaves in the twist.
Iq'mik	Alaska	Tobacco, punk ash	Users pinch off a small piece and chew the iq'mik. Often, the user may premasticate the iq'mik and place it in a small box for later use by others, including children and sometimes teething babies.	Alaska Natives (men, women and children). One study found that 52% of Yukon-Kuskokwim Delta Alaska Natives used iq'mik	Fire-cured tobacco leaves are mixed with punk ash (ash generated by burning a woody fungus that grows on the bark of birch trees). The ingredients are available at grocery stores and retail outlets, but are generally combined by the user before use.1 It is believed that the punk ash in the mixture raises the pH level in the mouth, increasing the dose and enhancing the delivery of nicotine to the brain.

Common name	Where used, Brand names	Contents	How used	Who uses	Processing
South America					
Chimo	Venezuela *Ambil*	Tobacco resin; alkaline ash; Paullinia yoco; banana peel; sugar; avocado seed	A very small amount of the paste is placed under the tongue and absorbed there. Saliva is traditionally spat out. Chimo is popular as a replacement for cigarettes and provides a similar bolus of nicotine.	No data	Tobacco and the other plants involved in manufacture are crushed and the juices extracted. The liquid is boiled until it becomes very thick. Ash is then added, which helps thicken the mixture further. The resulting product is a very thick paste
Dry snuff, Rapé	Brazil *Guarany*	Dry tobacco powder with peppery smell	Sniffed through nostrils	No data	
Indian subcontinent					
Gul	Central and Eastern India *Gadakhu*	Tobacco powder, molasses, other ingredients	Often used for cleaning teeth	Primarily women	Commercially manufactured. Since 1986, gul has been machine produced and sold in toothpaste-like tubes.
Gutkha	India, Southeast Asia, United Kingdom *Manikchand, Moolchand, Tulsi, Shimla, Sikandar, Pan Parag*	Areca nut, catechu, tobacco, lime, saffron, flavouring, saccharine, mint	Held in the mouth and chewed. Saliva is generally spit out, but sometimes swallowed.	Widely used by both sexes, even children	Commercially manufactured. Tobacco, areca nut and catechu are mixed together and sweetened. Product is sold in small brightly-coloured packets, which may appeal to children.
Khaini	India	Tobacco; slaked lime paste; sometimes areca nut	Paste is placed in the mouth and chewed	No data	Powdered tobacco and slaked lime paste

Common name	Where used, Brand names	Contents	How used	Who uses	Processing
Kiwam	India	Tobacco; slaked lime; spices	Placed in the mouth and chewed	No data	Tobacco leaves are processed by removing their stalks and stems, then boiled and soaked in water flavoured with spices and additives. The resulting pulp is mashed, strained, and dried into a paste.
Mawa	Bhavnagar, India; Gujarat	Tobacco; slaked lime; areca nut	Placed in the mouth and chewed for 10 to 20 minutes	No data	Small pieces of sun-cured areca nut and mixed with tobacco flakes and slaked lime (liquid calcium hydroxide). The mixture is rubbed together to combine. The resulting mixture is about 95% areca nut.
Tuibur, hidakphu	India: Mizoram, Manipur	Tobacco water	Sipped and held in mouth 5-10 min and then spat out	Widespread use in certain areas	Made by passing tobacco smoke through water
Mishri (masheri or misheri)	Maharashtra, India	Tobacco	Applied to the teeth and gums, often for the purpose of cleaning the teeth. Users then tend to hold it in their mouths (due to the nicotine addiction).	Predominantly women	Tobacco is baked on a hot metal plate until toasted or partially burnt, then powdered.
Nass (naswar, niswar)	Central Asia; Iran; Afghanistan; Pakistan; Baluchistan, India	Nass: tobacco, ash; cotton or sesame oil; water; sometimes gum. Naswar or niswar: tobacco, slaked lime; indigo; cardamom; oil; menthol; water	Held in the mouth for 10 to 15 minutes. Naswar is sometimes chewed slowly.	No data	Sun- and heat-dried tobacco leaves, slaked lime, ash from tree bark, and flavouring and colouring agents are mixed together. Water is added and the material is rolled into balls.

Common name	Where used, Brand names	Contents	How used	Who uses	Processing
Pan masala	India, Sri Lanka, Pakistan, Bangladesh, Myanmar, Thailand, Cambodia, Malaysia, Singapore, Indonesia, Philippines, New Guinea, Taiwan, China *Manikchand, Mahak, Pan Parag, Vimal, Crane, Patti, Rajdarbar, Kuber, Yamu, Badshah, Tulasi, Rahat, Pan King, Jubilee, Kanchan, Sir*	Tobacco; areca nuts, slaked lime, betel leaf. "Chewing tobacco" is sometimes used, and flavouring agents such as menthol, camphor, sugar, rosewater, aniseed, mint, or other spices are sometimes added in different regions.	A quid is placed in the mouth (usually between the gum and cheek) and gently sucked and chewed. Pan masala is sometimes served in restaurants after the meal.	Widely used by both sexes	Commercially prepared or assembled at home. Areca nut is boiled, roasted, or sun-dried. Tobacco may be used raw, sun-dried, roasted, then finely chopped, powdered and scented. Alternatively, the tobacco may be boiled (zarda), made into a paste and scented with rosewater or perfume. To assemble, slaked lime and catechu are smeared on a betel leaf. The betel leaf is folded into a funnel shape and tobacco, areca nut and any other ingredients are added. The top of the funnel is folded over, resulting in a quid, which is placed in the mouth for use.
Zarda	India	Processed tobacco	Along with betel quid	Both men and women in Indian sub continent and immigrants from there	Commercially manufactered. Processed tobacco leaves with spices flavouring agents and vegetable dyes
Creamy snuff	India *Ipco*	Tobacco, clove oil, glycerine, menthol, spearmint, camphor	Often used to clean teeth. The manufacturer recommends letting the paste linger in mouth	Primarily women	Commercially manufactured. Sometimes marketed as a dentifrice.
Red tooth powder	India	Tobacco			
Middle East					
Shammah	Saudi Arabia	Tobacco; ash; slaked lime			

Common name	Where used, Brand names	Contents	How used	Who uses	Processing
Africa					
Toombak	Sudan	Tobacco; sodium bicarbonate	Product is rolled into a ball of about 10g called a saffa. The saffa is held between the gum and the lip or cheeks, or on the floor of the mouth. It is sucked slowly for 10 to 15 minutes. Male users periodically spit, while female users typically swallow the saliva generated. The user usually rinses their mouth with water after the saffa is removed.	Among those over the age of 18, about 34% of men and 2.5% of women in Sudan use toombak.	Tobacco leaves are harvested and left in a field for uniform drying. The leaves are then tied into bundles, sprinkled with water and stored for a couple of weeks at 30 to 45ºC for fermentation. The leaves are then ground up and aged for up to a year. After aging, toombak vendors (in toombak shops) place the product in bowls and gradually add sodium bicarbonate until the mixture is approximately 2 parts tobacco to 1 part sodium bicarbonate. The mixture is blended by hand and constantly tested with the tips of the fingers until it becomes moist and hardened. The toombak is then placed in an airtight container for about 2 hours and sold. Toombak is frequently home grown.
Snuff	South Africa *Ntsu, Taxi Red, Singleton Menthol, and Tobacco-rette original (pre-packed in pouches).*	Tobacco	Sniffed through nostrils Portion bags introduced	Black women (13%) and black children (18%)	Commercially grown or home-grown

3.3.2. Chemical composition

3.3.2.1. General considerations

There is a choice of 60 *Nicotiana* species and 100 varieties of tobacco that can be used to prepare the final tobacco products. However, the majority of commercial tobacco products use *N. tabacum* species. Cured tobacco can contain between 0.2 and 4.75% nicotine by weight, depending on plant genetics, growing conditions, degree of ripening, fertilizer treatment and leaf position on the stalk (Stratton et al. 2001). The classification of leaf tobacco commonly used in smokeless tobacco products is primarily based on curing methods (e.g. air-, flue- and fire-cured tobacco) and tobacco types (e.g. burley, Wisconsin, Pennsylvania air-cured tobacco; dark fire-cured tobacco, fire-cured Virginia tobacco).

The number of chemicals identified in tobacco totals more than 3 000 (Roberts 1988). Major components are alkaloids (0.5–5.0%, Figure 1), with nicotine as the predominant compound (85–95% of total alkaloids), terpenes, (0.1–3.0%), polyphenols (0.5–4.5%), phytosterols (0.1–2.5%), carboxylic acids (0.1–0.7%) and alkanes (0.1–0.4%) (IARC 1985). Other constituents are aromatic hydrocarbons, aldehydes, ketones, amines, nitriles, N- and O-heterocyclic hydrocarbons, pesticides, alkali nitrates (0.01–5%) and at least 30 metallic compounds (Brunnemann and Hoffmann 1992, IARC 2007). Many smokeless tobacco formulations use plant extracts or chemicals as flavouring agents (Mookherjee and Wilson 1988, Roberts 1988, Sharma et al. 1991). Other additives, such as ammonium salts and sodium carbonate, are applied to increase the pH.

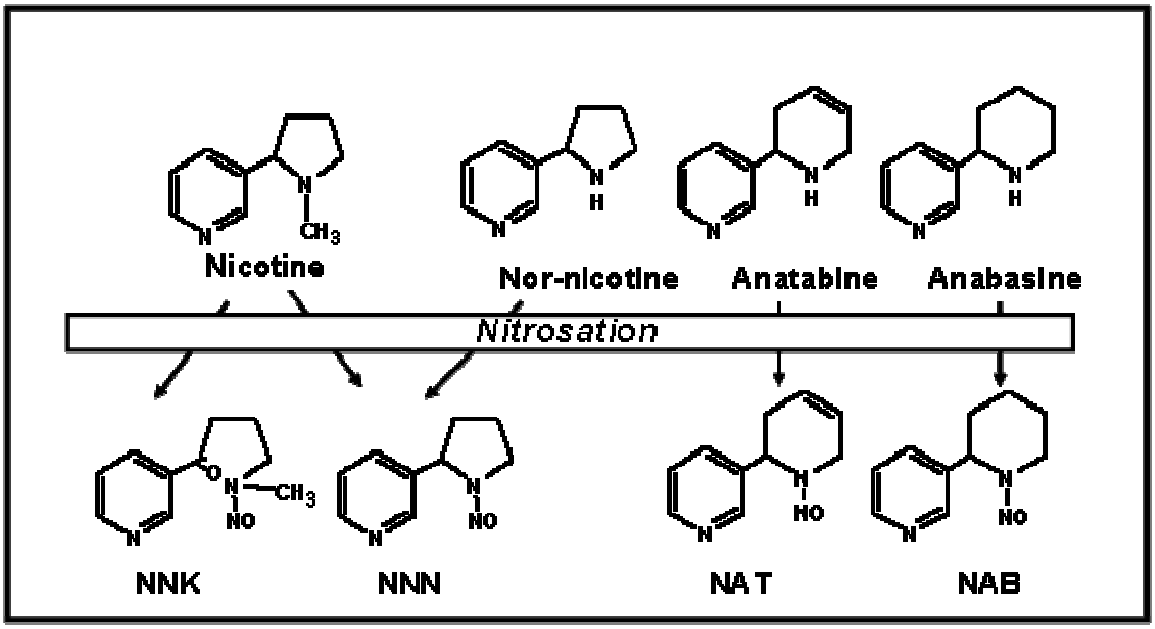

Figure 1. Structures of tobacco alkaloids and related tobacco specific nitrosamines.

3.3.2.2. Nicotine, pH and unionised nicotine

As in tobacco smoking, nicotine remains the main determinant of addiction for smokeless tobacco use (Henningfield et al. 1997, Hatsukami and Severson 1999). The level of unionised (free) nicotine increases with higher pH, facilitating nicotine absorption. The nicotine content in 17 brands ranged from 3.4 mg/g to 14.5 mg/g; the pH ranged from 5.39 to 7.99 and unionised nicotine ranged from 0.23% to 48.3% of total nicotine

(Djordjevic et al. 1995). Similar findings were reported by Henningfield et al. (1995) for products purchased at three locations. Among moist snuff brands the highest amount of nicotine was found to be 13.5 mg/g. Chewing tobacco had the lowest amount of nicotine (mean, 1.22%; range 0.45–4.65%). Moist snuff had, on average, the highest pH (7.43 versus 6.36 and 5.82 in dry snuff and chewing tobacco, respectively). Because of the high pH, the levels of unionised nicotine in moist snuff averaged 3.5 mg/g product, ranging from 0.03 to 8.6 mg/g.

The nicotine content of Zarda products was reported in the range 14 - 65 mg/g while that of gutkha was in the range 1.2 -11.4 mg/g (Stepanov et al. 2005a, McNeill et al. 2006). The moisture in the Zarda products ranged from 4.9-9% (w/w), pH ~5-6 and free nicotine 0.1-0.4 mg/g whereas in gutkha products the values were: moisture 1.3-1.5, pH ~9 and free nicotine 2.1-5.9 mg/g. Nasal tobacco contains up to 16 mg/g nicotine, and has a pH up to 10.1 (Ayo-Yusuf et al. 2004).

3.3.2.3. Carcinogenic compounds in smokeless tobacco products

To date, more than 28 carcinogens have been identified in tobacco leaves for smokeless use (Table 2 lists carcinogens classified by IARC and EU); (Brunnemann and Hoffmann 1992).

N-Nitrosocompounds

The major and most abundant group of carcinogens is the non-volatile alkaloid-derived tobacco-specific N-nitrosamines (TSNA) and N-nitrosoamino acids (Ohshima et al. 1985). Other carcinogens reportedly present in tobacco include volatile N-nitrosamines, certain volatile aldehydes, some polynuclear aromatic hydrocarbons such as benzo[a]pyrene (levels depending on curing process), certain lactones, urethane, hydrazine, metals, polonium-210 and uranium-235 and –238 (for reviews, see Weeks 1985, Roberts 1988, Brunnemann and Hoffmann 1992). There are three major types of nitroso compounds in STP: (a) non-volatile TSNA (Figure 1), including 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butan-1-ol (NNAL) and N.-nitrosonornicotine (NNN); (b) N-nitrosamino acids, including N-nitrososarcosine (NSAR), 3-(N-methylnitrosamino)propionic acids (NMPA) and 4-(N-methylnitrosamino)butyric acids (MNBA); and (c) volatile N-nitrosamines, including N-nitrosodimethylamine (NDMA), N-nitrosopyrrolidine (NPYR), N-nitroso-piperidine (NPIP) and N-nitrosomorpholine (NMOR). In addition to these three groups of compounds, smokeless tobacco contains N-nitrosodiethanolamine (NDELA), which is formed from diethanolamine, a residual contaminant in tobacco. Although there has been a decline in the concentrations of nitrosamines in STP in Sweden and the USA since the 1980s (Djordjevic et al. 1993a, Brunnemann et al. 2004, Österdahl et al. 2004), the trend may not apply to other products and countries. Two recent papers reported levels of TSNA in Zarda and gutkha products; McNeill et al. (2006) reported total TSNA levels 0.3 -1.4 µg/g in gutkha products and 0.7-29.7 µg/g in Zarda products. Stepanov et al. (2005a) reported NNN 0.9-1.09 µg/g, NNK 0.04-0.20 µg/g, NAT 0.01-0.08 µg/g and NAB 0-0.05 µg/g in gutkha products. The major carcinogenic TSNA and nitrosamino acid levels in different products from Europe, USA, and Canada are shown in Tables 3 and 4. For some of the Indian STP relatively high levels of TSNA have been reported (IARC 2007).

In recent years there has been a declining trend of NNN and NNK levels in moist snuff in Europe that the manufacturers attribute to selection of raw products with low levels of TSNA and inhibition of nitrosation reactions during the processing and storage of the products (Österdahl et al. 2004). The moist snuff produced and purchased in Sweden in this study had an average value of NNN and NNK 0.5 and 0.2 µg/g wet weight, respectively. In a recent analysis, snuff produced by conventional methods in USA had NNN 0.9-4.5 µg/g and NNK 0.17 -1.5 µg/g wet weight (Stepanov et al. 2006). Two brands with similar manufacturing process as the one used in Sweden to reduce harmful

nitrosamines, had mean levels of 0.98 and 2.2 µg NNN/g and of 0.18 and 0.26 µg NNK/g wet weight, respectively.

The median yield of TSNA in the mainstream smoke of cigarettes is estimated to be about 350 ng/cigarette (Borgerding et al. 2000, IARC 2007). An average smoker of 20 cigarettes/day would then be exposed to 7µg of TSNA. In comparison, the exposure of TSNA in an average moist snuff user will be about 6 times higher (40 µg/day) assuming the use of 20g of the product/day with a 2 µg/g concentration.

Other nitrosamines

N-Nitrosomorpholine, derived from nitrosation of morpholine used in packaging, was detected in some US STP at concentrations up to 0.7 µg/g, and N-nitrosodiethanolamine at 0.3 - 3.3 µg/g. The latter compound is thought to have originated from the agricultural use of diethanolamine as solubiliser for the growth inhibitor maleic hydrazide (Brunnemann et al. 1982). Today, the products found in the US as well as on the Swedish market are practically free from these nitrosamines (Brunnemann and Hoffmann 1991). The contents of volatile nitrosamines such as NDMA, NPYR and NPIP in Swedish moist snuff have generally been low (0.008 µg/g, mean of 14 samples from 1982; Österdahl and Slorach 1984).

Polycyclic aromatic hydrocarbons (PAH)

In flue- (fire) cured tobacco elevated concentrations of PAHs are found. PAHs in tobacco products originate primarily from ambient air and, in addition, from flue-curing. Benzo[a]pyrene (BaP), an indicator of PAH exposure, has a carcinogenic potency comparable to that of NNK (Nilsson 1998), and may be present in some U.S. snuff products at a concentration up to about 60 ng/g (Hoffmann et al. 1986) and up to 90.5 ng/g in dry snuff (Brunnemann and Hoffmann 1992). McNeill et al. (2006) reported the BaP levels in gutkha and Zarda products to be 0.3-8.9 ng/g. However, in comparison with NNK and NNN, the detectable levels of carcinogenic PAHs in American snuff from fire-cured tobacco must be considered as very low. Because Swedish snuff is not prepared from fire-cured tobacco, the levels of PAH in these products lie below the detection limit.

Radionuclides

The most important radionuclide in tobacco used for snuff is the alpha and gamma emitter 226Ra with a half-life of 1620 years, and to some extent also 210Pb with a half life of 19 years (USEPA 1979). Tobacco used for snuff has also been claimed to contain the alpha and gamma emitter 210Po that decays to stable 206Pb (Gregory 1965, Harley et al. 1980, Hoffmann et al. 1986). According to Hoffman et al. (1986), the average total activity of alpha emitters in 5 major brands of US snuff was found to be 0.16-1.22 pCi/g (0.006-0.045 Bq/g), which is in agreement with the activity measured by other researchers (Martell 1974). Daily consumption of 20 g snuff will thus result in an exposure of 0.12 – 0.9 Bq. Uranium-235 and -238 were reported only in Indian nasal snuff, each at about 2 pCi/g tobacco (Sharma et al. 1985). The dose of ionising radiation from these sources must be considered as negligible in comparison e.g. with the natural radiation background and other sources of ionising radiations.

Other compounds

Formaldehyde and other volatile aldehydes such as acetaldehyde and crotonaldehyde (IARC Group 3) are formed from amino acids and sugars by heating during tobacco processing (Coleman and Perfetti 1997). Urethane may be present in fermented tobacco at up to 375 ng/g).

Table 2. Levels of classified carcinogenic agents identified in smokeless tobacco products[9].

Agent	Type of product[10]	Concentration range (ng/g)	IARC classification[11]	EU classification[12]
Benzo(a)pyrene	MS,DS,Z,G	>0.1-90	1	Carc. Cat. 2
Urethane	CT	310-375	2A	Carc. Cat. 2
Formaldehyde	MS,DS	1600-7400	1	Carc. Cat. 3
Acetaldehyde	MS,DS	1400-27,000	2B	Carc. Cat. 3
N-Nitrosodimethylamine	MS,CT	ND-270	2A	Carc. Cat. 2
N-Nitrosopyrrolidine	MS,CT	ND-860	2B	-
N-Nitrodopiperedine	MS,CT	ND-110	2B	-
N-Nitrosomorpholine	CT,MS	ND-690	2B	-
N'-Nitrososarcosine	MS	ND-6300	2B	-
N-Nitrosonornicotine	MS,CT,Z,G	400-58000	1	-
4-(Methylnitrosamino)-1-(3-pyridyl)1-butanone	MS,CT,Z,G	ND-7800	1	-
Nickel	MS,G	180-2700	1	Carc. Cat. 3
Arsenic	Z,G	40-290	1	-

[9] In addition, radioactive polonium- 210, uranium-235 and -238 are present at pCi levels in moist snuff.
[10] Not all carcinogens are measured in each product (MS - moist snuff; DS - dry snuff; CT - chewing tobacco; Z - zarda product; G - gutkha product).
Adapted and updated from IARC (2007).
[11] Group 1: Carcinogenic to humans; Group 2A: Probably carcinogenic to humans; Group 2B: Possibly carcinogenic to humans (IARC, 2006)
[12] Category 1: Substances known to be carcinogenic to man; Category 2: Substances which should be regarded as if they were carcinogenic to man; Category 3: Substances which cause concern for man owing to possible carcinogenic effect (EC, 2007)

Table 3. Comparison of the levels of TSNA in smokeless tobacco products (µg/g tobacco) across countries[13].

Country	Type of product	NNN	NNK	Reference
USA	Moist snuff Chew Dry snuff	ND[14]–135 0.25–6.5 9.4–116.1	ND–17.8 0.08–1.05 0.88–84.4	Brunnemann et al. (1985, 1987a, 1987b, 2004); Ohshima et al. (1985); Hoffmann et al. (1986, 1988, 1991, 1995); Chamberlain et al. (1988); Tricker and Preussmann (1991); Adams et al. (1987); Andersen et al. (1989); Djordjevic et al. (1989a, 1993a, 1993b, 1995); Brunnemann and Hoffmann (1992); Prokopczyk et al. (1992, 1995); MDPH (2001); Österdahl et al. (2004)[15]
USA[16]	Moist snuff Moist snuff	2.4-6.4 0.9-4.5	0.6-1.6 0.17-1.5	Rodu and Jansson (2004) Stepanov et al. (2006)
Canada	Moist snuff Chew	15.6–88.9 2.09	1.94–15.2 0.24	Brunnemann et al. (1985, 1987a)
Sweden	Moist snuff Chew	0.15–20.9 0.7–1.7	0.03–10.4 0.01–0.46	Brunnemann et al. (1985); Ohshima et al. (1985); Hoffmannn et al. (1988, 1991); Österdahl and Slorach (1988); Tricker and Preussmann (1991); Brunnemann and Hoffmann (1992); Djordjevic et al. (1993b), MDPH (2001); Janssson et al. (2003), Österdahl et al. (2004)[15]
Sweden[16]	Moist snuff Moist snuff	1.0-1.1 0.98-2.2	0.4-1.6 0.18–0.26	Rodu and Jansson (2004) Stepanov et al. (2006)
Denmark	Chew	0.08–1.6	0.01–1.9	Österdahl et al. (2004)[15]
Norway	Moist snuff	21[17]	3.3[17]	Österdahl et al. (2004)[15]
United Kingdom	Moist snuff Chew Dry/nasal snuff	1.1–52.0 0.9 1.8–16.0	0.4–13.0 0.3 0.26–4.3	Hoffmann et al. (1988); Brunnemann and Hoffmann (1992); Österdahl et al. (2004)[15]
Germany	Chew Dry snuff	0.9–2.3 0.68–18.75	0.03–0.3 0.1–6.43	Brunnemann et al. (1985); Tricker and Preussmann (1991); Brunnemann and Hoffmann (1992); Österdahl et al. (2004)[15]
Belgium	Chew	7.38	0.13	Ohshima et al. (1985)

[13] Adapted and updated from IARC (2007)
[14] ND: Not Detected
[15] 13 out of 27 samples were provided by manufacturers, 2 ordered on the internet, the rest purchased from shops
[16] These have been published after the IARC-Monograph (2007)
[17] Sample from 1983

European (country, origin not reported)	Nasal snuff	2.4-18.8	0.6-6.43	Tricker and Preussmann (1991)
UK	Gutkha Zarda (no data on NNN/NNK)	0.3-29.7[18]		McNeill et al. (2006)
India	Gutkha Zarda	0.9-1.09 4.81-19.9	0.04-0.43 1.07-3.09	Stepanov et al. (2005a)

Table 4. Major carcinogenic N-nitrosamino acids in smokeless tobacco (µg/g dry wt)[19].

Country	Type of product	NSAR	NMPA	Reference
USA	Moist snuff Chew Dry snuff	ND[20]–6.3 ND ND	0.15–70.0 0.6 1.2–4.5	Ohshima et al. (1985); Djordjevic et al. (1989b, 1993a, 1993b); Hoffmann et al. (1991, 1995); Brunnemann and Hoffmann (1992)
Sweden	Moist snuff	0.01–0.68	1.0–3.28	Hoffmann et al. (1991); Tricker and Preussmann (1991); Brunnemann and Hoffmann (1992)
United Kingdom	Moist snuff Nasal snuff	0.03–1.1 0.04	1.36–19.0 1.0–2.8	Tricker and Preussmann (1991); Brunnemann and Hoffmann (1992)
European	Nasal snuff	ND–0.085	0.49–4.26	Tricker and Preussmann (1991)

[18] TSNA total
[19] Adapted from IARC (2007)
[20] ND: Not Detected

3.3.2.4. Adducts of tobacco specific nitrosamines in animal models

NNK and NNN – the major carcinogens present in smokeless tobacco – induce two types of primary DNA lesions: nucleotide methylations and pyridyloxo-butylations (HPB adducts). With respect to methylations, the highest yields of adducts in the target organs lung, liver and nasal mucosa of rats exposed to NNK have been found for 7-methylguanine (7-mGua), followed by O^6–methylguanine (O6-mGua), whereas very low levels of O^4–methylthymidine (O4-mTh) were present (Belinsky et al. 1986). O6-mGua is, on the other hand, a highly pro-mutagenic adduct that gives rise to GC to AT transitions (Tan et al. 1994, Pletsa et al. 1994, Jansen et al. 1996) of a type found in codon 12 of the Ki-*ras* oncogene from mouse lung tumours induced by NNK (Belinsky et al. 1989, Ronai et al. 1993).

O^6–methylguanine (O6-mGua)

Nasal mucosa

In rats, after administering subcutaneous NNK injections, 3 times per week for 4 weeks with doses ranging from 0.03 mg to 50 mg/kg (0.013 to 21.4 mg/kg/day), the adduct levels increased rapidly in the dose range 0.13 to 0.43 mg/kg/day, followed by a decline in alkylation efficiency at higher doses (Belinsky et al. 1990). No increase in O6-mGua was detected in the respiratory epithelium at the lowest dose of 0.013 mg/kg/day, although the limit of detection for O6-mGua was stated as 0.1 pmol/µmol guanine. At 1 mg/kg some necrotic changes were detected in the rat nasal olfactory epithelium that became increasingly severe at doses above 10 mg/kg. The respiratory epithelium was considerably less sensitive. After 20 weeks of treatment a significant increase in malignant tumours was found only at 50 mg/kg. The authors therefore concluded that cell proliferation secondary to toxicity is required for tumour induction by NNK in the rodent nose (Belinsky et al. 1987, Belinsky et al. 1990).

Liver

Repeated administration of 100 mg NNK/kg/day for 12 days resulted in an initial sharp increase in O6-mGua as well as of 7-meGua levels that subsequently declined markedly, evidently due to the induction of DNA repair enzyme 0^6-methylguanine-DNA methyltransferase (Swenberg et al. 1982). No increase in O6-mGua could be detected one day after single subcutaneous injections of low doses of NNK in the range 0.03 – 0.3 mg/kg/day, nor at 0.43 mg/kg/day during 4 weeks, reflecting efficient removal of the adducts by the DNA methyltransferase (Belinsky et al. 1990). As the dose was increased to 21.4 mg/kg/day, necrotic changes and subsequent development of hepatic neoplasia appeared after 20 weeks´ treatment.

Lung

In contrast to liver and nasal mucosa, repeated intraperitoneal administration of 100 mg/kg/day NNK during 12 days causes a progressive accumulation of O6-mGua and O4-mThd in the lung (Belinsky et al. 1986). It was found that O6-mGua is more slowly eliminated from Clara cells than from other cell types (Belinsky et al. 1990) probably due to low levels of O6-mGua DNA methyltransferase (Belinsky et al. 1988), of which the activity is drastically reduced at higher exposures. This effect is probably bound to augment DNA alkylation; 12 days of treatment with 100 mg/kg/day NNK was found to diminish the activity by 95% (Belinsky et al. 1986). Using radiolabeled NNK, Murphy et al. (1990) were unable to detect any increase in O6-mGua in either whole lung or liver below a dose of 0.6 mg/kg/day given by the i.p. route during 4 days.

For rats treated with NNK during 4 weeks by s.c. injections, 3 times per week, with doses ranging from 0.1 mg to 50 mg/kg (0.043 to 21.4 mg/kg/day) there is a sharp increase in the yield of adducts at a dose of 0.13 mg/kg/day for Clara cells, and above 4.3

mg/kg/day for whole lung. Correspondingly, there was a non-significant increase in benign lung tumours at 0.013 mg/kg/day after 20 weeks of treatment, with a steep increase of the slope of the dose-response curve in the range 0.13-0.43 mg/kg/day. For O6-mGua an excellent correlation was found between degree of alkylation in Clara cells (less so for other cell types or whole lung) after administration of NNK and the incidence of lung tumours in the mouse (Peterson and Hecht 1991) as well as in the rat (Belinsky et al. 1990). No data for induction of adducts in lung at the lowest dose, 0.013 mg/kg/day, were reported.

7-Methylguanine (7-mGua)

In comparison with O6-mGua, the levels of 7-mGua induced by NNK are between 4 (lung) to 8 (liver) times higher (Belinsky et al. 1986). For liver and lung the dose response for formation of this adduct was studied upon i.p. administration of tritiated NNK in the dose range 0.003 to 5 mg/kg/day during 4 days (Murphy et al. 1990). Above 0.075 mg/kg there was a steep increase in the yield of adducts that was virtually linear for liver. In this organ as well as in the lung, adduct concentrations of 0.22 and 0.23 pmol 7-mGua/µmole guanine could be detected at the lowest dose. Because radiolabeled NNK was used, background levels could not be determined. However, by employing the ^{32}P postlabeling assay, Zhao et al. (1999) found a background concentration in rats of 2.1-2.5 7-mGua/10^7 nucleotides (0.8-1.0 pmol/µmole guanine), implying that the adduct yield for NNK at 3 µg/kg/day approximately represents a 20% increase of the natural background.

O^4–methylthymine (O4-mT)

O4-mTh adducts are strongly pro-mutagenic. The concentrations induced by NNK in the rat are more than one order of magnitude below those for O6-mGua (Belinsky et al. 1986); however it cannot be excluded that they may contribute to a minor degree to the overall cancer risk from TSNA.

When comparing promutagenic activity of 3 above-mentioned NNK adducts it seems that, 7-mGua is a poorer inducer of point mutations than O6-mGua and O4-mTh (Jansen et al. 1996, Kaina et al. 1983, Saffhill et al. 1985, Wood 1996). Therefore, although the yield of 7-mGua is much higher than that of O6-mGua, 7-mGua adducts seem to be of secondary importance with respect to cancer induction by NNK. This assumption is strengthened by the observation that there is no correlation between 7-mGua adduct levels and incidence of tumours in rodent (Liu et al. 1992).

Exposure to NNK by the oral route may result in an adduct tissue distribution that is different from that from s.c. or i.p. injection, a fact that is underlined by the finding that in contrast to injection, pancreatic tumours can readily be induced by administering NNK by the oral route. As compared with i.p injection, the levels of O^6 and 7-mGua adducts induced by NDMA in rat kidney were significantly lower upon oral administration (Pegg and Hui 1978). NNAL has been suggested to induce pancreatic tumours, and one reason for this discrepancy may be a first pass metabolism in liver and small intestine yielding more NNAL. In the study conducted by Rivenson et al. (1988) male Fischer 344 rats were administered the TSNA in drinking water at 0.5, 1.0 or 5.0 ppm during the animals' lifetime. Clear dose response relationships were evident for tumours in lung, liver, and nasal cavities, out of which the induction of lung tumours appears to be the most sensitive end point that could conveniently be used for high-to-low dose risk extrapolation. At the lowest dose, there was a significant increase in pancreatic tumours but not in lung tumours. However, the unusually high incidence of lung tumors in controls (7.5%), as well as the fact that the pancreatic tumor incidence was less at the highest than at the lowest dose, represents an anomalous feature of this study.

Haemoglobin adducts have been explored as biomarkers of exposure to and metabolic activation of tobacco-specific nitrosamines. NNN and NNK form haemoglobin adducts in humans and experimental animals. These adducts release 4-hydroxy-1-(3-pyridyl)-1-

butanone (HPB) upon mild alkaline hydrolysis. HPB released from human haemoglobin can be quantified by gas chromatography–mass spectrometry (Hecht et al. 1991). For pathways see Figure 2.

Levels of HPB released from haemoglobin (fmol HPB/g haemoglobin) were 517 ± 538 (standard deviation) in snuff dippers, 79.6 ± 189 in smokers and 29.3 ± 25.9 in non-smokers (Carmella et al. 1990). Nasal snuff users also showed high levels of haemoglobin adducts; HPB-releasing adducts were not correlated with the amount or type of snuff used. Unlike in smokers, haemoglobin adducts from aminobiphenyl compounds were not elevated in users of nasal snuff (Schaffler et al. 1993).

Rats treated five times weekly for 5 weeks by i.p. injection of 0.5, 1 or 5 µg/kg NNK had 247, 517 or 1916 fmol/g Hb of HPB releasing adducts in their globin. The levels of HPB releasing adducts measured in humans were in the range expected based on the measurements in rats treated with NNK. The HPB adducts released in the DNA was 20 times greater than from the haemoglobin (Hecht et al. 1993, Murphy et al. 1990).

The interpretation of HPB adduct data is complicated by the fact that more than one adduct seems to be generated (Hecht et al. 2004), and reliable dose response relationships in the low-dose region that can be correlated to induction of cancer do not seem to be available. However, when investigating HPB released from liver and lung DNA in rats given daily i.p. injections of NNK during 4 days, no increase in the adduct concentration could be detected at a dose of 3 µg/kg/day (detection limit, 0.05 pmol HPB/µmol Gua). In the range 3 to 600 µg/kg/day the dose response relationship was roughly linear, whereas a non- linear response was seen in the upper dose range, an observation that was tentatively interpreted as saturation of the metabolic activation system involved (Murphy et al. 1990). For the nasal epithelia of the rat, a single dose of 3460 µg/kg NNK did not cause any detectible elevation of HPB adducts, neither in the respiratory nor in the olfactory mucosae (Trushin et al. 1994). The bulky HPB adducts, that can be expected to be repaired by the nucleotide excision pathway, have been reported to induce G to A transitions and G to T transversions (Ronai et al. 1993), and there is evidence that HPB DNA adducts are involved in the induction of tumors of the rodent nasal epithelium and oesophagus (Trushin et al. 1994, Hecht 1999). NNN and NNK, both of which induce HPB adducts at this site, have very similar carcinogenic potency with respect to induction of neoplasia in the rat nasal mucosa, whereas dimethylnitrosamine, which does not induce HPB adducts, but is a potent methylator, has a very low carcinogenic efficacy with respect to these target tissues.

Two recent studies (Lao et al. 2007a, Lao et al. 2007b) reported specific pyridyloxobutyl-DNA adducts in rats treated with NNK, NNAL and NNN respectively. Chronic treatment of rats with NNK, (R)-NNAL, or (S)-NNAL at low doses gave higher levels of pyridyloxobutyl-DNA adducts in the lung than in the liver. O2- O2-[4-(3-pyridyl)-4-oxobut-1-yl]thymidine was the major POB-DNA adduct found in vivo and accumulated over the course of treatment. The highly abundant O2- pyridyloxobutyl-deoxythymidine may be important for NNK and NNAL carcinogenicity. O6-[4-(3-Pyridyl)-4-oxobut-1-yl]-2¢-deoxyguanosine was found to persist in the lung, supporting its important role in NNK and NNAL lung carcinogenesis in rats. In the rat oesophagus, (S)-NNN treatment generated levels of pyridyloxobutyl-DNA adducts 3-5 times higher than (R)-NNN treatment. 7-[4-(3-Pyridyl)-4-oxobut-1-yl]guanine was the major adduct detected, followed by O2-[4-(3-pyridyl)-4-oxobut-1-yl]thymidine and O2-[4-(3-pyridyl)-4-oxobut-1-yl]cytosine. O6-[4-(3-Pyridyl)-4-oxobut-1-yl]-2¢-deoxyguanosine was not detected.

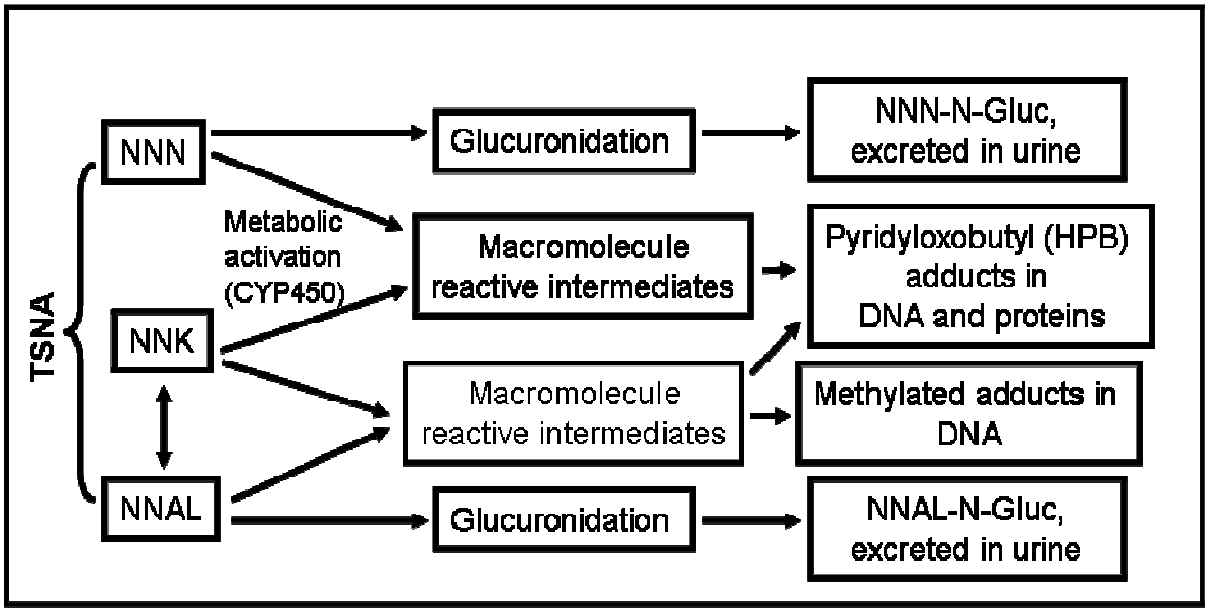

Figure 2. Summary pathways of activating metabolic reactions, adduct formation and excretion of TSNA in humans and rodents.

3.3.2.5. Conclusion on chemical composition

The major group of carcinogens in STP includes non-volatile tobacco-specific nitrosamines (TSNA) and *N*-nitrosamino acids. During the last two decades the levels of TSNA in moist snuff have been considerably lowered. One recent study documented total TSNA levels in one brand of Swedish snus to be 2.0 microgram/g product wet weight, whereas total TNSA levels in 6 American brands of moist snuff varied from 1.3 to 9.2 microgram/g. The average moist snuff user will be exposed to about 6 times more TSNA than the average smoker. NNK and NNN – the major carcinogens present in smokeless tobacco – induce two types of primary DNA lesions: nucleotide methylations and pyridyloxobutylations (HPB adducts). With respect to methylations, the highest yields of adducts in the target organs lung, liver and nasal mucosa of rats exposed to NNK have been found for 7-methylguanine (7-mGua), followed by O^6–methylguanine (O6-mGua), whereas very low levels of O^4–methylthymidine (O4-mTh) were present. O^6–methylguanine seems to play a major role in cancer formation. Some forms of STP contain polycyclic aromatic hydrocarbons depending on curing. STP also contain low levels of carcinogenic aldehydes. For some current Indian STP relatively high levels of TSNA have been reported.

3.3.3. Use and exposure: Experience in countries where smokeless tobacco products, in particular oral tobacco, are permitted

3.3.3.1. Experience with smokeless tobacco products, in particular oral tobacco, in Sweden

The smokeless tobacco market in Sweden is totally dominated by moist snuff called snus.

Snus has a long tradition in Sweden as manufacturing of snus started in the 1820's. In the beginning of the 20th century snus was used widely, predominantly among working class men. Production peaked in the 1920's at about 7,000 tonnes annually but the success of the cigarette later in the 20th century made snus less popular. By the end of the 1960's, production was down to 2,600 tonnes and the consumers were mainly elderly

men. Tobaksbolaget (now Swedish Match) decided to modify the product and its marketing to make it more palatable and fashionable to consumers. Intensive advertising campaigns promoted snus as the tobacco product for health-conscious but daring, sports-loving young males. In 2005, the annual production was again about 7,000 tonnes. The sale of cigars, roll-your-own and forms of oral tobacco other than snus in Sweden was negligible and declining.

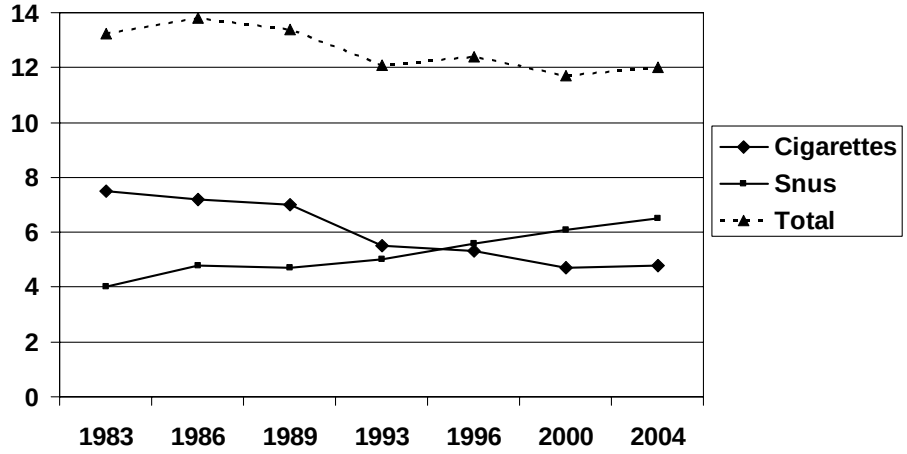

Figure 3. Annual sales of tobacco products (metric tonnes, thousands). (Tobaksfakta 2007)

Smoking rates among men in Sweden fell sharply from 1980 but have fallen similarly in men and women since the mid 1990´s. Since the early 1970s there has been an increase in snus use among men. Snus has traditionally not been acceptable for women in Sweden. The prevalence of snus use has been monitored since 1988-89 and the rise in consumption is a quite recent phenomenon (Figure 4a). In 2006 the national prevalence of daily snus users among men aged 16-84 years was 21% and among women 4%. Five percent of males and 3% of females reported occasional snus use (Statistics Sweden 2007).

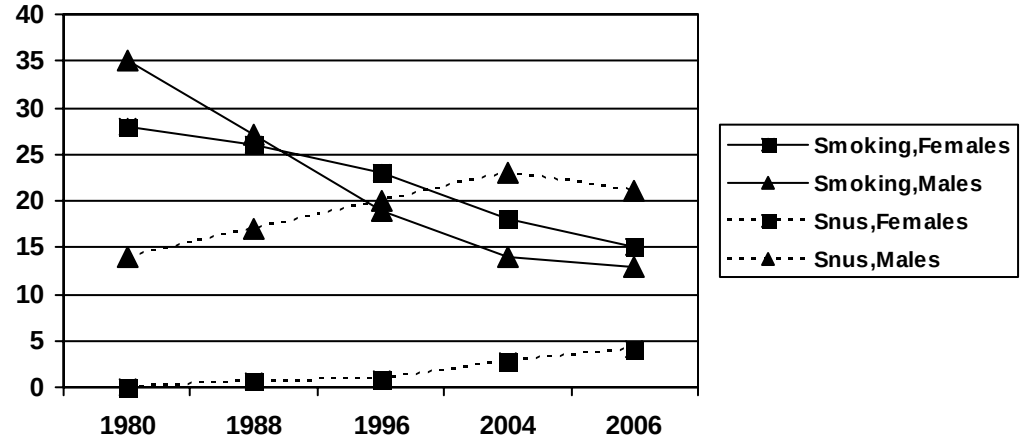

Figure 4a. Daily tobacco users, 16-84 years (percent). (Statistics Sweden 2007)[21]

[21] The results from the 1980 survey must be interpreted with caution. In that survey daily and less than daily snus use were not separated and the data in all the diagrams below present estimates based on extrapolation.

The frequency of use may vary between groups and regions. In the northern part of Sweden, where snus use is more prevalent, use by women may reach 10%. Due to the intensive marketing of snus in the 1970´s and 80´s, a strong cohort effect can be observed among Swedish males (Figure 4b). Among men with a university degree ("High"), 20 % of those aged 18-39 reported daily use, compared to 5 % among males aged 60-84. For males with shorter education ("Low"), the prevalence of use was 32 and 7 %, respectively. Marketing of snus to women is a much more recent phenomenon. Figure 4c shows data from urban regions: in the ages 30–69, females with a university degree smoked much less than those with shorter education (12 vs 25 %). Snus use, on the other hand, was more prevalent among women with a university degree (4 vs 2 %). Five percent of women with a university degree aged 30-39 used snus daily (Upmark 2003).

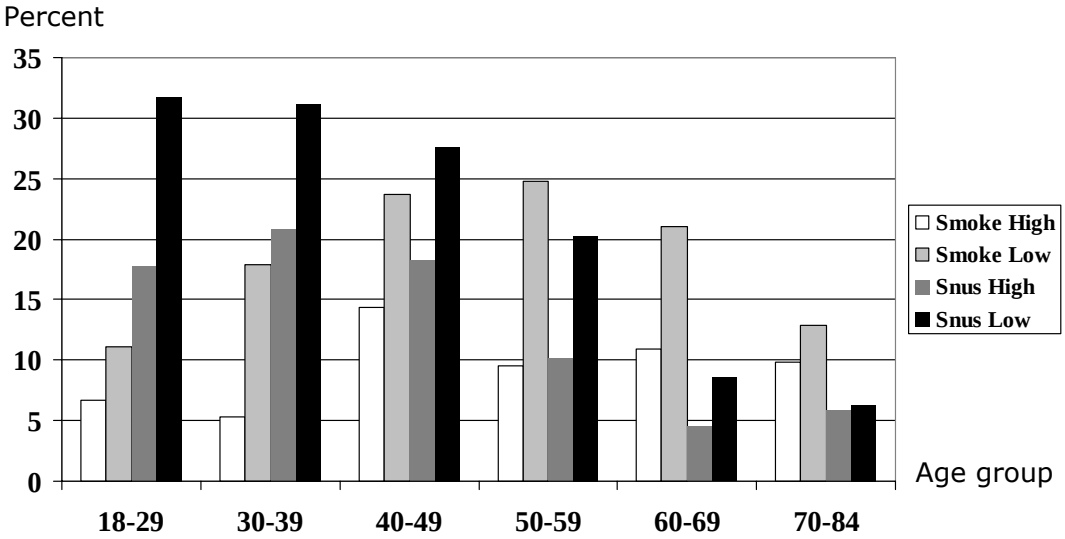

Figure 4b. Daily tobacco use among men in Stockholm according to age and education. "Smoke High" means smokers with higher education (Upmark 2003)

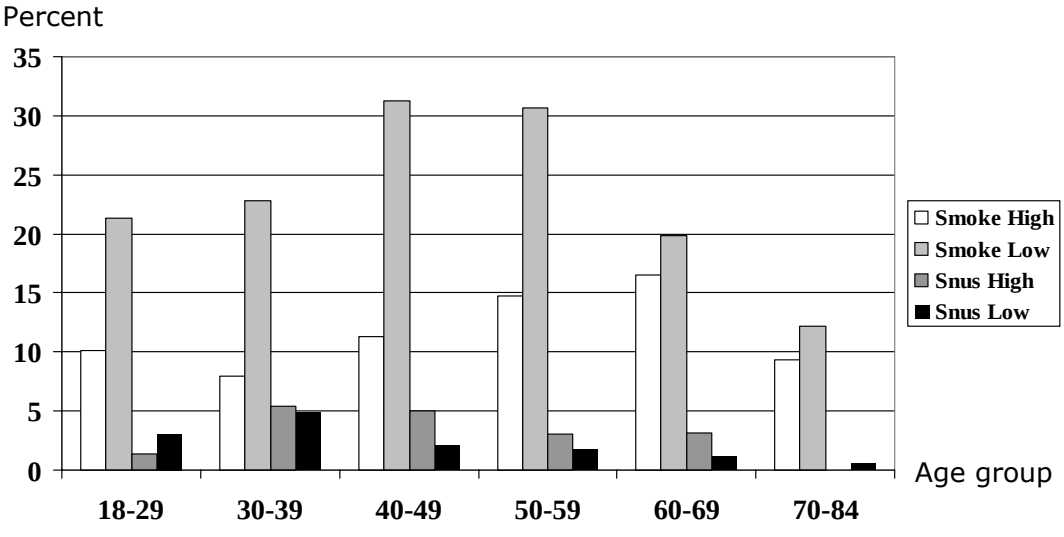

Figure 4c. Daily tobacco use among women in Stockholm according to age and education (Upmark 2003)

In 2005, among 16-24 year old men, 26% use snus daily. For 25-34 year old men, the prevalence of daily snus use was 33%. In men aged 35-44 years, 31% used snus daily and among 45-54-olds the prevalence was 24%. The corresponding changes in consumption of cigarettes can be seen below in Figures 5-8. One must keep in mind however, that the figures given here for all use (total use) may be slightly exaggerated as 1-3% may be using both products on a daily basis (Upmark 2003, Ramstrom and Foulds 2006).

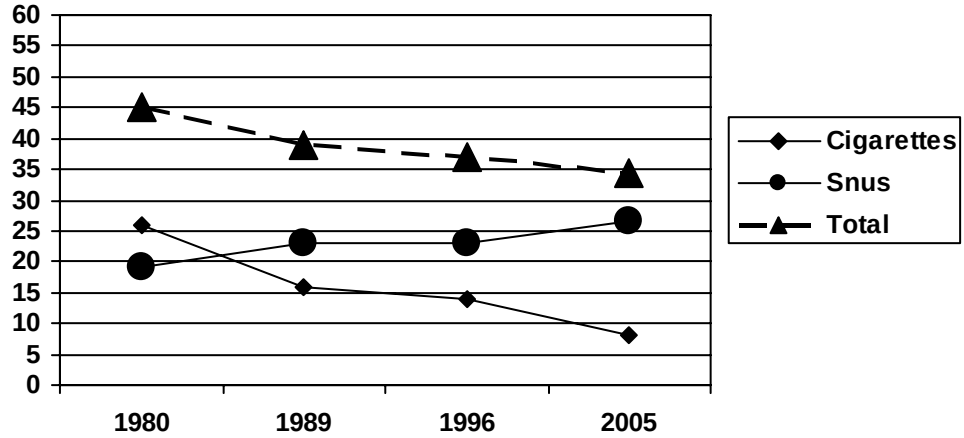

Figure 5. Prevalence of daily users, males, 16-24 years (percent). (Statistics Sweden 2007)

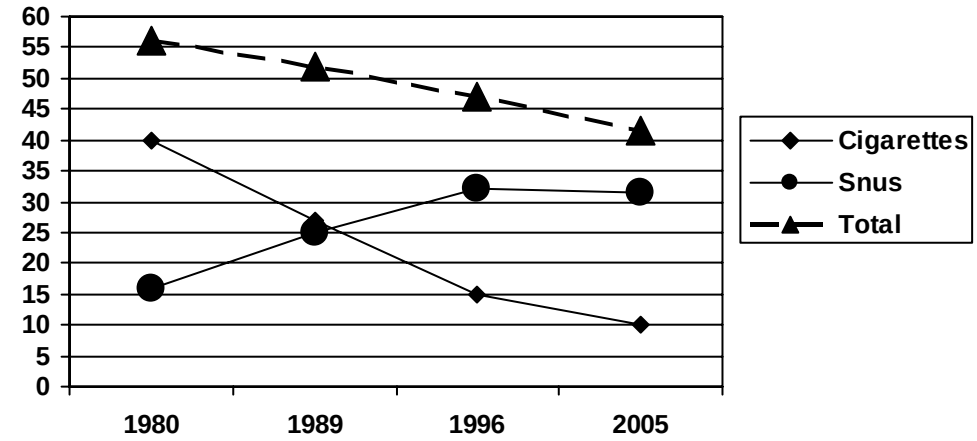

Figure 6. Prevalence of daily users, males, 25-34 years (percent). (Statistics Sweden 2007)

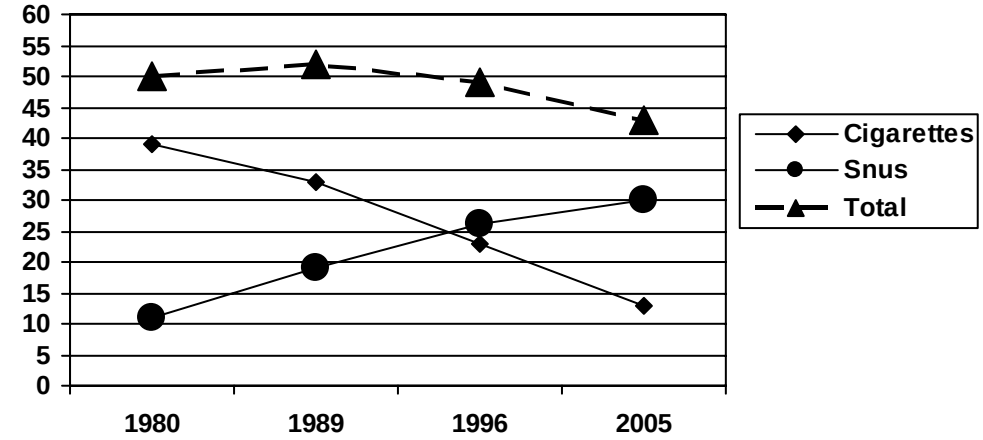

Figure 7. Prevalence of daily users, males, 35-44 years (percent). (Statistics Sweden 2007)

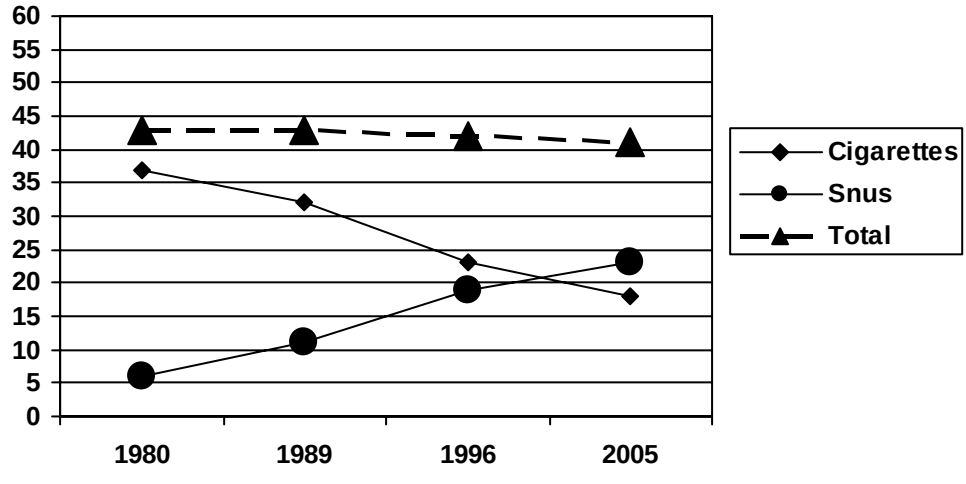

Figure 8. Prevalence of daily users, males, 45-54 years (percent). (Statistics Sweden 2007)

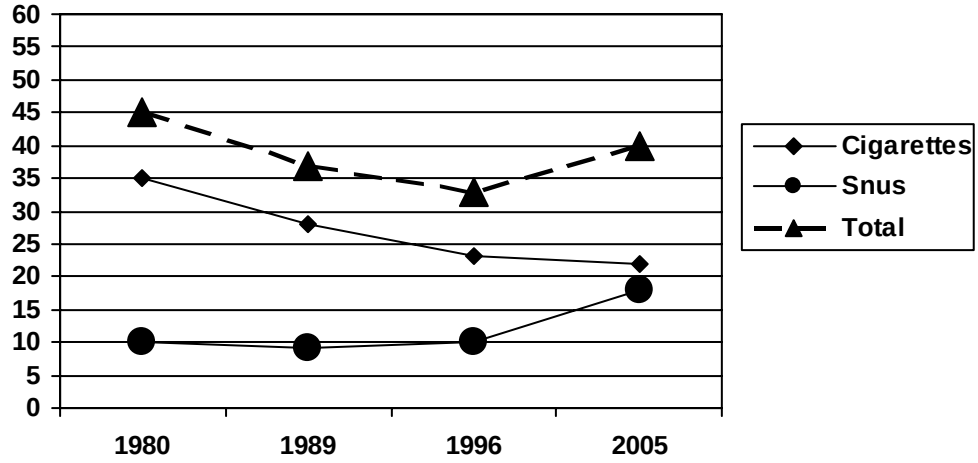

Figure 9. Prevalence of daily users, males, 55-64 years (percent). (Statistics Sweden 2007)

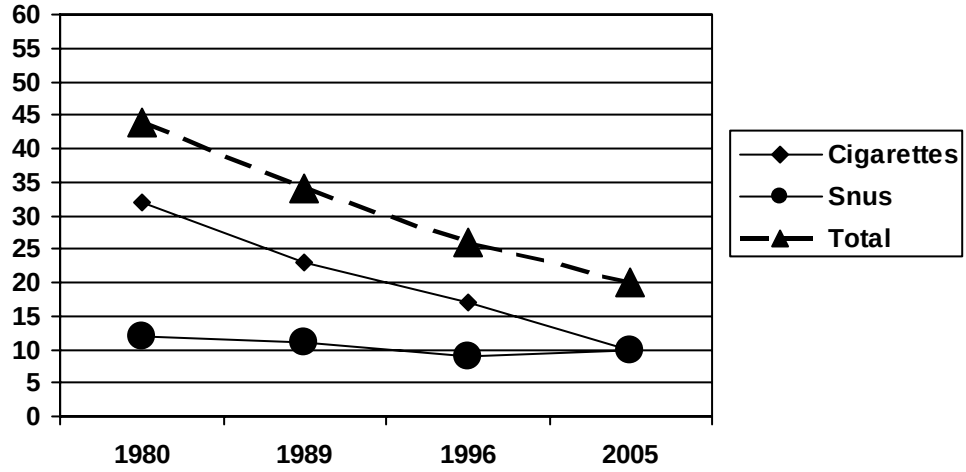

Figure 10. Prevalence of daily users, males, 65-74 years (percent). (Statistics Sweden 2007)

The patterns of snus use and cigarette smoking have not changed much over a 20-year period among 12-year old Swedish boys (Figure 11). Among 15-year olds, however, a trend of increasing snus use and declining cigarette smoking has been observed (Figure 12).

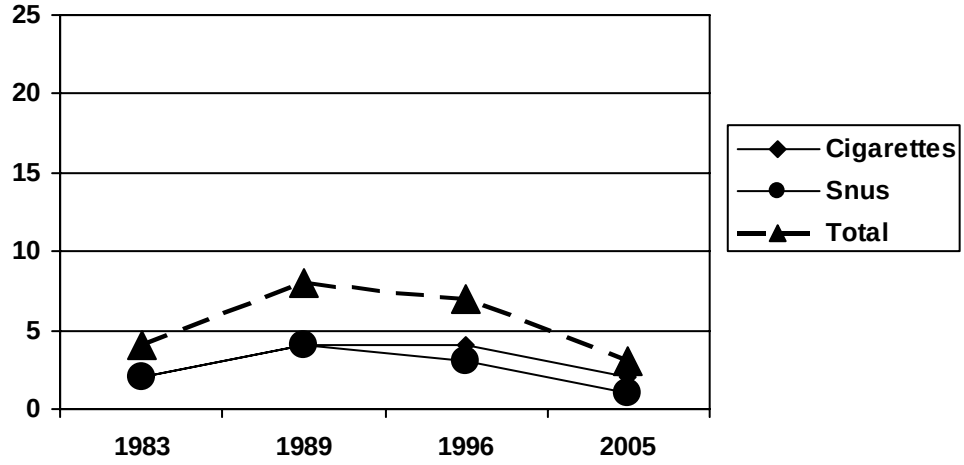

Figure 11. Prevalence of daily users, boys, 6th grade (percent). (CAN 2006)

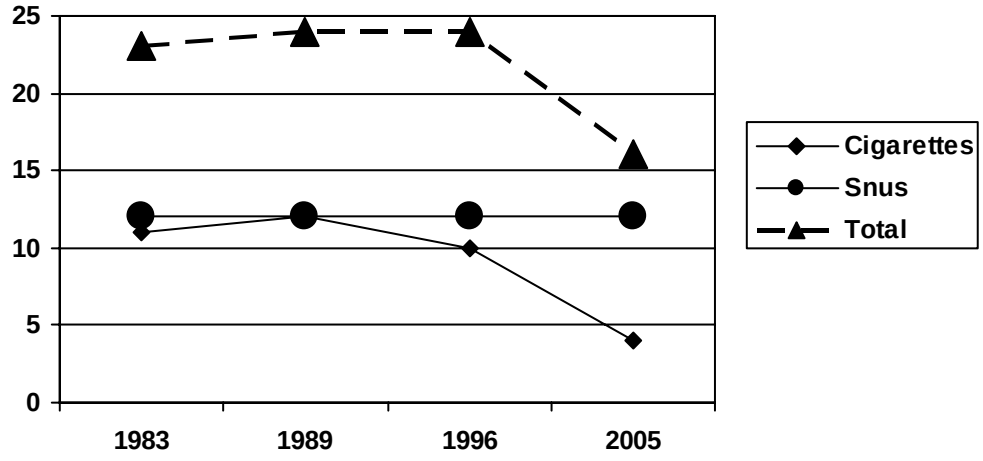

Figure 12. Prevalence of daily users, boys, 9th grade (percent). (CAN 2006)

The number of immigrants (born in other countries or born in Sweden where both parents were born abroad) in Sweden is currently 1.2 million, or 14% of the total population. Figure 13 shows that snus use in men born in Sweden by immigrant parents is more frequent than in men born abroad.

The tobacco habits in the different ethnic groups may vary considerably. The extent to which snus is used in the different groups is not known in detail.

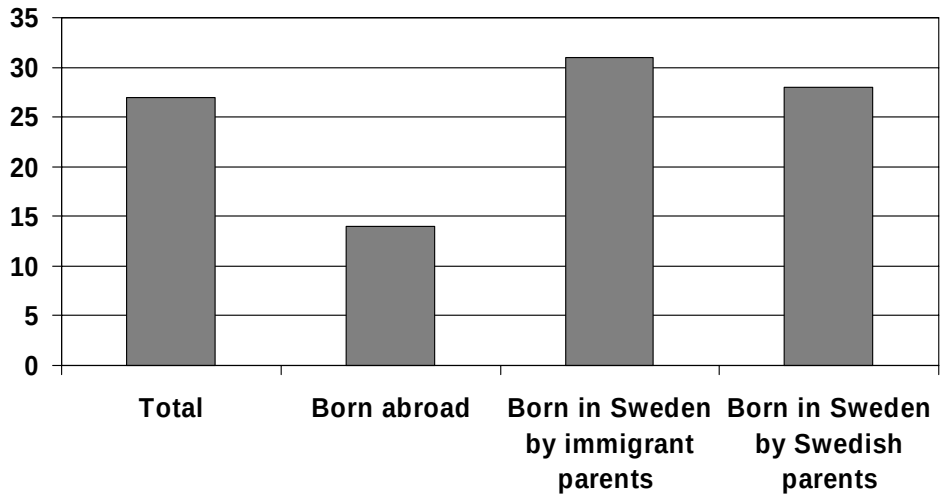

Figure 13. Snus use in men according to ethnic background, 16-84 years. (Statistics Sweden 2007)

3.3.3.2. Experience with smokeless tobacco products, in particular oral tobacco, in Norway

Tobacco use in Norway has been surveyed for more than 30 years through questionnaires of random national samples consisting of approximately 5.000 respondents (Statistics Norway 2007; Norwegian Directorate of Health and Social Affairs 2007). The figures 14-26 and tables 5-7 below were derived from data made available from the two sources.

Whereas smoking was much more prevalent in Norwegian men compared to women 30-40 years ago, smoking prevalence has been similar in both sexes during the last decade and was 24% in both men and women in 2006 (Figure 14). 10% of 16-74 year olds were occasional smokers in 2006. Overall, the prevalence of daily smoking has been reduced by almost 10 percentage points since 1997.

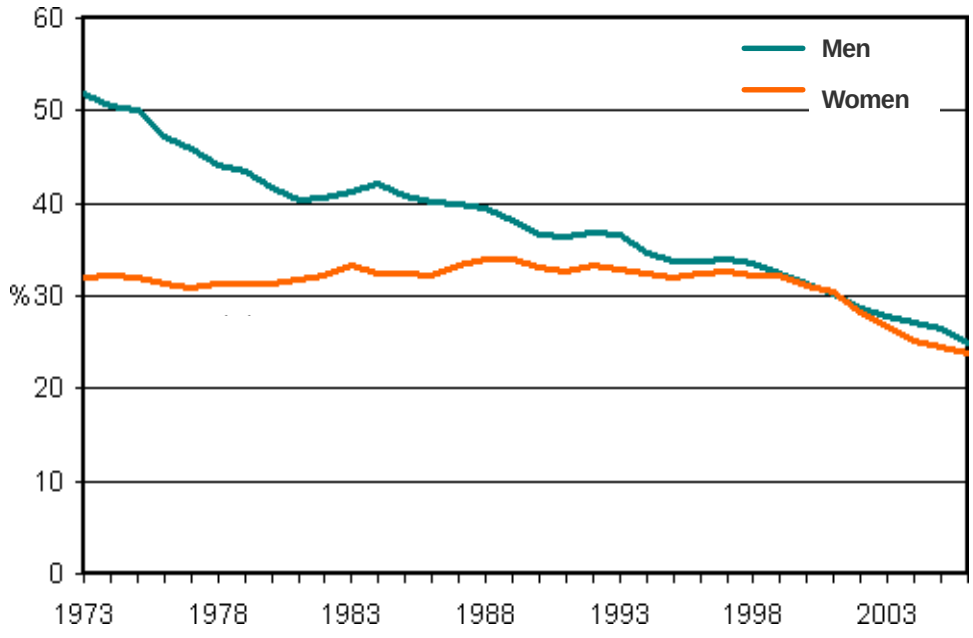

Figure 14. Prevalence of daily smoking among Norwegian men and women, 16-74 years, 1973-2006. (Statistics Norway 2007)

Smoking is quite similar between the sexes in all age groups in 2005-2006 (Figure 15).

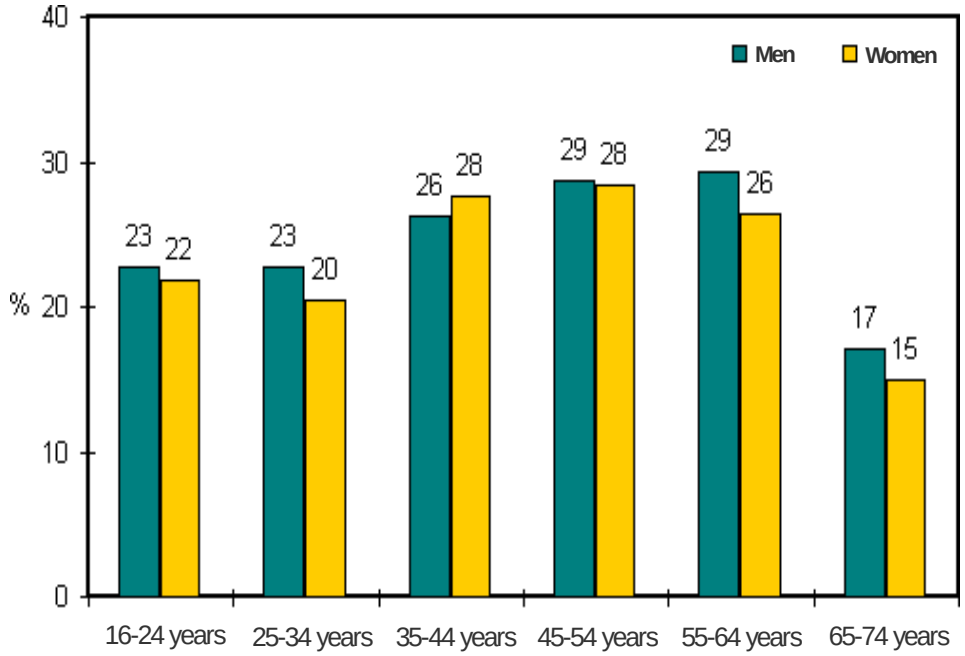

Figure 15. Age- and sex-dependent daily smoking among Norwegian men and women, 16-74 years, 2005-2006. (Statistics Norway 2007)

Smoking in young male and female Norwegians aged 16-24 years occurred in more than 40% of this population in the early 1970s. The decline has been parallel and at the same rates so that both sexes show similar smoking prevalence in 2006, 23% in males and 22% in females, respectively (Figure 16).

In Norwegians aged 25-34 years, smoking prevalence between sexes has been similar for more than 20 years and has decreased during this time period (Figure 17).

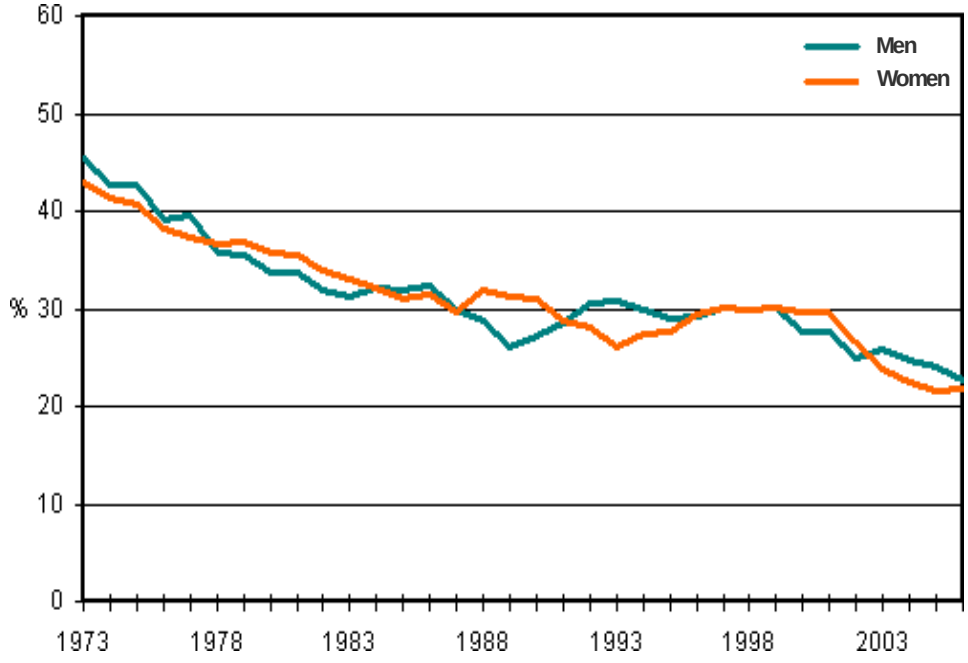

Figure 16. Prevalence of daily smoking among Norwegian men and women, 16-24 years, 1973-2006. (Statistics Norway 2007)

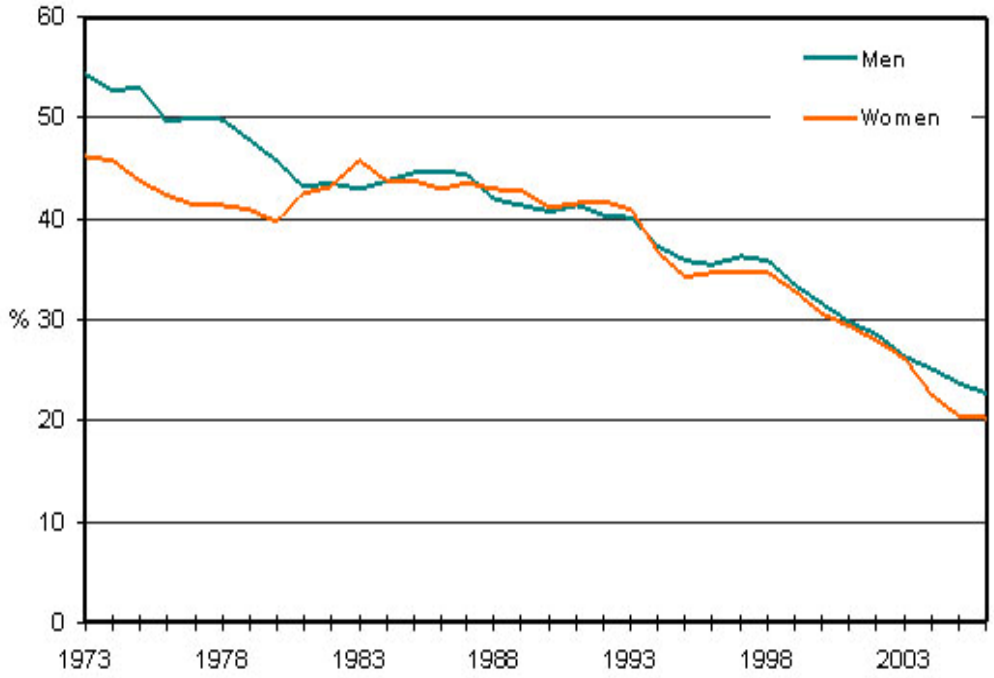

Figure 17. Prevalence of daily smoking among Norwegian men and women, 25-34 years, 1973-2006. (Statistics Norway 2007)

The use of moist snuff in Norway is almost exclusively in the form of Swedish snus. 11% of Norwegian men use snus daily in 2006, 7% of men use snus occasionally, whereas less than 1% of women use snus. Amongst 16-24 year old males, 18% use snus daily and 17% use snus occasionally. For 25-34 year old men, the prevalence of snus use is 21% (daily) and 7% (occasionally), respectively. Most of the snus users stated that they used cigarettes before they started using snus; however, one quarter reported that they used snus before they started smoking (Kunnskapssenteret 2005).

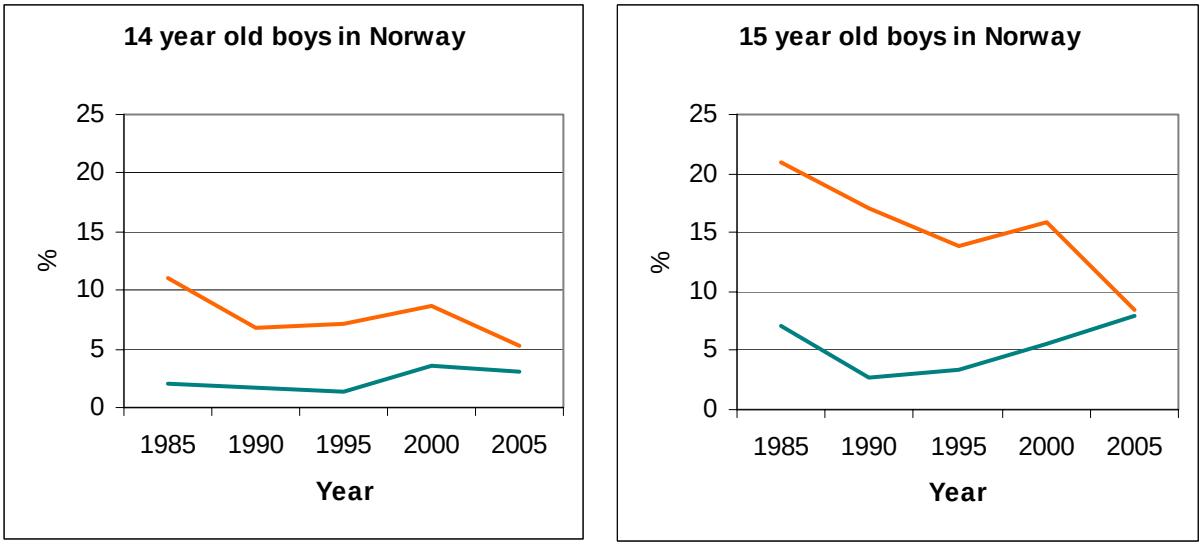

Figure 18. Daily use of cigarettes (upper lines) and snus (lower lines) among 14 and 15 year old boys in Norway (Norwegian Directorate of Health and Social Affairs 2007)

The use of snus in 14 and 15 year old boys has increased slightly between 1985 and 2005, whereas the prevalence of cigarette use especially in the 15 year olds has decreased markedly (Figure 18). The decline in smoking prevalence in this age-group is not matched by a clear compensatory increase in snus use.

Overall prevalence of daily smoking, snus use and all tobacco use in Norwegian men 16-74 years of age, as well as prevalence of daily smoking, snus use and all tobacco use in men in the age groups 16-24 years, 25-34 years, 25-44 years, 45-54 years, 55-64 years and 65-74 years is presented in the figures 19-22, respectively. Total tobacco is the sum of daily smoking and snus use; these figures do not take dual use into account. This is addressed in Tables 5 and 6 below.

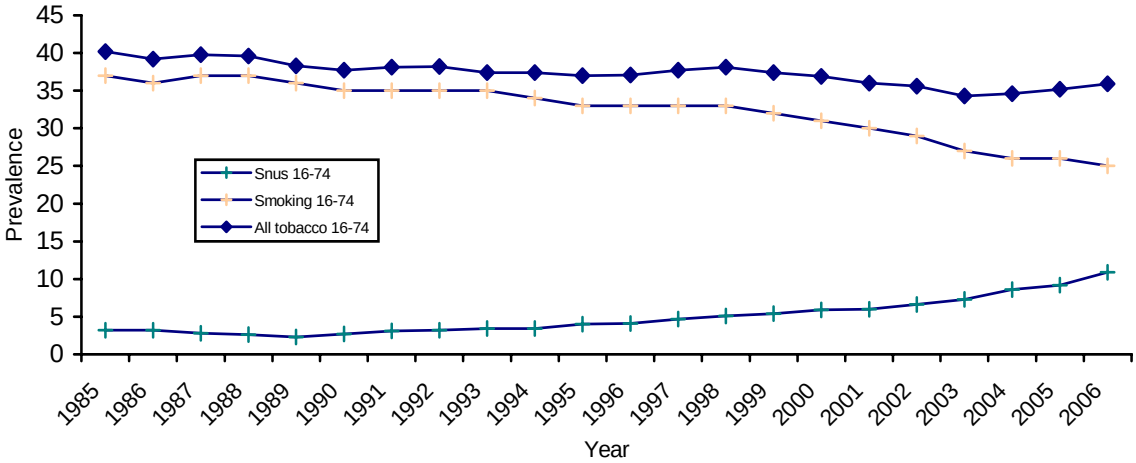

Figure 19. Prevalence of daily smoking, snus use and all tobacco use in men aged 16-74, Norway. (Statistics Norway 2007)

Figure 19 shows the data for all ages 16-74, and that the overall prevalence of snus use has increased in this time, use of smoking has fallen, whereas total tobacco use has remained nearly constant.

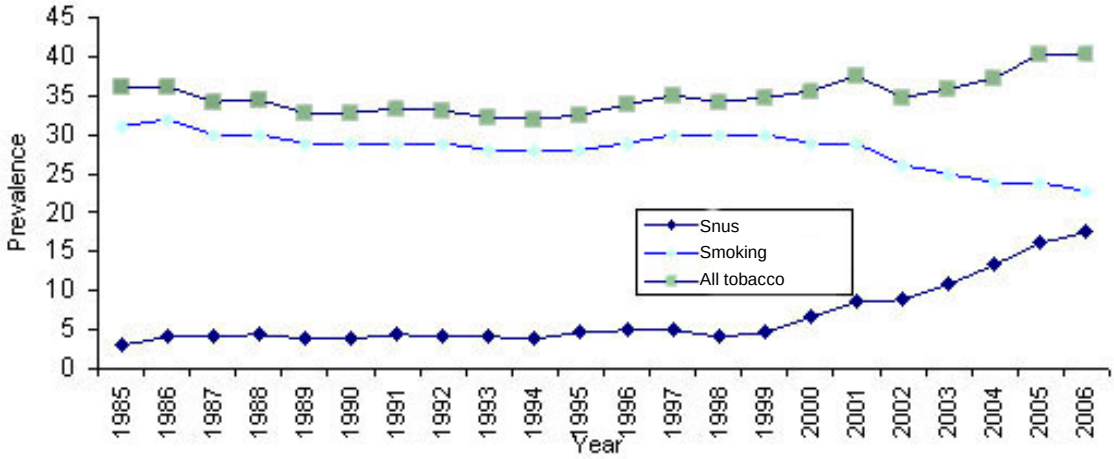

Figure 20. Prevalence of daily smoking, snus use and all tobacco use in men aged 16-24, Norway. (Statistics Norway 2007)

Among 16-24 year old men in Norway, there has been gradual, slow reduction in prevalence of cigarette smoking, whereas the use of snus has markedly increased from the year 2000 onwards (Figure 20). The rate of increase in snus use is larger than the rate of decrease in cigarette use, and the indicator of all tobacco use has increased (dual use is addressed below). Relative to Sweden in 2005, smoking prevalence in this age-group is approximately twice as high, and snus use approximately 30% lower.

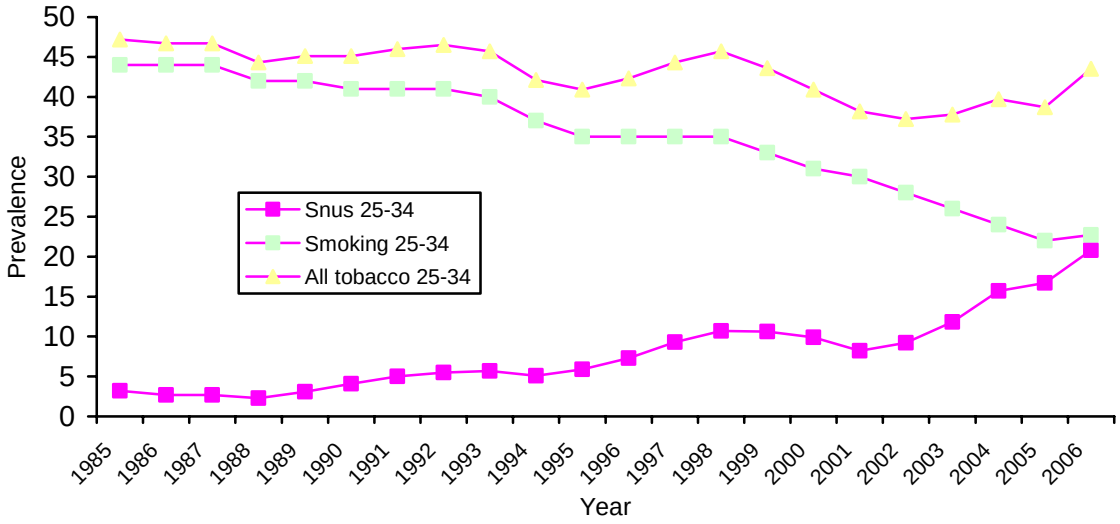

Figure 21. Prevalence of daily smoking, snus use and all tobacco use in men aged 25-34, Norway. (Statistics Norway 2007)

In 25-34 year old males a more marked increase in the prevalence of snus use has occurred since 1990, from 4.1% to 20.8% in 2006, and there has been a continuous and substantial decline in smoking prevalence from 41% to 23% respectively (Figure 21). The prevalence of any tobacco use has fallen slightly.

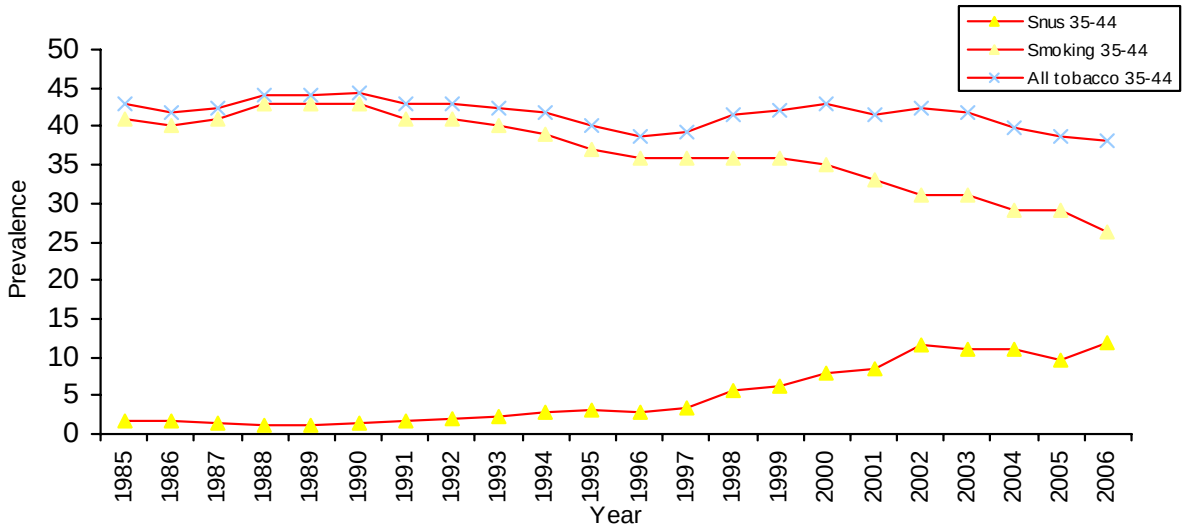

Figure 22. Prevalence of daily smoking, snus use and all tobacco use in men aged 35-44, Norway. (Statistics Norway 2007)

Snus use among 35-44 year old male Norwegians increased particularly from 1995 until 2002, and thereafter it has levelled off. Smoking prevalence for this age-group has steadily decreased during the last twenty years (Figure 22). Overall tobacco use has also fallen slightly.

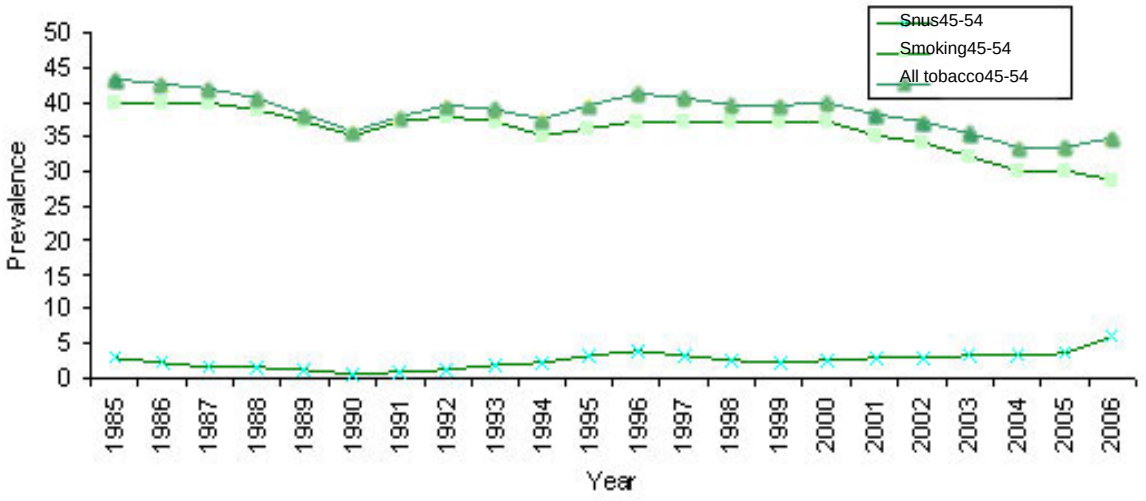

Figure 23a. Prevalence of daily smoking, snus use and all tobacco use in men aged 45-54, Norway. (Statistics Norway 2007)

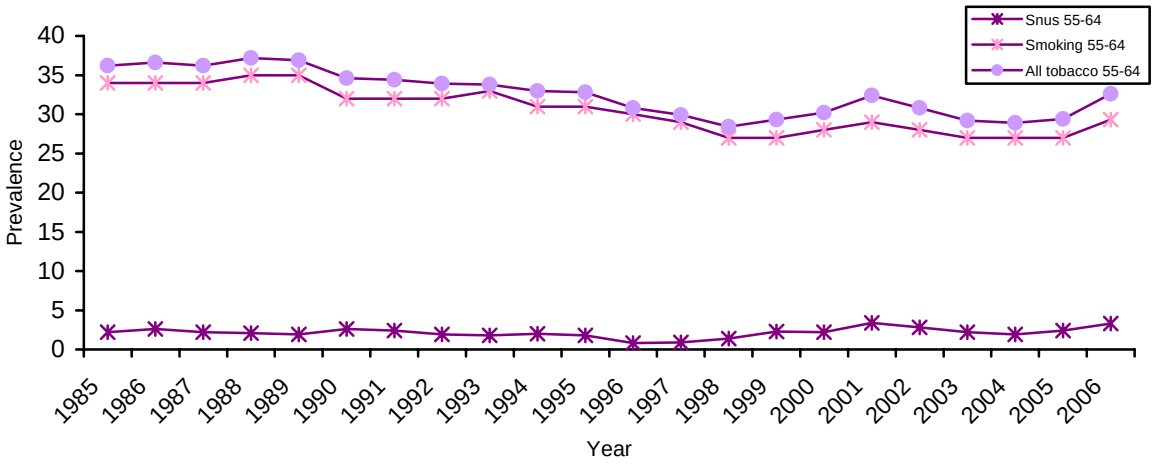

Figure 23b. Prevalence of daily smoking, snus use and all tobacco use in men aged 55-64, Norway. (Statistics Norway 2007)

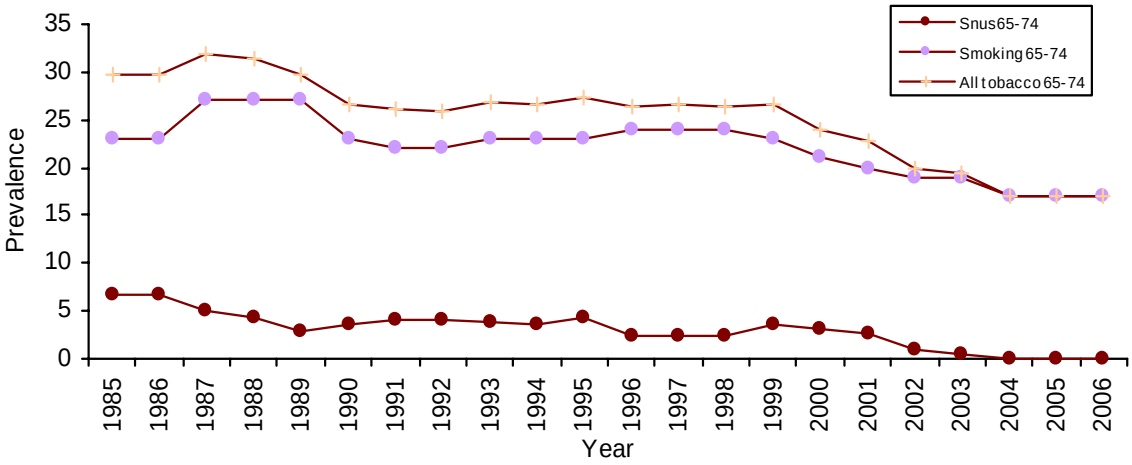

Figure 23c. Prevalence of daily smoking, snus use and all tobacco use in men aged 65-74, Norway. (Statistics Norway 2007)

Snus use in older age-groups has been relatively uncommon throughout this period. The prevalence of smoking and of all tobacco use has declined progressively (Figures 23a-c).

Daily smoking among Norwegian males aged 16-24 years has decreased markedly over the last 20-25 year period, whereas daily snus use in this group has increased considerably during the last 10-15 years (Figures 24 and 25).

Among daily Norwegian users of snus aged 16-74 years (pooled data from 2003-2004, n=105), 31% were never smokers, 24% were occasional smokers, 23% former daily smokers, 12% daily smokers and 11% former occasional smokers. National surveys of tobacco use in Norway showed that among smokers who managed to quit between 1990 through 2006, snus was the most commonly reported cessation aid (17%), compared to nicotine gum (10%), nicotine patch (4%), bupropion (3%) and contact with a telephone quit line (1%) (Directorate of Health and Social Affairs, 2007).

Whereas smoking prevalence in recent years has clearly fallen in all male age-groups, the use of snus has increased markedly only in the younger age-groups: 16-24 years, and 25-34 years, and 35-44 years (Figure 26). On the other hand, the group reporting occasional smoking has remained constant at a prevalence of approximately 10% during the later years (Figure 24). Occasional snus use in men has also risen in the younger groups (Figure 25). It is difficult to envision any significant impact of snus use on smoking cessation in Norway, since the decline in smoking prevalence rates are similar in both sexes, whereas the increased snus use has occurred almost exclusively in men.

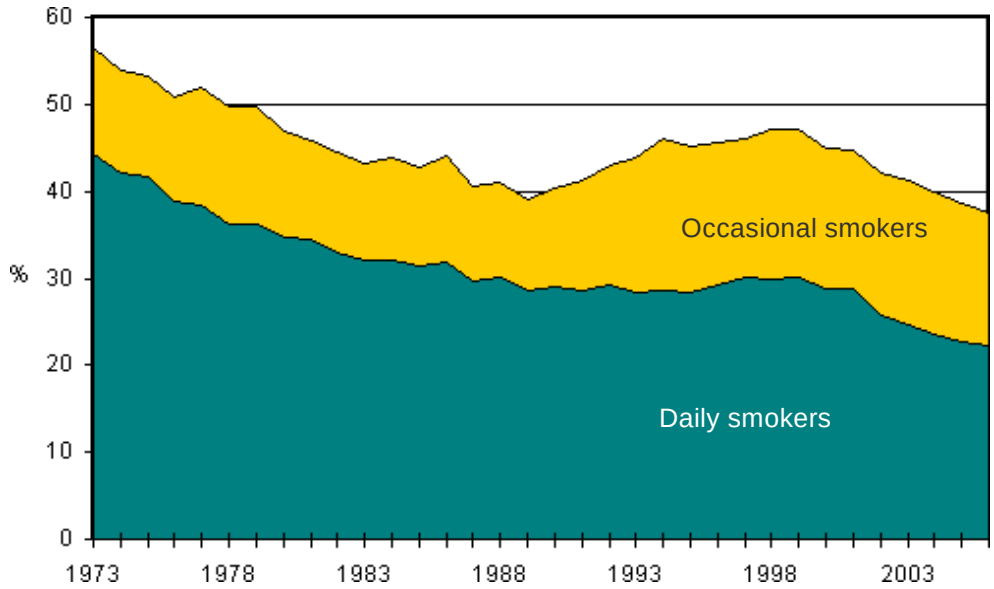

Figure 24. Prevalence of daily or occasional smoking among Norwegian men and women, 16-24 years, 1973-2006. (Norwegian Directorate of Health and Social Affairs 2007)

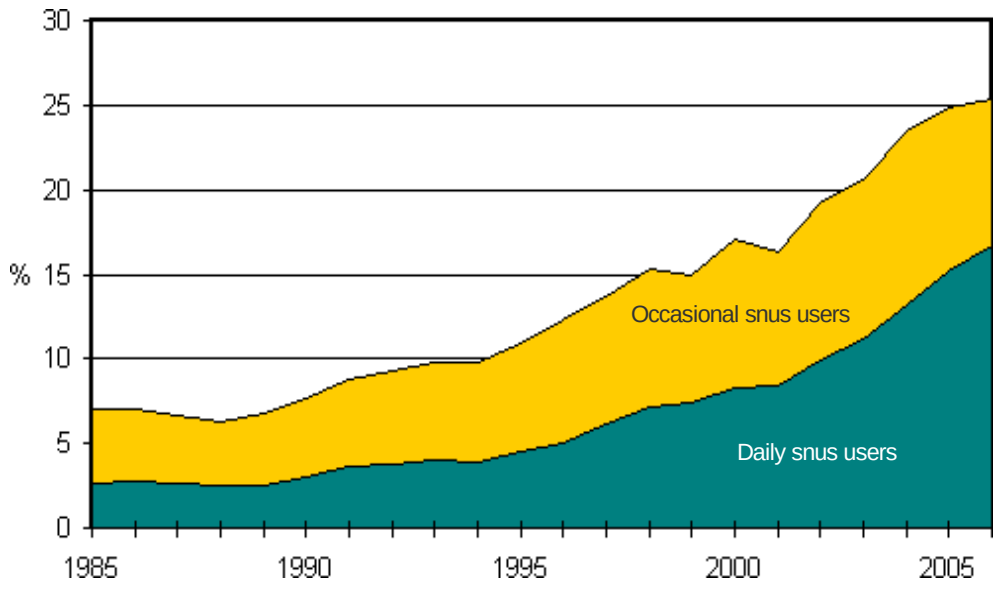

Figure 25. Prevalence of daily or occasional snus use among Norwegian males, 16-44 years, 1985-2006. (Norwegian Directorate of Health and Social Affairs 2007)

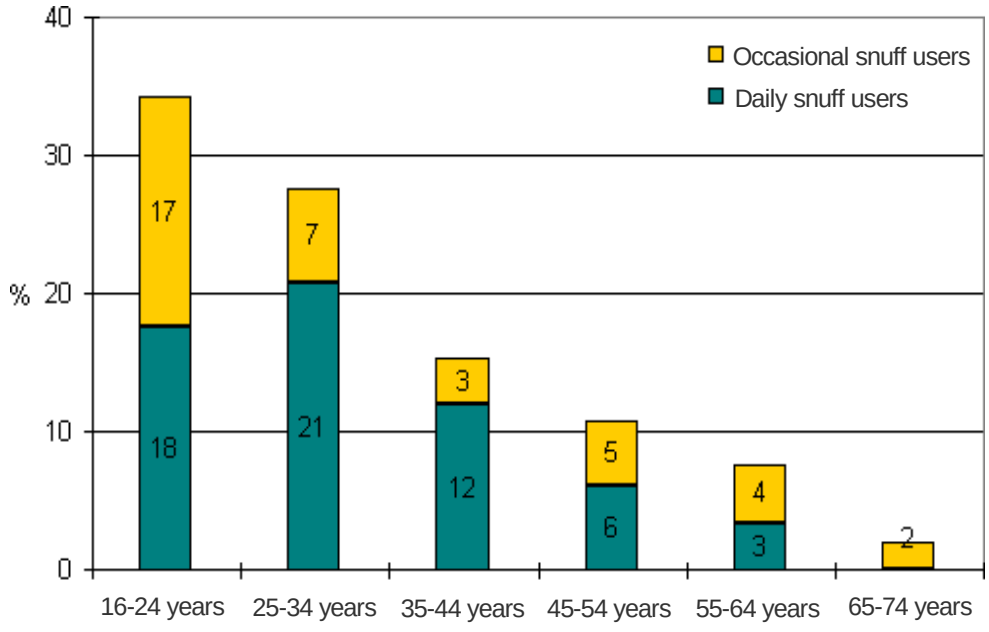

Figure 26. Prevalence of snus use according to age among Norwegian males in 2005-2006. (Norwegian Directorate of Health and Social Affairs 2007)

Dual use of snus and smoking in Norwegian men is depicted in Tables 5 (16-74 years) and 6 (16-44 years) in 2002-2006 (mean prevalence) from a statistically selected sample of 3145 respondents. Among the whole age-group (16-74 years), 27% smoke but never use snus, 8% use snus but never smoke, 7% use both snus and smoke, whereas 58% never use any form of tobacco. Among the 16-44 year olds, 26% smoke but never use snus, 11% use snus but never smoke, 11% use both snus and smoke, whereas 52% never use any form of tobacco.

Table 5. Dual use of snus and smoking in Norwegian men aged 16-74, mean prevalence for 2002-2006. (Norwegian Directorate of Health and Social Affairs 2007)

Snus use	Prevalence	Daily smoking	Occasional smoking	No smoking	Total
Daily snus use	Number of respondents	25	66	184	275
	% among snus users	9.1	24.0	66.9	100.0
	% among smokers	3.2	22.1	8.9	8.7
	% of total	0.8	2.1	5.9	8.7
Occasional snus use	Number of respondents	105	36	65	206
	% among snus users	51.0	17.5	31.6	100.0
	% among smokers	13.3	12.1	3.2	6.6
	% of total	3.3	1.1	2.1	6.6
No snus use	Number of respondents	657	196	1811	2664
	% among snus users	24.7	7.4	68.0	100.0
	% among smokers	83.5	65.8	87.9	84.7
	% of total	20.9	6.2	57.6	84.7
Total	Number of respondents	787	298	2060	3145
	% among snus users	25.0	9.5	65.5	100.0
	% among smokers	100.0	100.0	100.0	100.0
	% of total	25.0	9.5	65.5	100.0

Table 6. Dual use of snus and smoking in Norwegian men aged 16-44, mean prevalence for 2002-2006. (Norwegian Directorate of Health and Social Affairs 2007)

Snus use	Prevalence	Daily smoking	Occasional smoking	No smoking	Total
Daily snus use	Number of respondents	22	65	149	236
	% among snus users	9.3	27.5	63.1	100.0
	% among smokers	5.1	30.0	13.3	13.3
	% of total	1.2	3.7	8.4	13.3
Occasional snus use	Number of respondents	84	28	50	162
	% among snus users	51.9	17.3	30.9	100.0
	% among smokers	19.4	12.9	4.5	9.1
	% of total	4.7	1.6	2.8	9.1
No snus use	Number of respondents	328	124	924	1376
	% among snus users	23.8	9.0	67.2	100.0
	% among smokers	75.6	57.1	82.3	77.6
	% of total	18.5	7.0	52.1	77.6
Total	Number of respondents	434	217	1123	1774
	% among snus users	24.5	12.2	63.3	100.0
	% among smokers	100.0	100.0	100.0	100.0
	% of total	24.5	12.2	63.3	100.0

Table 7. Prevalence of daily snus use among Norwegian women 1986-2006, in percent (triannual means, numbers of respondents in parenthesis). (Norwegian Directorate of Health and Social Affairs 2007)

Age group	1986-1988	1991-1993	1996-1998	2001-2003	2004-2006
16-24 years	0.2 (542)	0.2 (517)	0 (310)	0.3 (303)	0.7 (304)
25-34 years	0.1 (750)	0.2 (627)	0.2 (440)	0.8 (371)	0.5 (376)
16-74 years	0.1 (3521)	0.1 (2925)	0.2 (1950)	0.3 (1940)	0.4 (1846)

The prevalence of daily snus use among Norwegian women is very low (Table 7). However, there has been an increase in prevalence of use during the last decade.

3.3.3.3. Experience with smokeless tobacco products, in particular oral tobacco, in other countries

Marketing of snus is banned in all EU countries except Sweden, but is available through the internet. The amount sold to other countries is not known. The use of smokeless tobacco appears to be very limited across Europe and these products and their use is rarely surveyed. An inventory from 'International Smoking Statistics' (Forey et al. 2002) found sufficient information on oral tobacco consumption for the study of only 10 European countries (Austria, Denmark, Finland, France, Iceland, Ireland, Italy, Norway, Sweden, and United Kingdom). However, STP as commonly used in Venezuela, Alaska and Sudan may be found and used in Europe by a fraction of migrants from these countries.

Finland: Although moist snuff (snus) sales are banned in Finland, snus use is increasing whereas chewing tobacco or use of other forms of smokeless tobacco has become extremely rare (Huhtala et al. 2006). According to the 2005 national survey (National Public Health Institute 2005) snus was predominantly used by younger males (15-44 yrs). The highest prevalence was observed among 25-34 year olds - 5.3% daily and 5.3% occasional users. Less than 1% of elderly men use snus in Finland and among women it was barely measurable. The total annual consumption has been estimated to 100 tonnes. **Denmark**: In Denmark, the use of oral tobacco has been very limited since the second world war. In spite of the proximity to Sweden, snus has never become a significant source of nicotine here. In recent years, medicinal nicotine has emerged as the substitute of choice when Danes are not permitted to smoke. **Germany:** STP, mainly nasal snuff, has traditionally been used in the southern regions (i.e. Bavaria) but available information suggests that its use is declining. There is limited production (230 tonnes) of nasal snuff from a handful of producers under a plethora of brand names. Hence, there is reason to believe that smokeless tobacco plays a very minor role in Germany. There are no data on the number of users. **Switzerland**: Although not an EU member state, Switzerland has adopted the EU sales ban on moist snuff. The consumption is allowed as is bringing up to 1.2 kg of moist snuff every second month into the country. It appears that the use of dry snuff (taken up by the nasal passages) and chewing tobacco plays a minor role. In the **USA** the use of STP has recently been seen to decrease (Nelson et al. 2006). In California both the prevalence of smoking and smokeless tobacco use have decreased concurrently (CDHS 2008, Nelson et al. 2006).

Products used by the Asian community in United Kingdom

The use of chewing tobacco is largely restricted to members of the Indian, Pakistani and especially Bangladeshi communities, which, for example, in the UK, make up 4.5% of the

population, slightly over two million people. Many types of smokeless tobacco are used among the South Asian population. Chewing tobacco is common among the Bangladeshi community. 19% of Bangladeshi men and 26% of Bangladeshi women use chewing tobacco. Tobacco is often consumed in combination with other products. Betel pepper leaf is used to wrap the fillings to form a quid. The leaf has a mint flavour and is considered a mouth freshener. The leaf (paan) itself is considered as relatively harmless: the health risks arise from the tobacco and other ingredients contained in the paan. Ready-made mixtures of smokeless tobacco are known as gutkha or paan masala which are chewed on their own.

3.3.3.4. Conclusion on use and exposure

The use of STP in Europe is significant only in the form of snus (oral tobacco or moist snuff) in Sweden, Norway and to some extent, Finland. UK immigrants from the Indian subcontinent continue to use the traditional products from their native countries. In the rest of Europe, smokeless tobacco is a minor problem from a public health point of view, as has been exemplified above. Nothing is known about the countries that have joined the EU more recently.

3.4. Biological Effects of Smokeless Tobacco Constituents

3.4.1. Nicotine

3.4.1.1. Toxicokinetics

Nicotine, the main addictive substance in tobacco products, is a weak base with a pKa of 8.0 (Fowler 1954). At pH 6.5 and higher, a considerable part of nicotine is in its unionised, free base form which readily crosses biological membranes. Chewing tobacco and snuff are buffered to alkaline pH to facilitate absorption of nicotine through the oral mucosa (Benowitz 1999a). Nasally applied snuff will be absorbed through the nasal mucosa, whereas swallowed nicotine from STP will be absorbed from the small intestine. The nicotine-dosing potential of snuff is determined by at least three factors: the amount of nicotine in the product, the pH level of the product, and the size of the tobacco cutting (Henningfield et al. 1995, Tomar and Henningfield 1997a).

Nicotine absorption

Absorption of nicotine from moist snuff is rapid and becomes maximal at 30 minutes, but absorption is less rapid than from cigarette smoke (Benowitz 1988a, Benowitz et al. 1988b, Fant et al. 2000, Holm et al. 1992, Russell et al. 1983, Stratton et al. 2001) The maximal plasma nicotine concentration is higher for cigarettes compared to smokeless tobacco, but nicotine plasma concentrations are higher after smokeless tobacco than after use of nicotine replacement products (Figure 1). Blood levels of nicotine fall more slowly after removing the smokeless tobacco compared to after smoking a cigarette. This is presumably due to absorption of nicotine that has been swallowed and also nicotine remaining in the buccal epithelium. The absorbed dose of nicotine was found to be at least twice as great from smokeless tobacco compared to cigarettes, with estimated absorbed doses of nicotine of 1.8, 3.6 and 4.5 mg from cigarette, snuff and chewing tobacco respectively (Benowitz et al. 1988b). When moist snuff is used throughout the day, venous blood nicotine concentrations are similar to those seen with cigarette smoking. There is considerable individual variation in the amount of nicotine absorbed from smokeless tobacco.

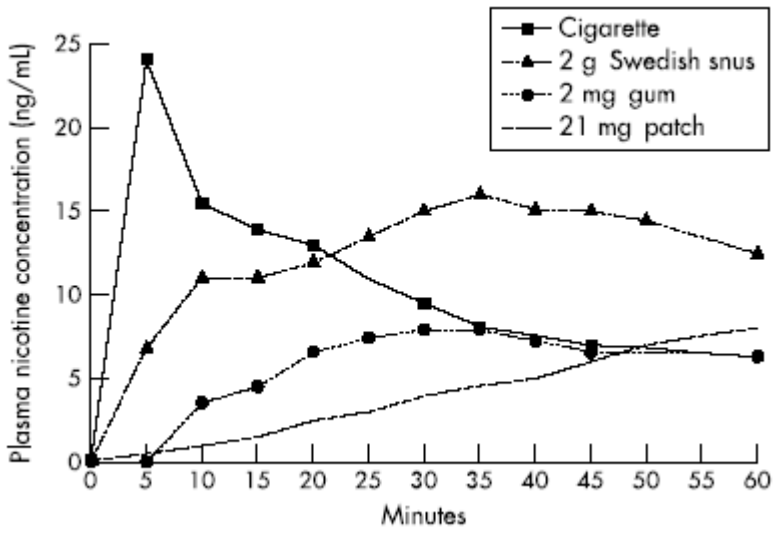

Figure 27. Venous blood concentrations in nanograms of nicotine per millilitre (ng/ml) of plasma as a function of time for various nicotine delivery systems; all plasma nicotine concentrations have been reconfigured such that the pre-absorption level starts at 0 ng/ml (that is, to take out the baseline differences). Cigarette, and 2 mg nicotine gum, adapted from Russell et al. (1983), and 21 mg patch adapted from Stratton et al. (2001). Swedish snus plasma nicotine concentrations in 10 Swedish snus users from a single 2 g pinch of loose snus adapted from Holm et al. (1992). (Figure from Foulds et al. 2003, Tobacco Control, 2003, 12, 349-59, reproduced with permission from the BMJ Publishing Group)

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

Absorption of nicotine from a single 2 g pinch of Swedish moist snuff in 10 users resulted in average plasma nicotine concentrations of 9.9$\pm$6.5 ng/ml after 10 minutes and peaked at 14.5$\pm$4.6 ng/ml shortly after discarding at 30 minutes (Holm et al. 1992). Among groups of habitual snuff takers and cigarette smokers, peak blood nicotine levels after use were similar, averaging 36.6$\pm$14.4 ng/ml and 36.7$\pm$16.1 ng/ml, respectively.

Nicotine plasma levels related to one day's use of four Swedish brands of snus have been compared with those from Nicorette chewing gum in a cross-over study (Lunell and Lunell 2005). The mean extracted amounts were 2.74$\pm$0.80, 1.55$\pm$0.68, 2.00$\pm$0.56 and 1.08$\pm$0.94 mg/sachet for General (1 g, pH 8.4), Catch Licorice (1 g, pH 8.5), Catch Mini (0.5 g, pH 8.4) and Catch Dry Mini (0.3 g, pH 7.3) snus, respectively. The approximate bioavailable dose of nicotine from snus was 40-60% of the extracted amounts. Nicotine plasma levels with General portion snus were sustained at higher levels than current nicotine replacement products, peaking at 29.0$\pm$8.5 ng/ml, and more closely mimicking cigarette smoker's nicotine plasma levels. The area-under-the-curve (AUC) and

maximum concentration (Cmax) for Catch Licorice 1 g and Catch Mini 0.5 g portion snus were twice those for the 2 mg Nicorette gum. For the strongest brand, General, these values were 2.5 times those for Nicorette gum.

Nicotine distribution

After nicotine is absorbed into the systemic circulation, it is rapidly distributed to all areas of the body including the brain. Whereas high levels of nicotine reach the brain in 10-20 seconds after a cigarette puff, the rise in brain nicotine is slower after the use of chewing tobacco and snuff (Benowitz et al. 1988b). The volume of distribution of nicotine averages 180 L (2.6 times body weight; Benowitz et al. 1982). The distribution half-life of nicotine is estimated to be 9 minutes (Feyerabend et al. 1985). The plasma half-life of nicotine after intravenous infusion or cigarette smoking averages about 2 hours and with a range of 100-150 minutes (Benowitz and Jacob 1993, Benowitz and Jacob 1994, Benowitz and Jacob 2000, Benowitz et al. 1999c, Benowitz et al. 2002). After administration of STP, plasma levels of nicotine decline at a slow steady rate that was parallel to the slope of the elimination phase that followed intravenous nicotine administration. As a consequence of the differences in absorption and distribution of nicotine after smoking or administration of smokeless tobacco, brain tissue is confronted with a steady concentration of nicotine after smokeless tobacco as opposed to the pulsed increases seen after each cigarette puff (IARC 2007).

Cotinine (the main primary metabolite of nicotine) is present in the blood of tobacco product users in much higher concentrations than of nicotine because of its longer half-life. Cotinine blood concentrations average about 250 to 300 ng/ml in groups of cigarette smokers, in some smokers even up to 900 ng/ml (Benowitz et al. 1983, Gori and Lynch 1985). After stopping smoking, levels of cotinine in plasma decline in a log linear fashion with an average half-life of about 16 hours and with a range of 12.8-18.8 hours (Benowitz and Jacob 1993, Benowitz and Jacob 1994, Benowitz et al. 1999c, Benowitz and Jacob 2000, Benowitz et al. 2002).

Swallowing of the juice from STP is prevalent (Ebbert et al. 2004a). Nearly 80% of nicotine that is absorbed from the intestine is metabolised to cotinine in the first pass through the liver and never reaches the systemic circulation. Thus, the level of plasma cotinine may not be as strong an index of consumption in users of smokeless tobacco as it is in cigarette smokers (IARC 2007).

Nicotine metabolism

Nicotine is extensively metabolised to a number of metabolites by the liver (recently reviewed by Hukkanen et al. 2005). Six primary metabolites have been identified (Figure 2). About 90% of a systemic dose of nicotine can be accounted for as nicotine and metabolites in urine. In humans, about 70 to 80% is converted to cotinine. This transformation occurs in two steps, first by cytochrome P450, thereafter by aldehyde dehydrogenase. Cotinine is excreted in the urine to a small degree (10 to 15% of the nicotine and metabolites in urine). Nicotine *N'*-oxide is another primary metabolite of nicotine, about 4 to 7% of nicotine absorbed by smokers is metabolised via flavin monooxygenase 3 to this metabolite. The remainder of nicotine is converted primarily to nicotine glucuronide (3-5%), cotinine glucuronide (12-17%), *trans*-3'-hydroxycotinine (33-40%) and *trans*-3'-hydroxycotinine glucuronide (7-9%). Although nicotine is primarily metabolised in the liver, nicotine may be metabolised to a small extent in extrahepatic organs such as lung, kidney, nasal mucosa and brain.

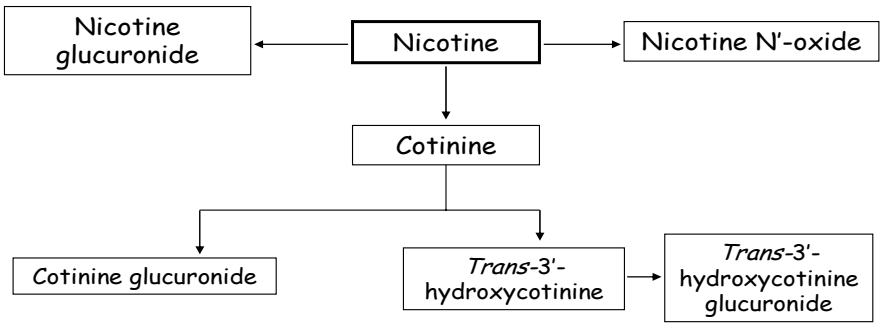

Figure 28. Main pathways of nicotine metabolism.

Total clearance of nicotine averages about 1200 ml/min, about 70% of nicotine undergoes first pass metabolism in the liver (Hukkanen et al. 2005). The metabolism of cotinine is much slower than that of nicotine, cotinine clearance averages about 45 ml/min. Also the clearance of *trans*-3'-hydroxycotinine is quite slow, about 82 ml/min.

In vitro and *in vivo* studies have shown that CYP2A6 is the enzyme that is primarily responsible for the oxidation of nicotine and cotinine (Hukkanen et al. 2005). CYP2B6 is the second most active hepatic P450 enzyme in nicotine C-oxidation. In humans, CYP2D6 poor-metaboliser and extensive-metaboliser phenotypes have similar nicotine and cotinine kinetics, although an ultrarapid-metaboliser phenotype caused by amplification of *CYP2D6* gene may be associated with accelerated nicotine metabolism (Saarikoski et al. 2000). CYP2E1 has some activity toward nicotine in *in vitro* systems at high nicotine concentrations.

A large-scale twin study with intravenous infusions of nicotine and cotinine demonstrated that their clearances were higher in women compared with men, being 13 and 26% higher, respectively, in women not using oral contraceptives compared with men (Benowitz et al. 2004c). Oral contraceptive use further accelerated nicotine and cotinine clearances in women. Pregnancy has a marked influence on nicotine and especially cotinine clearance, being increased by 60 and 140%, respectively, in pregnancy compared to after birth (Dempsey et al. 2002). Clearance of nicotine has been shown to be decreased in elderly persons (age>65) compared with younger adults (Molander et al. 2001). Menthol in cigarettes inhibits nicotine oxidation and glucuronidation thereby enhancing systemic nicotine exposure (Benowitz et al. 2004b). The effects of menthol on nicotine kinetics in users of STP appear not to have been studied.

Nicotine excretion

Nicotine is excreted by glomerular filtration and tubular secretion in the kidney, with variable reabsorption depending on urinary pH (Hukkanen et al. 2005). With uncontrolled urine pH, renal clearance averages about 35 to 90 ml/min. In acid urine, nicotine is mostly ionised and tubular reabsorption minimised so that renal clearance may be as high as 600 ml/min. In alkaline urine, a larger fraction of nicotine is unionised, which may result in a renal clearance as low as 17 ml/min.

Studies with cannulated rats show that a few percent of radioactivity is excreted in bile after intravenous injection of labelled nicotine, and studies with dogs and rats have detected 4 to 5% of radioactivity in faeces (Schievelbein 1982, Schepers et al. 1993). No human study has tried to quantify the excretion of nicotine and metabolites via the bile into faeces (Hukkanen et al. 2005).

3.4.1.2. Neurobiological effects including mechanisms of addiction

Evidence that nicotine is the primary addictive constituent of tobacco

Nicotine is an alkaloid present in concentrations of 1-3% in cultivated tobacco, and many of the pharmacological effects of tobacco consumption reflect the actions of nicotine (Henningfield and Fant 1999). It is a potent and powerful agonist of nicotinic receptors in the cholinergic nervous system, and upregulation of nicotinic acetylcholine receptor binding is observed in brains of both human cigarette smokers and animals chronically exposed to nicotine (Buisson and Bertrand 2002). Short-term exposure accelerates heart rate and alters mood, although the half-life of nicotine is short (approximately 2 hours), resulting in rapid clearance. These primary effects of nicotine are reviewed elsewhere (see 3.4.1.3). It is widely accepted that nicotine is the primary addictive constituent of tobacco, and there is a growing body of evidence that nicotine demonstrates the properties of a drug of abuse (Balfour 2004). However, definitions of tobacco dependence, such as those in the DSM-IV-TR and ICD-10, typically assume (implicitly) that nicotine in tobacco is delivered in the form of tobacco smoke, usually by cigarette. All commercially successful tobacco products, regardless of delivery mechanism, deliver psychoactive levels of nicotine to users, while denicotinised tobacco products are typically not widely accepted by or palatable to chronic tobacco users and are commercially marginal (Henningfield and Fant 1999).

Self-administration of nicotine

Behavioural experiments with laboratory animals demonstrate that nicotine has psychostimulant properties similar to those of amphetamine and cocaine (Balfour 2004). In common with other psychostimulant drugs, nicotine can serve as a reinforcer in self-administration models, suggesting that nicotine has rewarding properties in common with other drugs of abuse (Balfour et al. 1998). Studies of nicotine self-administration in various species, including humans, indicate that nicotine can serve as an effective positive reinforcer (i.e., is rewarding), although in a more restricted range of conditions than for some other positively reinforcing substances such as cocaine (Henningfield and Fant 1999). The pattern of self-administration appears to be more similar to stimulants than that of other drug classes. Nicotine delivered by cigarette appears to provide a particularly effective means of maximising the observed reinforcing effects of nicotine, in part due to the rapid delivery of the bolus of nicotine delivered by cigarette smoke via the lungs, but it is clear that nicotine itself is the primary positively reinforcing constituent of tobacco (Henningfield and Fant 1999).

Evidence for nicotine self-administration is reviewed by Perkins (Perkins 1999), and concludes that nicotine alone, isolated from tobacco, is self-administered by animals and humans, although environmental cues can substantially influence rate of self-administration. It should be noted that some authors disagree with the strength of empirical evidence that human smokers will self-administer pure nicotine (Dar and Frenk 2004). Recent evidence in rats suggests that nicotine-induced excitation of reward systems, reflected in alterations of intracranial self-stimulation thresholds, persists for at least 36 days after cessation of nicotine self-administration (Kenny and Markou 2006). Daily pre-nicotine and post-nicotine reward thresholds remained stable and unaltered in control rats previously unexposed, while post-thresholds assessed 15 min after each daily nicotine self-administration session were lowered compared with pre-thresholds in nicotine self-administration rats. In addition, there was a progressive lowering of pre-thresholds in nicotine self-administration rats that resulted in a gradual downward shift in both pre-thresholds and post-thresholds, compared with pre-thresholds obtained prior to the first nicotine self-administration session (Kenny and Markou 2006).

Evidence of tolerance

Evidence for tolerance to the effects of acute administration of nicotine following acute exposure exists for various effects, such as cardiovascular effects, and is also suggested by the gradual increase in the number of cigarettes smoked per day by regular smokers over the course of their smoking careers, in particular in the early stages (Henningfield and Fant 1999). Tolerance may be related to the upregulation of nicotinic acetylcholine receptors (Buisson and Bertrand 2002), but the usual aversive consequences of nicotine administration in nicotine naïve individuals (e.g., nausea and vomiting) typically dissipate within a few hours and are rarely experienced again, possibly due to both the individual becoming more skilled in self-administration (thereby avoiding overdosing), and the development of tolerance (Henningfield and Fant 1999). Laboratory studies in humans have demonstrated greater sensitivity to the behavioural and psychoactive effects of nicotine administration in individuals previously unexposed compared to those chronically exposed to nicotine (Heishman and Henningfield 2000).

Evidence of withdrawal effects

Nicotine withdrawal symptoms in humans include elevated irritability and aggression, depression, restlessness, impaired concentration, increased appetite, light-headedness, sleep disturbance and craving, while withdrawal signs include decreases in heart rate, adrenaline and cortisol release, and resting metabolic rate (American Psychiatric Association 2000). While the broad symptoms and signs associated with withdrawal are similar across most individuals, the degree of severity varies substantially between individuals. Animal models of nicotine withdrawal have been developed, primarily as models to evaluate medications for treating withdrawal, and include measures of the frequency of observed signs such as writhes and gasps, wet shakes and tremors, ptosis, and chewing (Malin et al. 1992). This suggests that a component of the dependency potential of nicotine operates via negative reinforcement processes (i.e., the amelioration of withdrawal symptoms following resumption of nicotine consumption) as well as positive reinforcement processes.

Dopamine

Although the molecular mechanisms that lead to and maintain nicotine addiction are not fully understood, they are known to involve the regulation of brain monoamines, and in particular dopamine (DA) (Balfour 2004). Experimental evidence indicates that nicotine induces DA release partly by binding directly to nicotinic acetylcholine receptors located within the mesolimbic system, specifically within the ventral tegmental area (Watkins et al. 2000). In the rat brain, nicotinic acetylcholine receptors have been identified on the cell bodies and dendrites of dopamine neurones in the ventral tegmental area, as well as their terminal fields in the nucleus accumbens (Watkins et al. 2000). Rodent models also indicate that there may be critical sensitive periods during development where exposure to nicotine has more pronounced effects than at other times. Exposure to nicotine in adolescent animals has been reported to be associated with greater preference for nicotine and nicotine-induced arousal (Adriani et al. 2002), as well as different neurochemical adaptations to nicotine exposure, such as increased dopamine transporter density (Collins et al. 2004), compared to adult animals.

Nicotine and stimulation of DA release

Nicotine increases DA release in the ventral tegmental area, which is thought to play a central role in the reinforcing effect of the drug. Experimental impairment of DA function by lesion or antagonist challenge indicates that DA neurotransmission is involved in nicotine's discriminative stimulus properties, nicotine-induced facilitation of intracranial self-stimulation, intravenous nicotine self-administration, nicotine conditioned place preference, and nicotine-induced disruption of latent inhibition (Di Chiara 2000). The conclusion, therefore, is that nicotine depends on DA for those behavioural effects that are most relevant for its reinforcing properties, and that are likely to be the basis of the

abuse liability of tobacco (Di Chiara 2000). Nevertheless, the role that mesolimbic DA pathways play in responding to both natural and drug rewards, including nicotine, remains somewhat controversial (Balfour 2004).

It has been hypothesised that stimulation of DA projections to the medial shell and core of the nucleus accumbens (NAcc) play complementary roles in the development of nicotine dependence (Balfour 2004). That is, increased DA overflow in the NAcc medial shell confers hedonic properties on the response that the animal makes in order to receive the drug, and this in turn increases the probability that the animal will learn to make this response. By comparison, the primary role of increased DA overflow in the NAcc core is the attribution of incentive salience to cues associated with delivery of the drug, and the transition to Pavlovian responding to these conditioned behaviours (Balfour 2004).

Associative learning and cue responding

Behaviours associated with nicotine delivery will persist following removal of the contingency between nicotine and self-administration behaviours (Baker et al. 2004). In humans, for example, environmental cues may trigger craving for cigarettes several years after smoking cessation. In particular, after extensive self-administration, cues associated with nicotine can, by themselves, influence self-administration behaviours (Baker et al. 2004). The associative learning processes which accompany nicotine self-administration mean that nicotine serves as a conditioned stimulus when paired with a non-drug reward, acquiring new appetitive and affective properties as a result (Bevins and Palmatier 2004). It also appears to amplify the salience of other high incentive stimuli, resulting in enhanced nicotine self-administration and conditioned reinforcement processes (Bevins and Palmatier 2004). This goes some way to explain the apparent discrepancy between the relatively subtle psychoactive effects of nicotine, and its potent abuse liability.

Other neurotransmitter pathways

While the majority of research has focussed on the role of DA in mediating the positive reinforcing and hedonic effects of nicotine, there is evidence for the implication of other neurotransmitter pathways. In particular, non-DA pathways may modulate nicotine reinforcement processes, and neurochemical adaptations associated with tolerance and withdrawal effects following chronic nicotine exposure.

Acetylcholine

Nicotine produces its central and peripheral actions by binding to the nicotinic acetylcholine receptor complex. Evidence suggests that cholinergic input to the mesolimbic DA pathway may provide a system through which nicotine may increase DA release (Watkins et al. 2000), and self-administered nicotine may directly stimulate nicotinic acetylcholine receptors within the ventral tegmental area (Watkins et al. 2000).

Serotonin

Evidence for the involvement of the serotonergic system in the positive reinforcing effects of nicotine is limited, although acute systemic administration of high nicotine dose has been reported to increase the release of serotonin in the frontal cortex of rats (Ribeiro et al. 1993). Nevertheless, the functional role of serotonin in mediating the positive reinforcing effects of nicotine remains unclear (Watkins et al. 2000).

Glutamate

Recent evidence indicates a role for glutamatergic receptor in the increases in the acoustic startle response, a measure of reactivity to environmental stimuli, associated with nicotine withdrawal (Helton et al. 1997). There is also some evidence that glutamate is involved in some behavioural changes and neuroadaptations occurring following

chronic nicotine administration, such as the development of sensitization and tolerance to nicotine (Watkins et al. 2000).

Noradrenaline

Nicotine increases cortical noradrenaline in rats, and increases in hypothalamic noradrenaline levels correlate with nicotine self-administration in rats (Cryan et al. 2003). Furthermore, noradrenergic autoreceptors are markedly down-regulated in smokers, suggesting that the nicotine-induced noradrenaline release might result in adaptive processes in feedback mechanisms that regulate noradrenaline function (Cryan et al. 2003).

3.4.1.3. Cardiovascular effects

Studies in animals

A number of animal studies have investigated the effects of nicotine on the cardiovascular system (reviewed in Cnattingius et al. 2005). Increases in blood pressure and heart rate have been observed, both as a direct effect after intravenous injection in dogs (Jain et al. 1997, Mehta et al. 1998, Mehta et al. 2001) and after 2 weeks exposure from subcutaneous nicotine pellets in rats (Swislocki et al. 1997). Injection of 50 µg nicotine/kg bodyweight induced cardiac arrhythmias in dogs, whereas lower doses did not (Mehta et al. 1997). In addition, nicotine has been shown to increase the sensitivity towards arrhythmias and induce ventricular fibrillation in hearts with healed myocardial infarction (Yashima et al. 2000).

Two studies in dogs have investigated the effect of nicotine exposure on myocardial infarction (Sridharan et al. 1985, Villarreal et al. 1999). In one study, there was poorer myocardial healing one week after infarction in those animals who had been exposed to nicotine-patches during one week before the infarction. In the other study, the volume of damaged tissue in the cardiac muscle was larger in those animals that had been exposed to nicotine; the effect was dose-dependent.

Some animal studies have investigated the metabolic effects of nicotine (Swislocki et al. 1997, Swislocki 2003). Rats exposed for 2.5 weeks subcutaneously with nicotine were compared to a placebo group. There were no observed effects amongst others on insulin and glucose intolerance. Mice exposed orally to nicotine for 20 weeks showed a more extensive plaque formation in blood vessels compared to the placebo group (Heeschen et al. 2001).

Studies in humans

Any form of tobacco affects acutely both heart rate and blood pressure in humans, and results in an increase of approximately 10-20 mm Hg in systolic blood pressure and 6-12 mm Hg in diastolic pressure (Benowitz et al. 1988b, Asplund et al. 2003b, Wolk et al. 2005, reviewed in Royal College of Physicians 2000). This is presumably due to an effect of nicotine since also nicotine replacement therapy results in similar effects (Asplund 2003a). However, it has been shown that there is no change in resting blood pressure during chronic exposure to nicotine from STP (Eliasson et al. 1991, Wennmalm et al. 1991, Hirsch et al. 1992, Bolinder et al. 1997b, Bolinder and de Faire 1998, Wallenfeldt et al. 2001).

Human studies have demonstrated that if nicotine is administered orally to non-smokers, this will result in changes in the plasma concentration of triglycerides (Quensel et al. 1989). In animal models, nicotine has been shown to affect lipid metabolism through increasing LDL-levels and reducing HDL-levels (Cluette-Brown et al. 1986). In experiments in rabbits administered nicotine, this resulted in increased levels of total cholesterol, glucose and LDL-cholesterol (Booyse et al. 1981). High doses of nicotine

given to rabbits have been found to induce endothelial damage and this appears to accelerate development of atherosclerosis in the carotid arteries and aorta (Kilaru et al. 2001).

3.4.1.4. Reproductive toxic effects

High, intravenous doses of nicotine in experimental animals have been shown to reduce placental and foetal perfusion (Suzuki et al. 1971). However, it is assumed that there is a considerable reserve capacity in human placental circulation and nicotine administration to pregnant women has not given indication of hypoperfusion (Lambers and Clark 1996). Exposure of pregnant rats has been demonstrated to result in insufficient development of nicotinic cholinergic receptors in the brains of the offspring, with documented altered behaviour and ability to handle hypoxic stress (Slotkin 1998). It is not clear from evidence in experimental animals whether nicotine has potential adverse effects on the human developing foetus. Studies of the acute effects of nicotine replacement therapy in pregnant humans indicate that nicotine alone has minimal effects upon the foetus.

3.4.1.5. Other effects

Nicotine has a number of cellular effects in various *in vitro* systems (reviewed in Cnattingius et al. 2005). Many of these effects are related to binding and activation of nicotinic acetylcholine receptors in non-nervous tissue and are associated with stimulated division of epithelial and endothelial cells (Waggoner and Wang 1994, Heeschen et al. 2001, West et al. 2003, Ye et al. 2004). Receptor activation is seen at nicotine concentrations similar to those measured in plasma during tobacco use (10-100 nM). Receptor activation can also increase cellular survival and inhibit apoptosis under various cell culturing conditions and exposure to toxic stimuli (Minna 2003, Yildiz 2004). It is believed that nicotine leads to a redistribution of receptor subunits in the cell membranes resulting in downstream alterations of signalling involved in cellular proliferation and apoptosis (Zia et al. 1997, Takahashi et al. 1999, Zia et al. 2000, Arredondo et al. 2001, Ye et al. 2004).

Cellular apoptosis has been observed at low concentrations of nicotine (0.06-0.8 µM) (Wu et al. 2002, Crowley-Weber et al. 2003). At higher concentrations (0.01-2 mM) cellular proliferation and premature differentiation have been noted (Konno et al. 1991, Kwon et al. 1999, Hakki et al. 2000), whereas very high concentrations of nicotine (2-10 mM) lead to growth inhibition and necrotic cell death (Konno et al. 1991, Lahmouzi et al. 2004). Plasma levels of nicotine related to STP are in the order of 0.1-0.2 µM (Benowitz et al. 1988b, Holm et al. 1992, Fant et al. 1999, Lunell and Lunell 2005).

Dependent on concentration, nicotine can function as an antioxidant in incubations with mitochondria (Soto-Otero et al. 2002). In cell culture, a low concentration (10 µM) of nicotine can inhibit oxidative stress caused by hydrogen peroxide, whereas higher concentrations of nicotine alone (1-10 mM) will induce oxidative stress (Guan et al. 2003).

Nicotine administration *in vitro* (200 µg/ml, i.e. 1.2 µM) and *in vivo* (20 µg 3 times per week for 4 weeks by topical injection) has been shown to promote angiogenesis, tumour invasion and metastasis in sponge implantation and Matrigel membrane models of gastric cancer (Shin et al. 2005).

3.4.2. Other constituents

3.4.2.1. Toxic effects of tobacco-specific nitrosamines (TSNA)

The outcome of bioassays for various TSNA and volatiles nitrosamines has been adequately covered in the IARC monographs (IARC 1985, IARC 2007).

In brief, NNN, the most prevalent N-nitrosamine in STP, induces tumours of the oesophagus in rats (Hecht and Hoffmann 1989). NNK is a strong systemic lung carcinogen in rodents, inducing lung tumours independently of its route of administration (Hecht 1998). The strength of NNK is particularly great in the rat, in which total doses as low as 1.8 mg/kg induce a significant incidence of lung tumours (Belinsky et al. 1990). NNK is the only pancreatic carcinogen known to be present in tobacco products (Rivenson et al. 1988). Long-term, repeated oral cavity swabbing with NNK produced only one papilloma in the oral cavity in 29 rats. However, significant tumour formation was found in the lungs, the nasal cavity and the liver (Prokopczyk et al. 1991). Combined application of NNK and NNN induced oral tumours in F 344 rats (Hecht et al. 1986). The IARC working group on the evaluation of NNN and NNK concluded that there is sufficient evidence of carcinogenicity of these compounds in experimental animals (IARC 2007).

3.4.2.2. Toxic effects of other constituents

Other nitrosamines

As described in section 3.3.2.3, the products found to-day on the US as well as on the Swedish market are practically free from other nitrosamines than TSNA (Brunnemann and Hoffmann 1991, Brunnemann et al. 2001, Brunnemann et al. 2004) and their toxic properties will not be reviewed in this context.

Polycyclic aromatic hydrocarbons (PAH)

Benzo(a)pyrene (BaP) is an indicator of PAH exposure and has a carcinogenic potency comparable to that of NNK (Nilsson 1998). However, in comparison with NNK and NNN, the levels of carcinogenic PAHs in American snuff must be considered as very low (see 3.3.2.3.) The levels of PAH in Swedish snuff lie below the detection limit.

Flavouring agents

Several brands of snuff are flavoured with commonly used food flavouring agents, such as menthol that are generally recognized as safe. However, one of these ingredients, liquorice obtained from the roots of Glycyrrhiza glabra, has long been recognized as an aldosterone antagonist in humans affecting mineral corticosteroid homeostasis. However, the intake required to induce symptoms of mineral corticosteroid imbalance in sensitive individuals requires a daily dose orders of magnitude above the intake due to use of liquorice flavoured snuff (Störmer et al. 1993).

Radionuclides

As discussed in 3.3.2.3, the dose of ionizing radiation from STP must be considered as negligible in comparison e.g. with the natural radiation background and other sources of ionizing radiations (Chruścielewski and Kaminski 1999).

3.4.2.3. Addictive effects of other constituents

Other constituents of tobacco

While nicotine is widely regarded as the primary addictive constituent of tobacco (see 3.4.1.2.), it is also the case that, compared with other addictive drugs, nicotine alone has relatively weak psychoactive and positive reinforcing properties, and there is some evidence that smokers will not self-administer pure nicotine (Dar and Frenk 2004). This can be partially explained with reference to the complementary role of the NAcc core and shell in nicotine dependence, and the importance of associative learning processes.

Nevertheless, there is evidence that tobacco dependence (as opposed to nicotine dependence) may result in part from monoamine oxidase (MAO) inhibition as well as from the positive reinforcing properties of nicotine (Berlin and Anthenelli 2001). For example, pharmaceutical nicotine delivery devices lack the dependency potential of tobacco (Pickworth et al. 1994), while denicotinized cigarettes are able to partially ameliorate craving and withdrawal associated with abstinence (Pickworth et al. 1999).

MAO is involved in the degradation of physiologically active monoamines, and MAO inhibitors in tobacco may themselves be involved in the positive reinforcing properties of tobacco. Preclinical and clinical studies have indicated that current smokers have lower brain MAO activity than non-smokers, which is normalized during prolonged abstinence (Guillem et al. 2005). Furthermore, it has been shown that an as yet unidentified component of tobacco smoke which is not nicotine, inhibits MAO activity (Rommelspacher et al. 2002), although some progress has recently been made in identifying candidate MAO inhibitors from extracts of tobacco leaves (Khalil et al. 2000).

Experimental inhibition of MAO has been reported to increase the motivation to self-administer nicotine in rats (Guillem et al. 2005), and while nicotine-naïve rats do not readily self-administer nicotine, robust self-administration occurs in the presence of MAO inhibitors (Villegier et al. 2006), so that nicotine and MAO inhibitors may act synergistically. In other words, the inhibition of MAO activity by compounds present in tobacco may combine with nicotine to produce the positive reinforcing effects of tobacco, and MAO inhibition by compounds in tobacco may therefore serve to potentiate the effects of nicotine (Berlin and Anthenelli 2001). Reductions in the rewarding effects of nicotine have also been observed in MAO knockout mice (Agatsuma et al. 2006).

In humans, brains of smokers show a 40% reduction in MAO activity relative to non-smokers and ex-smokers (Fowler et al. 1996a, Fowler et al. 1996b), and these differences are also observed in peripheral organs (Fowler et al. 2003). Smoking behaviour has been reported to be negatively correlated with platelet MAO activity (Rose et al. 2001). Moreover, MAO activity appears to increase following cessation, but this process occurs over several weeks, suggesting that the constituents in tobacco smoke responsible for MAO inhibition may have a half-life of several days (Rose et al. 2001).

Additives with direct effects

There is also limited evidence that additives introduced into cigarettes during the manufacturing process and not endogenously present in tobacco may contribute to the addiction potential of tobacco products. To date, however, relatively little research attention has been paid to the processes whereby tobacco additives may promote tobacco use initiation and subsequent dependence, although ammonia is known to increase the pH of smoke and thereby increase the delivery of free nicotine. Levulinic acid is a known cigarette additive, and a recent review of internal tobacco industry documents indicates that levulinic acid has been used as an additive to increase nicotine yields while enhancing perceptions of smoothness and mildness in cigarettes (Keithly et al. 2005). Levulinic acid also reduces the pH of cigarette smoke and desensitizes the upper respiratory tract, increasing the potential for cigarette smoke to be inhaled deeper

into the lungs, and may also enhance the binding of nicotine to neurons that ordinarily would be unresponsive to nicotine (Keithly et al. 2005).

Additives with indirect effects

Additives that increase the palatability of tobacco products may contribute to initiation and subsequent dependence indirectly, by increasing the likelihood of use and level of consumption. For example, menthol is used as an additive in some cigarettes (including, at reduced levels, in non-menthol brands), with the effect of altering subjective perceptions of tobacco smoke and its constituents via cooling, smoothing, and aesthetic effects (Ferris Wayne and Connolly 2004), while theobromine dilates the airway and increases inhalation. No data exist in the public domain regarding the potential of additives to STP, but it is possible that similar processes may occur with respect to the palatability of STP.

3.4.3. Conclusion on biological effects of smokeless tobacco constituents

Nicotine in STP is rapidly absorbed from the oral cavity and from the gastro-intestinal tract after swallowing, but less rapidly than from cigarette smoke. The pH of STP in solution is a significant factor for nicotine bioavailability. Increases in pH lead to increases in nicotine blood concentrations. The rise in brain nicotine is slower after using STP than after smoking. Nicotine is extensively metabolised, with cotinine as the main primary metabolite. Metabolic products of nicotine are chiefly excreted via the kidneys.

It is widely accepted that nicotine is the primary addictive constituent of tobacco, although there is also evidence that other constituents may play a role. The effects of nicotine appear to operate primarily via the modulation of neurotransmission in the dopamine pathway of the brain, and in particular via the release of dopamine in the nucleus accumbens, although other neurotransmitter pathways may play a role.

Experimental studies in both animals and humans show that nicotine acutely increases blood pressure and heart rate. There is no change in resting blood pressure associated with chronic exposure to nicotine from STP. There is experimental evidence that nicotine may affect lipid metabolism.

It is not clear from evidence in experimental animals whether nicotine has potential adverse effects on the human developing foetus.

Nicotine has a number of cellular effects in various *in vitro* systems, often demonstrated at much higher concentrations than those achieved after smokeless tobacco product use. Many of these effects are related to binding and activation of nicotinic acetylcholine receptors in non-nervous tissues. Nicotine may lead to redistribution of receptor subunits in cell membranes resulting in downstream alterations of signalling involved in cellular proliferation and apoptosis.

Constituents other than nicotine in tobacco may contribute to the addiction potential of tobacco. These include substances which may directly potentiate the effects of nicotine (e.g. constituents acting as monoamine oxidase inhibitors) and additives which have indirect effects (e.g. flavourings which increase the palatability of tobacco).

4-Hydroxy-1-(3-pyridyl)-1-butanone (HPB), a metabolite of NNN and NNK capable of forming a DNA adduct, has been detected as an haemoglobin adduct in rats (surrogate of DNA adduct) upon treatment of with very low doses of NNK.

The major tobacco-specific nitrosamines in STP, NNN and NNK, are carcinogenic in rodents inducing tumours of oral cavity, oesophagus, lung and pancreas. In products made from fire-cured tobacco, carcinogenic PAHs have been detected.

3.5. Experimental Studies with Smokeless Tobacco Products

3.5.1. Toxicokinetics of constituents other than nicotine

3.5.1.1. Adducts of N-nitrosamines

DNA and haemoglobin adducts formation after exposure to TSNA was described in section 3.3.2.4. In this section additional data related to understanding the role of TSNA adducts in carcinogenesis are presented.

In non-exposed individuals 7-mGua levels between 2.5 per 10^7 nucleotides (1 pmol/µmol Gua) in lymphocytes (Mustonen and Hemminki 1992) and 8.3 /10^7 nucleotides (3.3 pmol/µmol Gua) in non-tumour larynx tissue (Szyfter et al. 1996) have been reported.

In contrast to 7-methylguanine, relatively few studies on the background levels of *O6-methylguanine* have been conducted. Using a monoclonal antibody specific for O6-methyldeoxyguanosine (O6-MeGua) in a competitive enzyme-linked immunosorbent assay with a lower limit of detection of 0.5 pmol O6-mdGuap/µmol deoxyguanosine, placental DNA from smoking and non-smoking women was analysed (Foiles et al. 1988). Two of 10 DNA samples from smoking women and three of 10 from non-smoking women had detectable concentrations of O6-MeGua. Thus, this study failed to reveal any significant differences. With the development of novel and more sensitive ^{32}P postlabeling and radioimmunological techniques, the background concentrations of O6-mGua in liver was found to be in the range 0.1 – 0.7 pmol/µmol guanine. In peripheral leukocytes from healthy volunteers the median adduct concentrations were about an order of magnitude lower (range, 0.07 – 0.46 pmol/ µmol Gua) than in liver (Kang et al. 1995, Haque et al. 1997), or colon. In normal colorectal tissues O6-mGua was detected in 27 out of 62 samples (detection limit 0.01 pmol/µmol Gua) where the concentrations ranged from 0.01 to 0.94 pmol/µmol Gua (Povey et al. 2000). This adduct was found in 83-86% in samples of maternal and cord blood leukocyte DNA from healthy smoking and non-smoking women at levels up to 0.2 pmol/µmol guanine (Georgiadis et al. 2000). Similar to rats treated with NNK, the concentrations of O4-mTh in human tissues appears to be low. Thus, in human liver the mean value of the ratio between O6-mGua and O4-mThd was about 6 (Kang et al. 1995).

Although HPB Hb adducts can obviously be used as a measure of exposure, the HPB releasing DNA adducts constitute the relevant biomarkers for induction of cancer. HPB DNA adducts are most probably involved in the induction of tumours of the rodent nasal epithelium and oesophagus (Trushin et al. 1994), and could also be important for the induction of human cancer. Foiles et al. (1991) reported differences between 9 smokers and 8 non-smokers by measuring the release by acid hydrolysis of HPB DNA adducts from human peripheral lung and tracheobronchial tissues collected at autopsy. However, the employed methodology was not sufficiently sensitive to permit any definite conclusions. In non-smokers a mean HPB DNA adduct level of 50+/- 42, 130+/-148, and 130+/-110 fmol HPB/mg DNA, was detected in lung, oesophagus and cardia, respectively. Although the average concentrations of DNA HPB adducts in lung were increased in 49 smokers (91+/-133 fmol HPB/mg) as compared with 34 non-smokers (50+/-42 fmol HPB/mg), this difference was not statistically significant. The concentration of HPB-releasing lung DNA adducts was significantly higher ($p<0.0001$) in 21 self-reported smokers compared to 11 self-reported non-smokers (404+/-258 fmol versus 59+/-56 fmol HPB/mg DNA, respectively) (Hölzle et al. 2007).

The presence of appreciable levels of HPB releasing adducts in haemoglobin as well as in DNA from non-exposed subjects has been a cause for concern, because it indicates that other sources for HPB adducts than tobacco are important, and where myosmine present in various foods represents a possible candidate (Zwickenpflug et al. 1998, Wilp et al. 2002) However, in a recent study, HPB-releasing Hb- and DNA-adducts were clearly

detected in the rats treated with NNN or NNK, but no evidence was found for production of these adducts from the combination of myosmine plus $NaNO_2$ (Hecht et al. 2007).

Murphy et al. (1990) determined HPB released from lung as well as liver DNA from rats treated with NNK (i.p.) in the dose range 0.003 – to 5 mg/kg/day during 4 days. In the low dose region, the amount released was similar for the two tissues and characterized by a slope factor of approximately 3 pmol HPB/µmol guanine per mg/kg/day of NNK (250 fmol/mg DNA). In this context it is assumed that both NNK and NNN contribute to an equal extent in the induction of HPB adducts.

In a study by Hecht et al. (1991), the mean HPB haemoglobin adduct levels were 517+/-538 (SD), 79.6+/-189 and 29.3+/-25.9 fmol HPB/g haemoglobin for users of snuff, smokers and non-smokes, respectively. However, the increase of HPB adducts exhibited large individual variations, where some non-smokers had higher HPB values than the mean value for smokers. Falter et al. (1994) reported median concentrations of 34 and 61 fmol/g globin in smokers and non-smokers, respectively. However, they found significantly elevated levels of HPB-releasing Hb adducts in users of nasal dry snuff (median 236 fmol/g globin).

Measurement of urinary metabolites indicate striking differences between users of tobacco and non-exposed, but the measured increase in HPB haemoglobin adducts in smokers and users of snuff appears to be elevated above background only in a subset of individuals (Hecht 1996). Measured concentrations of HPB haemoglobin adducts in humans agree rather well with the levels expected from rodent studies.

Immunoassays for O^6-methyldeoxyguanosine, a DNA adduct that could arise from NNAL and NNK, have shown negative results in exfoliated oral cells from snuff dippers (Hecht et al. 1987).

As described in section 3.3.2.4, NNN and NNK form haemoglobin adducts in humans and experimental animals. These adducts release 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) upon mild alkaline hydrolysis. Nasal snuff users also showed high levels of haemoglobin adducts; HPB-releasing adducts were not correlated with the amount or type of snuff used.

3.5.1.2. N-Nitrosamines in saliva of smokeless tobacco users

Carcinogens derived from STP have been detected in the saliva of users of these substances. The tobacco-specific nitrosamines (TSNA), NNN, NNK N'- NAT and NAB as well as the volatile nitrosamines, N-nitrosodimethylamine and N-nitrosodiethylamine, were detected in the saliva of tobacco chewers and snuff dippers. The volatile nitrosamines are probably also tobacco-derived.

High levels of TSNA (NNN, NNK, NAB) and volatile nitrosamines were detected in saliva samples collected from India. The saliva of men who chewed tobacco with lime contained higher levels of TSNA than that of men who chewed betel quid with tobacco and lime (Bhide et al. 1986). NNN and NNK were also reported to be present in saliva in several other studies (Wenke et al. 1984, Nair et al. 1985, Nair et al. 1986). Volatile nitrosamines and TSNA in the saliva of chewers could be from the leached-out nitrosamines present in the tobacco or could be formed endogenously from abundant precursors during chewing. Levels of TSNA, nicotine and cotinine were measured in the saliva of 20 snuff dippers. Levels of NNN, NNK and NAT plus NAB found in the saliva following a 15-min period of keeping 0.5–1.5 g moist snuff in the gingival groove were considerable: NNN, 115–2610 ppb; NAT plus NAB, 123–4560 ppb; and NNK, up to 201 ppb. The salivary level increases with the duration of keeping snuff in the mouth. The total amount of TSNA was estimated to be 444 µg per use, a large part of which may be swallowed (Brunnemann et al. 1987b).

Levels of TSNA were analysed every 10 min in the saliva of habitual snuff dippers. Detectable levels of at least two TSNA were found in all samples collected between 10 and 30 min after the snuff had been placed in the mouth. Total concentrations of TSNA, up to 241 ng/g, were found in the saliva. Trace levels of TSNA were still found in the saliva 20 min after the snuff had been removed (Hoffmann and Adams 1981, Österdahl and Slorach 1988, Prokopczyk 1992).

Levels of salivary TSNA were measured in Indian smokeless tobacco users, who placed a mixture of Khaini (tobacco and slaked lime) in the oral cavity. Among these tobacco chewers, up to 1580 ng/mL NNN, 690 ng/mL NAT, 90 ng/mL NAB and 180 ng/mL NNK were measured (Stich et al. 1992).

3.5.1.3. Endogenous nitrosation

Tobacco contains secondary and tertiary amines that can be nitrosated in the saliva during the chewing of tobacco when they react with available nitrite in the presence of nitrosation catalysts such as thiocyanate. The N-nitrosoproline (NPRO) test measures the potential for intragastric formation of carcinogenic nitrosamines in humans (Ohshima and Bartsch 1981).

The role of poor oral hygiene in the formation of N-nitroso compounds was investigated by means of the NPRO assay. Endogenous nitrosation is significantly higher in tobacco chewers with poor oral hygiene (determined by dental plaque) compared with those with good oral hygiene (Nair et al. 1996).

Among subjects dosed with proline, NPRO was significantly elevated in the urine of individuals who chewed tobacco plus lime (Nair et al. 1987, Chakradeo et al. 1994).

Measurable concentrations of all tobacco alkaloids (nicotine, nornicotine, anabasine, and anatabine) were excreted in the urine of subjects using smokeless tobacco. These compounds could be substrates for endogenous nitrosation in tobacco chewers (Jacob et al. 2002).

3.5.1.4. Absorption and excretion of TSNA

Absorption of TSNA as NNN, NAT and NAB by smokeless tobacco users has been demonstrated by detection of their -N-glucuronides. Levels of NNN and NNN-Gluc in 11 users were 0.03–0.58 pmol/mg creatinine (mean ± SD, 0.25 ± 0.19 pmol/mg) NNN and 0.091–0.91 pmol/mg creatinine (mean ± SD, 0.39 ± 0.27 pmol/mg) NNN-N-Gluc; not detectable to 0.11 pmol/mg creatinine (mean ± SD, 0.0037 ± 0.034 pmol/mg) NAB and 0.021–0.44 pmol/mg creatinine (mean ± SD,0.19 ± 0.16 pmol/mg) NAB-N-Gluc and 0.020–0.15 pmol/mg creatinine (mean ± SD, 0.069 ± 0.046 pmol/mg) NAT and 0.084–2.78 pmol/mg creatinine (mean ± SD, 1.36 ± 1.06 pmol/mg) NAT-N-Gluc respectively (Stepanov and Hecht 2005b). Absorption and metabolism of NNK has been demonstrated in smokeless tobacco users by measuring its metabolites NNAL and NNAL-Gluc which were detected in the plasma of smokeless tobacco users (Hecht et al. 2002b). Glucuronidation of NNAL at the pyridine nitrogen gives NNAL-N-Gluc while conjugation at the carbinol oxygen yields NNAL-O-Gluc (Carmella et al. 2002). The NNAL glucuronides are collectively referred to as NNAL-Gluc. Both NNAL and NNAL-Gluc are excreted in human urine and are very useful biomarkers because they are derived from NNK that is specific to tobacco products (Hecht 2002a). Because NNAL is not usually present in tobacco, NNAL and NNAL-Gluc in urine originate largely from the metabolism of NNK. Most investigations to date have demonstrated a correlation between NNAL plus NNAL-Gluc and cotinine (Hecht 2002a). In 13 male smokeless tobacco users, the distribution half-lives of NNAL and NNAL-Gluc were determined. Baseline levels in urine as well as

renal clearance of the NNK metabolites correlated with number of tins or pouches of smokeless tobacco consumed. Ratios of (S)-NNAL:(R)-NNAL and (S)-NNAL-Gluc:(R)-NNAL-Gluc in urine were significantly higher 7 days after cessation than at baseline. Urinary NNAL plus NNAL-Gluc also provides a good approximation of carcinogen dose of snuff dippers. A correlation between the number of tins or pouches of smokeless tobacco consumed per week and NNAL plus NNAL-Gluc in urine was observed, as well as a correlation between salivary cotinine and NNAL plus NNAL-Gluc in the urine of smokeless tobacco users (Hecht et al. 2002b).

In 47 male smokeless tobacco users, urinary NNAL and NNAL-Gluc levels were similar to those in smokers. The ratio of NNAL-Gluc/NNAL was higher in snuff dippers than in tobacco chewers. A significant association between levels of NNAL plus NNAL-Gluc in the urine of smokeless tobacco users and the presence of oral leukoplakia was observed, supporting the potential role of NNK as a causative factor for this lesion (Kresty et al. 1996).

NNAL, NNAL-N-Gluc and NNAL-O-Gluc were analysed in the urine of 14 smokeless tobacco users. NNAL-N-Gluc in the urine comprised 24 ± 12% of total NNAL-Gluc and demonstrated that NNAL-N-Gluc contributes substantially to NNAL glucuronides in human urine (Carmella et al. 2002).

Pyridine-N-oxidation of NNK and its major metabolite NNAL produces NNK-N-oxide and NNAL-N-oxide, respectively, which are detoxification products of NNK metabolism and are excreted in the urine of rodents and primates. Pyridine-N-oxidation is a relatively minor detoxification pathway of NNK and NNAL in humans (Carmella et al. 1997).

In a randomised study from USA, Hatsukami and co-workers (Hatsukami et al. 2004) have investigated differences in carcinogen uptake between Swedish snus and nicotine replacement, with US moist snuff. The test persons were men who regularly used US moist snuff. Individuals who concurrently smoked or used other tobacco products were excluded form the analysis. During the first two weeks of the study period the participants used their usual US brand. The participants were then randomly assigned to one of two groups. In the first group the participants received the test product (Swedish snus), in the second group the participants received nicotine replacement (nicotine patch). The analysis was conducted in 41 individuals after four weeks with test product or nicotine replacement. After switching from US moist snuff to Swedish snus or nicotine replacement, the mean levels of the NNK metabolite NNAL [4-methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronide] in urine were significantly reduced ($p<0.001$) in both groups. The group which received nicotine replacement had lower mean levels of total NNAL than that which receiving Swedish snus (1.2 and 2.0 pmol NNAL/mg creatinine, respectively). Those switching from US moist snuff to Swedish snus had a mean reduction of 52% in total urinary NNAL, 11/19 had more than 50% reduction, 5/19 had 15% to 50% reduction, whereas 2/19 had an increase (17% and 28%, respectively).

Excretion of NNAL in the urine is reported to be at similar levels in some of the new tobacco products produced under new heat treatment techniques to reduce TSNA levels (Hatsukami 2006).

3.5.1.5. Conclusion on toxicokinetics of constituents other than nicotine

Adducts of tobacco-specific nitrosamines (TSNA) to haemoglobin have been detected in snuff dippers. TSNA were detected in the saliva of chewers of smokeless tobacco and snuff users. Additional exposure to nitroso-compounds could occur in the oral cavity and in the body due to endogenous nitrosation of secondary and or tertiary amines from tobacco including nornicotine. Systemic absorption and metabolism of TSNA have been demonstrated in the smokeless tobacco product users.

3.5.2. Addiction

There are no current animal models of smokeless tobacco self-administration. Consequently, since animal models of addiction rely on indexing an increase in self-administration of a substance relative to placebo, no literature exists which directly addresses the question of the addiction potential of STP in animals.

3.5.3. Cancer

3.5.3.1. Genotoxicity

Numerous studies in different types of prokaryotic and eukaryotic cells *in vitro* have reported on the mutagenicity and clastogenicity of aqueous and organic extracts of a variety of STP, including Swedish snus and American moist snuff, and various types of American and Indian chewing tobacco (IARC 2007).

Jansson et al. (1991) investigated the genotoxicity of aqueous and methylene chloride extracts of Swedish moist oral snuff using both microbial and mammalian assays. The methylene chloride extract contained much more nicotine (9.1 mg/mL) than the aqueous extract (2.4 mg/mL). The aqueous extract was found to induce sister chromatid exchanges in human lymphocytes *in vitro* and chromosomal aberrations in V79 Chinese hamster ovary cells *in vitro* (both with and without a metabolism system. However, no mutation induction in *Salmonella typhimurium* or V79 cells was observed. Micronuclei in mouse bone marrow cells were also not found. The methylene chloride extract showed genotoxic activity and gave positive results in the *Salmonella* mutagenicity test, and induced chromosomal aberrations and sister chromatid exchanges in V79 cells in the presence of a metabolism system. However, no induction of mutation was observed in the V79 cells. The results suggested that metabolism is required for genotoxic activity. The *in vivo* administration of methylene chloride extract did not cause micronuclei formation in mouse bone marrow cells, or sex-linked recessive lethal mutations in *Drosophila melanogaster*.

The mutagenic activity was determined in the *Salmonella* mutagenicity test of extracts of two leading brands of American chewing tobacco, treated with or without sodium nitrite under acidic conditions. Mutagenic activity was found only for nitrite-treated chewing tobacco extracts in the tester strains TA98 and TA100, and was independent of metabolism (Whong et al. 1985). However, in a previous study these authors had also reported mutagenic activity of tobacco snuff treated under acidic conditions in the *Salmonella* test with and without a metabolism system (Whong et al. 1984).

High concentrations of nicotine (0.3-0.6 mg/mL) have been reported to cause DNA damage in explant cultures of human nasal epithelia (Sassen et al. 2005).

3.5.3.2. Animal data

The following studies that relate to applications of snuff in experimental animals have been identified in the literature:

Table 8. Summary of studies on carcinogenic effects in experimental animals after snuff application.

Study No. Author	Species (No. animals per group)	Relevant oral tumours	Study length months	Comments
1. Peacock and Brawley 1959	Hamster (pouch) (50)	None	12-18	Control pouch with sand/chewing gum; > 50% mortality.
2. Peacock et al. 1960	Hamster (pouch) (60)	None	12-18	Control pouch with sand/chewing gum; > 50% mortality.
3. DiPaolo 1962	Rats (40) Mice (50)	None None	18 15	Feeding study, evidence of toxicity, MTD exceeded, few details provided.
4. Dunham et al. 1966	Hamster (pouch) (7) + alkali (6)	None	Lifetime	No changes with snuff alone. Lesions from Ca-hydroxide (atypical cells).
5. Smith et al. 1970	Rhesus monkey (12)	None	7 years	No experimental details provided.
6. Homburger 1971	Hamster (pouch) (84); webbing cartridge attached to the incisors.	None	8-12	Detailed study; signs of high overt toxicity including high mortality; 9,10-dimethyl-1,2-benzanthracene positive control.
7. Dunham et al. 1974	Hamster (pouch) (4)	None	16	Only 4 animals.
8. Homburger et al. 1976	Hamster (50)	None	24	Feeding study. Toxicity, reduced body weight increase (15-20%).
9. Hirsch and Thilander 1981	Rat (oral canal) (4)	None	9 – 22	High degree of nicotine absorption. Mild to moderate hyperplasia of the epithelium, hyperkeratosis at 18-22 months. Changes about same as at 9 to 12 months. Depressed body weight gain in males. Low number of animals; 2 controls.
10. Hirsch and Johansson 1983	Rat (oral canal) (10)	1 carcinoma	18-22	Hyperplasia, keratosis of oral epithelium. 6 papillary squamous epithelial hyperplasias in the forestomach vs. none in controls. 1 carcinoma in the oral cavity.
11. Hirsch et al. 1984	Rat (oral canal) snuff (10); snuff +HSV (10)	Snuff 1 Snuff + HSV 2 carcinomas	9-22 (snuff – 18 months)	Pronounced depression of body weight gain in snuff + HSV. Hyperplasia of the forestomach in 50% of snuff exposed. 2 carcinomas in the oral cavity.
12. Antoniades et al. 1984	Hamster (pouch) (20)	None	5	No histopathological effects
13. Park et al. 1985	Mouse (labial mucosa) snuff water extract (20)	None	2	Snuff water extract + HSV caused marked increase in hyperplasia and atypical cells. Acetone was almost as effective.
14. Shklar et al. 1985	Hamster (pouch) mucosa (20)	None	5	No premalignant changes in pouch mucosa. Increased mitotic activity.

Study No. Author	Species (No. animals per group)	Relevant oral tumours	Study length months	Comments
15. Hecht et al. 1986	Rat (oral canal) (32)	2 papillomas, 1 carcinoma	29	Snuff enriched up to double the amount of TSNA gave 1 papilloma in oral cavity, but significant increase in liver tumours; controls only subjected to surgery, no irritating control material. Snuff extract showed a protective effect against TSNA.
16. Park et al. 1986	Hamster pouch mucosa (20) snuff/HSV	None (snuff only)	6	Hyperplasia from mock snuff dipping. Invasive buccal carcinoma in 50% of animals on snuff + HSV.
17. Hirsch et al. 1986	Rat (oral canal) (10)	None	13	Hyperplasia; markedly reduced, or absent, after a recovery period of 1 or 4 months.
18. Mendel et al. 1986	Rat (direct application) (30)	None	1	Increased mitotic activity, very short treatment; no exptl. details; abstract
19. Mendel et al. 1987	Rat (lower lip pouch)	None	3	Pre-keratinisation changes; no exptl. details given; abstract.
20. Park et al. 1987	Mouse (labial mucosa) snuff water extract; snuff+HSV (20)	None	2-3	In combination with HSV, acetone was as effective as snuff extract to induce hyperplasia and hyperkeratosis. No effects of extract alone.
21. Chen 1989	Rat (oral application) (15)	None	12	Keratotic changes; increased incidence of polyploidal buccal cells.
22. Larsson et al. 1989	Rat (oral canal) (13)	1 carcinoma (snuff only)	Life-time	1 additional nasal tumour in snuff group. Snuff+HSV and NQO+HSV increased tumours at distant sites. High content of NNN and NNK in the Swedish snuff used (33 μg/g NNN and 4.6 μg/g NNK; Cotton pellet dipped in saline as control material. Effects on weight gain. Moribund animals. Inflammatory changes of the lip
23. Johansson et al. 1989	Rat (oral canal) (30)	1 lip, 2 hard palate carcinomas, 1 hard palate carcinoma in situ	Life-time	1 nasal cavity tumour; 1 forestomach carcinoma; Hyperplasia of lip, hard palate, forestomach; MTD exceeded. Marked effects on weight gain, moribund animals. Spectrum of tumours like NQO. Much lower TSNA levels than in the Larsson study No. 23 (NNN = 5.1μg/g). Cotton with propylene glycol as control material.
24. Johansson et al. 1991a	Rat (oral canal) (19) Effect on T-cells in peripheral blood	No tumours	15 weeks	Toxicological endpoint of questionable relevance.

Study No. Author	Species (No. animals per group)	Relevant oral tumours	Study length months	Comments
25. Johansson et al. 1991b	Rat (oral canal) (38) Snuff only, or Initiation by NQO or dimethyl-benzanthracene +snuff	10 lip sarcomas, 2 lip papillomas, 3 carcinomas, hard palate; no lung tumours	Life-time	Moribund animals, MTD exceeded; marked effects on weight gain. Spectrum of tumours like NQO. Cotton pellet dipped in saline as control material. Inflammatory changes in the lip
26. Worawongvasu et al. 1991	Hamster (pouch) (8)	None	6	Only 2 controls. Unspecific histopathological changes
27. Summerlin et al. 1992	Hamster (pouch) (20) Snuff/ethanol (15%)	None	6.5	Marked acanthosis (thickening) of the pouch epithelium for snuff alone, and for alcohol alone. Short duration of the study, advanced age of the animals at the beginning of the experiment
28. Ashrafi et al. 1992	Hamster (pouch)	None	24	Hyperkeratotic mucosal changes.

One major problem in designing an experimental model that mimics human use of snuff is the failure of the rat and mouse to retain the snuff for a longer period in the oral cavity. In this respect the cheek pouch of the hamster has offered a suitable option, which is the reason why a number of studies have been performed in this animal species. All in all, 186 hamsters were exposed to snuff and no malignant tumours were observed in any of the animals. However, except for the well designed Homburger (1971) study, no solid conclusions can, on the other hand, be drawn from these experiments due to various defects in experimental design, or lack of description of relevant methodological details.

The only indications for a potential carcinogenic effect from snuff in experimental animals derive from exposure to snuff that has been inserted into a surgically created canal of the lower lip of the rat. The method was first developed by the Swedish dental surgeon Jan-Michael Hirsch in the early 1980s, and was used in 8 subsequent studies. Out of these studies, two gave an indication of an increase in incidence of tumours in the oral cavity (Johansson et al. 1989, Johansson et al. 1991b).

In the first pilot study conducted by the group of Hirsch (Hirsch and Thilander 1981) in 4 animals and 2 controls, where the effects from exposure to snuff only were studied, the surgically created canal of Sprague Dawley rats was filled with a fresh standard snuff twice a day for 9 months. Nicotine levels were determined in blood in two exposed and one control. In the second study (Hirsch and Johansson 1983), rats were exposed twice per day, 5 days per week, to standard (n=42) as well as alkaline snuff (n=10) where the pH had been raised to 9.3 by addition of sodium carbonate, with histopathological evaluation after 9-22 months' exposure. Even in case of prolonged exposures that covered a major part of the rat's lifetime only relatively mild reactions were found, described as mild to moderate hyperplasia of the epithelium, with hyper-orthokeratosis (striated horny changes) and acanthosis (thickening). In a few rats dysplastic changes developed in the crevicular epithelium. The results from the animals treated with alkaline snuff were essentially the same. There was no clear evidence for neoplastic progression, in as much as the epithelium of rats exposed for 18-22 months differed only slightly from that of rats exposed for 9 to 12 months, lesions that were found to be reversible upon cessation of exposure (Hirsch et al. 1986). A single squamous cell carcinoma of the

buccal mucosa was observed among 52 exposed animals (Hirsch and Johansson 1983). Further, the treated animals had hyperplasia of the forestomach.

In one study by Hirsch et al. (1984) designed mainly to study interaction with herpes virus, one single oral tumour was found in the group of 42 rats in which test canals had been exposed to snuff for 9 months. Using the protocol developed by Hirsch and Thilander (1981), Hecht et al. (1986) exposed 32 Fischer 344 rats every 24 hrs for 116 weeks to snuff of unspecified origin. Among the 32 animals, one developed an oral cavity squamous cell carcinoma, while 2 papillomas were detected in two other rats. Snuff enriched with NNN and NNK induced a lower number of oral lesions than snuff only. However, rats exposed to the enriched snuff had a higher incidence of liver tumours. No control material was inserted in the lip canal of sham operated rats.

While the studies of Hirsch and co-workers were essentially negative with respect to induction of oral tumours by snuff alone, the two studies by Johansson et al. (Johansson et al. 1989, Johansson et al. 1991a) indicated a tumorigenic effect of snuff when administered into artificially created lip canals twice daily, 5 days per week, up to 104 weeks.

The overall incidences of tumours in snuff-treated animals were clearly significantly higher than in controls where cotton had been inserted in the lip canal. The following localised tumours were found: 4 squamous cell carcinomas of the lip and hard palate, as well as 2 papillomas at these sites, none of which were found in controls. In addition, the following neoplasms were observed distant from the site of application: 4 malignant lymphomas, 2 hepatomas, and 4 skin tumours (Johansson et al. 1989).

In the second study with US snuff (Johansson et al. 1991b), where the similar experimental model was used, 10 sarcomas and 2 papillomas of the lip as well as 3 squamous cell carcinomas of the palate were found. However, these results should be interpreted with caution because the surgical intervention could create a tissue that would be more sensitive to unspecific irritation, and the manner in which snuff was inserted and removed from the lip canal of the rat will have caused additional trauma. The snuff was applied and removed with a metal spatula 2 times a day for up till 104 weeks. This led to marked inflammatory changes that were seen in 92% of the rats. Although the survival did not seem to have been affected by the snuff treatment, the studies demonstrated a significant reduction in weight increase during treatment, amounting to 100 g after 40 weeks in the study of Johansson et al. (1989), i.e. about 20%. There were no significant differences in food intake.

The tumour promoting effects of snuff was further studied by the group of Hirsch (Larsson et al. 1989) in rats that had been initiated with 4-nitroquinoline-N-oxide (4-NQO), or inoculated with herpes simplex virus type 1 (HSV-1). The previously described protocol was used, but with a treatment period was extended until 70-94 wk (moribund animals). In the group treated with snuff only, 4 tumours were found in 3 rats among 13 surviving animals; one squamous cell carcinoma in the oral cavity, one in the nasal cavities, one was a colon adenocarcinoma, and one a skin fibroma (benign) of the skin.

In the group exposed to snuff plus HSV-1, 13 tumours were found in 8 animals, out of which 7 were malignant, whereas in the rats only exposed to HSV-1, there were 3 tumours. However, except for one salivary gland sarcoma and one gingival haemangioma, there were no oral cavity tumours in the animals with combined exposures. A cotton pellet dipped in saline represented the control material used in the sham operated animals. The cited contents of NNN and NNK in the Swedish snuff used, were also significantly higher (33 µg/g NNN and 4.6 µg/g NNK) than reported elsewhere for Swedish snuff from this time period (Larsson et al. 1989).

Another experiment with 7,12-dimethylbenz(a)anthracene (DMBA) (Johansson et al. 1991b) provided some evidence for a potential promoting effect caused by snuff in the rat. Groups of 40 rats were given a low dose of DMBA (dose not specified) 3 times/wk for

4 wk. In one group a cotton pellet was used as control material and the other received snuff. While there were only 3 tumours in the DMBA treated animals, there were 1 squamous cell carcinoma and 9 sarcomas of the lip, 2 squamous cell carcinomas of the palate, and 2 squamous cell carcinomas of the forestomach in the animals with a combined DMBA/snuff treatment. However, the incidences were not significantly different from the effects from snuff alone.

The study by Park et al. (1986) with HSV-1 and HSV-2 in the hamster appears to be the only study that provides convincing data supporting a promotive effect by snuff. Whereas no increase in tumours was found for inoculation either with HSV-1, HSV-2, or exposure to snuff only (twice a day, 5 days/wk, 6 moths), there was a 50% incidence (10/20; 11/20) of invasive squamous cell carcinoma of the buccal cell pouch of hamsters after combined HSV – snuff treatments.

3.5.3.3. Conclusion on cancer (experimental studies)

The majority of animal studies of snuff-associated carcinogenesis are old and the results are difficult to interpret. The experimental groups tended to be small and/or the animal models used were invasive, with tissue trauma possibly confounding the results. Most of the studies with snuff have been negative or equivocal. Studies with snuff inserted into a surgically created canal of the lower lip of the rat do, however, indicate that snuff has a carcinogenic potential in this model.

These data, coupled with evidence of genotoxic effects of extracts of moist snuff in various in-vitro systems, and the presence of carcinogenic nitrosamines in the products, lead to the conclusion that moist snuff is carcinogenic in experimental animals.

3.5.4. Cardiovascular effects

3.5.4.1. Animal data

A long-term study (2 years) in rats exposed to snus administered in the feed resulted in an increase in blood glucose, cholesterol and LDL levels compared to the group not exposed to snus (Cluette-Brown et al. 1986).

3.5.4.2. Human data

Heart rate and blood pressure were studied in 10 healthy men aged 24-61 years who were regular smokers, when they used either one of two brands of American snuff or three brands of American chewing tobacco (Benowitz et al. 1988b). Their cardiovascular responses were compared with smoking their usual brands of cigarettes. The maximal increases in heart rate were similar for all forms of tobacco. The integrated (AUC) heart rate and systolic blood pressure responses to smokeless tobacco tended to be greater than for cigarette smoking.

Short-term haemodynamic effects of Swedish snuff were studied in a randomised, controlled investigation of 9 healthy volunteers (8 males and 1 female, mean age 27 years) of which 8 of 9 were habitual users of snuff (Hirsch et al. 1992). The study population refrained from snuff use at least 9 hours before experiment. Recordings were performed at 0, 15 and 30 min after snuff intake on 2 different days separated by 2 to 3 weeks (1 day with snuff intake, 1 day served as control). Snuff intake induced a significant increase in heart rate and blood pressure, and a decrease in stroke volume during rest. Haemodynamic changes in this study were not found to be correlated with nicotine and cotinine concentrations. Resting levels of noradrenaline and neuropeptide Y-like immunoreactivity did not differ between the days subjects received snuff and the days they received placebo. In contrast, maximum workload was associated with a slight increase in circulating adrenaline after snuff intake.

Acute haemodynamic and autonomic effects of smokeless tobacco were investigated in sixteen healthy, male habitual snuff tobacco users (aged 22 ± 1 year) using a randomised, double-blind, placebo-controlled, crossover design (Wolk et al. 2005). American smokeless tobacco (Copenhagen moist tobacco snuff) increased mean blood pressure by 10 ± 1 mm Hg and heart rate by 16 ± 2 beats/min. Peripheral vascular resistance, muscle sympathetic nerve activity and plasma noradrenaline concentration did not change, whereas adrenaline increased by approximately 50%. It was concluded that smokeless tobacco is a powerful autonomic and haemodynamic stimulus with catecholamine release from the adrenal medulla being likely to contribute to this response.

Twenty healthy middle-aged (sex not specified) Swedish snuff users underwent ultrasound assessment of endothelial-dependent flow-mediated dilatation of the brachial artery (Rohani and Agewall 2004). A statistically significant decrease of dilatation (an endothelial dysfunction predicting cardiovascular morbidity) was found after snuff administration.

Two Swedish studies have used ultrasound to measure carotid and femoral artery endothelium-media thickness and to detect atherosclerotic changes in moist snuff users (Bolinder et al. 1997a, Wallenfeldt et al. 2001). There were no significant increases in carotid or femoral lesions compared to non-tobacco users, whereas smokers showed evidence of atherosclerotic changes.

As reviewed by Westman (1995), across various studies, administration of smokeless tobacco acutely increases systolic blood pressure up to 21 mm Hg, diastolic blood pressure up to 14 mm Hg and heart rate by 19 beats per minute. These increases can occur within 3-5 minutes after tobacco is placed in the mouth and persist for 90 minutes after its removal (Benowitz 1999b).

3.5.4.3. Conclusion on cardiovascular effects (experimental studies)

Human experimental studies show that smokeless tobacco use leads to short term increases in blood pressure and heart rate. Snus use may cause endothelial dysfunction; other moist snuff products have not been studied.

3.5.5. Reproductive toxic effects

3.5.5.1. Animal data

Most animal experiments have shown that nicotine administration at high doses (1-2 mg/kg bw i.v.) reduces blood flow in the uterine artery and thereby placental blood flow (Lambers and Clark 1996, Suzuki et al. 1971, Suzuki et al. 1974, Suzuki et al. 1980). Nicotine presumably also induces foetal hypoxia and foetal acidosis.

Aqueous extracts of smokeless tobacco equivalent to 8 mg extract/kg bodyweight administered to pregnant CD-1 mice three times per day on gestational days 6-15 were shown to decrease foetal body weights by 13% (Paulson et al. 1992). This treatment did not affect litter size, incidence of resorptions, deaths and/or malformations.

3.5.5.2. Human data

In studies of pregnant women exposed to nicotine from nicotine gum (4 mg or 8 mg), there was an increase in maternal blood pressure and heart frequency, but no change in

foetal heart frequency or blood flow in the umbilical artery (Dempsey and Benowitz 2001, Benowitz and Dempsey 2004a).

3.5.5.3. Conclusion on reproductive toxic effects (experimental studies)

There are not enough studies available to draw any firm conclusions regarding reproductive toxic effects of smokeless tobacco.

3.5.6. Local effects

3.5.6.1. Animal data

No animal studies have been identified which have specifically investigated oral lesions. Hyperplasia and keratosis of the oral epithelium and inflammation of connective tissues have been observed in the animal carcinogenicity studies of smokeless tobacco (see section 3.5.3.2).

3.5.6.2. Human data

Human volunteer studies

Several groups have experimented on humans by short-term application of smokeless tobacco on oral mucosa (Johnson et al. 1998, Payne et al. 1998). The study group (19 males; mean age 25 ± 1.4 years) were regular snuff users but placed moist snuff on a new mucosal site during the experiment. The authors reported erythema, ulceration and white striae at the place of application in as few as 2-7 days. By 7 days, 56% of subjects displayed white striated lesions (Johnson et al. 1998). Rapid development of STP lesions in human volunteers is somewhat contrasting to reported lesions in chronic users. Significantly increased mucosal concentrations of Interleukin-1 and PGE2 were also reported at new sites of snuff placement, both molecules with immune and inflammatory functions. These data are similar to what was earlier reported on 18 male STP users exhibiting increased gingival inflammation at new placement sites of STP (Poore et al. 1995).

Healthy volunteers (n=20) switching to a snuff brand with a lower pH and nicotine content of snuff demonstrated significantly less pronounced clinical and histological changes at experimental sites (Andersson and Warfvinge 2003).

Exposure of human buccal mucosa to 1.5-2.5g of smokeless tobacco (in Ringer's solution) caused dilatation of intercellular spaces of the epithelium and altered barrier function suggesting that STP may facilitate buccal transport of substances at application sites (Tobey et al. 1988).

3.5.6.3. Conclusion on local effects (experimental studies)

It appears that human volunteers who are regular users of snuff when experimentally exposed to moist snuff at sites not previously used for placement of tobacco, rapidly develop mucosal alterations at new sites of placement.

3.5.7. Other effects

3.5.7.1. Animal data

Male Wistar rats were orally dosed by gavage with an aqueous extract of gutkha (96 mg extract/kg bodyweight/day) for up to 32 weeks and examined for effects on the antioxidant defence status and histopathological changes in liver, lung and kidney. A decrease in the antioxidant defence system and mild to moderate inflammatory changes in liver and lungs were observed (Avti et al. 2006).

3.5.7.2. Human data

The acute effects of Swedish moist snuff on insulin sensitivity were investigated in a randomised treatment study of 7 healthy smokers (4 females and 3 males, mean age 31 years) with the normoglycaemic clamp technique (Attvall et al. 1993). Measurements were performed while either smoking one filtered cigarette (1.2 mg nicotine) per hour, one sachet of snus (1 mg nicotine) per hour or after 2 days of total tobacco abstinence. The steady-state plasma nicotine levels were similar during smoking and use of snus. The insulin and glucose levels were also similar during all three sessions. Smoking, but not use of snus, impaired insulin action, mainly due to a lower peripheral glucose uptake.

3.5.7.3. Conclusion on other effects (experimental studies)

There are very few experimental studies available investigating smokeless tobacco on endpoints other than cancer, cardiovascular effects, reproductive effects, and local effects.

3.5.8. Conclusion on experimental studies

Adducts (covalently bound products) of tobacco-specific nitrosamines (TSNA) to haemoglobin have been detected in users of various STP. TSNA were detected in the saliva of chewers of smokeless tobacco and snuff users. Additional exposure to nitroso-compounds could occur in the oral cavity and in the body due to endogenous nitrosation of secondary and/or tertiary amines from the tobacco, including exposure to nornicotine. Systemic absorption and metabolism of TSNA have been demonstrated in smokeless tobacco users.

There are no current animal models of smokeless tobacco self-administration. Consequently, no literature exists which directly addresses the question of the addiction potential of STP in animals.

Numerous studies in different types of prokaryotic and eukaryotic cells *in vitro* have reported on the mutagenicity and clastogenicity of aqueous and organic extracts of a variety of STP, including Swedish snus and American moist snuff, and various types of American and Indian chewing tobacco.

The majority of animal studies of snuff-associated carcinogenesis are old and the results are difficult to interpret. The experimental groups tended to be small and/or the animal models used were invasive, with tissue trauma possibly confounding the results. Most of the studies with snuff have been negative or equivocal. Studies with snuff inserted into a surgically created canal of the lower lip of the rat do, however, indicate that snuff has a carcinogenic potential in this model. These data, coupled with evidence of genotoxic effects of extracts of moist snuff in various *in vitro* systems, and the presence of carcinogenic nitrosamines in the products, lead to a conclusion that moist snuff is carcinogenic in experimental animals.

Human experimental studies show that smokeless tobacco use leads to short-term increases in blood pressure and heart rate. Snus may cause arterial endothelial dysfunction, other moist snuff products have not been studied with respect to such an effect.

Human experimental studies on volunteers who are regular users of snuff when experimentally exposed to moist snuff at sites not previously used for placement of tobacco, rapidly develop mucosal alterations at new sites of placement.

There are not enough studies available to draw any firm conclusions regarding reproductive toxic effects of smokeless tobacco.

There are very few experimental studies available investigating smokeless tobacco on endpoints other than cancer, cardiovascular effects, reproductive effects, and local effects.

3.6. Adverse Health Effects in Humans

3.6.1. Addiction potential of smokeless tobacco

The dependence liability of nicotine is a function of nicotine dose and speed of delivery. The same general principles apply to Nicotine Replacement Therapy (NRT) products and smokeless tobacco.

3.6.1.1. Levels of nicotine exposure and speed of delivery

Smokeless tobacco

Smokeless tobacco contains and delivers quantities of nicotine comparable to those typically absorbed from cigarette smoking. A dose of Swedish snus typically provides a venous nicotine "boost" of around 15 ng/ml after half an hour, with steady state levels around 35 ng/ml being typical (Holm et al. 1992). These nicotine levels are very similar to those found in cigarette smokers, with the main difference from smoked tobacco being the slightly slower nicotine absorption and the lack of a higher concentration arterial "bolus" that results from nicotine inhalation (Benowitz 1999b). These nicotine levels obtained from snus are about twice as high as the nicotine concentrations typically obtained from nicotine replacement therapy.

Other forms of smokeless tobacco than snus have been shown to produce similar blood nicotine levels, some producing higher peak levels than snus (Fant et al. 1999).

Dry nasal snuff delivers nicotine very rapidly compared to moist snuff (Figure 29) (Russell et al. 1980). Although there is no high-nicotine boli (arterial blood levels) with the use of snuff, as usually observed in smokers, the peak and trough venous blood levels are very similar.

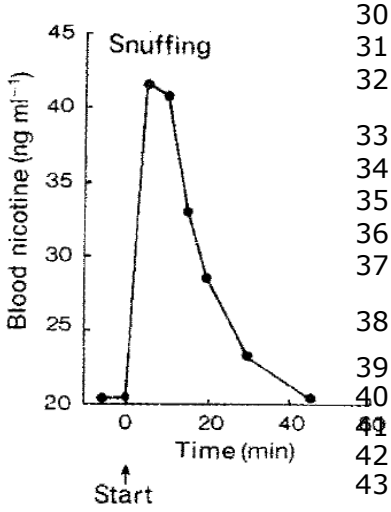

Figure 29. Blood nicotine levels during and after using dry nasal snuff (single pinch used by an experienced user). The subject has been taken snuff before the experiment (last dose about 1 h before), which explains the baseline blood nicotine level of 20.3 ng/ml (Russell et al. 1980) [22].

[22] Reprinted from The Lancet, 1(8166), Russell MA, Jarvis MJ, Feyerabend C, A new age for snuff?, 474-5, © 1980, with permission from Elsevier for English version

Nicotine Replacement Therapy

NRT is available as gum, transdermal patch, nasal spray, inhaler, sublingual tablet and lozenge. NRT has been shown to relieve withdrawal symptoms and improve abstinence rates (Balfour and Fagerström 1996, Fagerström et al. 1993, Fiore et al. 1994, Silagy et al. 2001). However, efficacy of NRT products may be limited by their pharmacokinetic profiles (slow absorption) and by insufficient dosage (Schneider et al. 2001).

Compared to cigarette smoking (the fastest route of delivery of nicotine to the brain), absorption from NRT products is appreciably slower. All existing oral administration formulations (gum, inhaler, tablet, lozenge) have similar absorption profile with a concentration peak (Cmax) around 20 minutes after start of use. Since absorption from nicotine gum is slow and persists even after the chewing stops, adjustments of the dose cannot be as precise as when smoking cigarettes (Benowitz 1988a). Ex-smokers usually chew fewer pieces of gum than they smoke cigarettes. Therefore, plasma nicotine concentrations attained are approximately one-third (with the 2 mg gum) and two-thirds (with the 4 mg gum) of those obtained after smoking (Fagerström 1988). More recent products like nicotine inhaler or nicotine tablet, with similar pharmacokinetic profile as the nicotine gum (buccal absorption) have been developed to improve compliance and to provide alternative administration forms to satisfy individual needs.

Because nicotine is readily absorbed through the skin, transdermal delivery systems (nicotine patches) have been developed for use in smoking cessation therapy. The transdermal system eliminates dosage and compliance problems by producing steady-state levels of nicotine. However, the percentage of nicotine replaced is an important issue; high doses are recommended for highly dependent smokers (Dale et al. 1995). As of yet, the patch's slow release (3 to 8 hour peak) and passive administration does not respond to urges to smoke (Fant et al. 2000).

Absorption of nicotine through the nasal route results in kinetic profiles more similar to absorption from tobacco smoke (Sutherland et al. 1992). The nasal spray is intended to treat highly dependent smokers, even though dosing and compliance problems may occur. Its pharmacokinetic profile, with a peak of 5 to 10 minutes, is closer to smoking, and this property permits a rapid response to urges to smoke (Schneider et al. 1995). Oral forms of NRT and transdermal patches release nicotine more slowly and produce much less reinforcement than smoking does because tolerance develops as nicotine blood levels rise.

3.6.1.2. Addiction potential

As mentioned above (section 3.4.1.1), nicotine absorption through cell membranes is pH dependent. The pH of the smoke of most cigarettes on the market (made of blond flue-cured tobacco) is acidic (pH = 5.5 – 6.0) making buccal absorption very low. Inhalation into the lungs is thus required to allow nicotine to be absorbed by the huge surface of the alveolar capillary interface. From there, nicotine reaches the brain in 9 to 19 seconds, faster than when nicotine is given intravenously (Le Houezec and Benowitz 1991). Considering that the addiction potential of a drug is related to the speed at which it reaches the brain, cigarette smoking is considered to be the highest addictive form of tobacco use. With oral forms of tobacco (smokeless) or nicotine (nicotine replacement therapy like gum, tablet, inhaler) the pH of the product would have to be alkaline in order to allow nicotine to be absorbed from the buccal mucosa (Le Houezec 2003).

Smokeless tobacco

Given the pattern of nicotine absorption described above there can be no doubt that smokeless tobacco is addicting in much the same way as other forms of tobacco consumption. However, considering the speed of nicotine delivery to the brain, one would expect non-inhaled forms of nicotine delivery to be proportionately less addictive than

inhaled tobacco smoke which delivers rapidly nicotine to the brain with each single puff (Henningfield and Kennan 1993, West et al. 2000). Cigarettes also contain additives that maximize the rate of delivery, such as ammonia (which increases the pH of smoke, speeding delivery of free nicotine) and theobromine (which dilates the airways, facilitating inhalation).

Nicotine Replacement Therapy

Because, in contrast with cigarettes, NRT does not produce rapid, high arterial plasma nicotine concentrations and, in contrast with both cigarettes and STP, produces lower blood nicotine levels, its potential for abuse is considered to be low (Hughes 1998). West et al. (2000) compared the abuse liability and dependence potential of nicotine gum, transdermal patch, nasal spray and oral inhaler. The study recruited 504 male and female smokers seeking help with stopping smoking who were randomly allocated to the four products. Measures were taken at the designated quit date, then 1 week, 4 weeks, 12 weeks and 15 weeks later. Smokers were advised to use the product for up to 12 weeks. Those still using the product at the 12-week visit were advised to cease use by week 14. Average ratings of pleasantness were low. The nicotine patch was rated as less unpleasant to use than all other products. There were no significant differences between the products in terms of satisfaction or subjective dependence except at week 15 when no patch users rated themselves as dependent. Continued use of NRT at week 15 was related to rate of delivery of nicotine from the products – 2% for patch, 7% for gum and inhaler, 10% for spray ($P<0.05$ for linear association). The authors conclude that abuse liability from all four NRT products was low. Subjective dependence was moderate and did not differ across products. Behavioural dependence was modest and was positively related to rate of nicotine delivery.

3.6.1.3. Evidence of tolerance

Both acute and chronic tolerances are experienced by smokeless tobacco users (Hatsukami and Severson 1999). The heart rate and blood pressure effects of smokeless tobacco appear to be of the same magnitude as with cigarette smoking (Benowitz et al. 1988b). The decline in heart rate despite persistently high levels of nicotine after smokeless tobacco use indicates rapid and substantial development of acute tolerance to nicotine effects with use of smokeless tobacco, consistent with studies with intravenous exposure to nicotine (Benowitz et al. 1982).

There are no specific studies of chronic tolerance with STP. However, increased use of such products observed over time by individuals indicates chronic tolerance (Riley et al. 1996).

3.6.1.4. Evidence of withdrawal effects

Upon cessation of tobacco products withdrawal symptoms occur. The withdrawal signs and symptoms observed in cigarette smokers as listed in the DSM-IV-TR (American Psychiatric Association 2000) include: (1) irritability, frustration or anger; (2) anxiety; (3) dysphoric or depressed mood; (4) insomnia; (5) restlessness; (6) difficulty of concentrating; (7) decreased heart rate; and (8) increased appetite. These symptoms involve a combination of negative affect, cognitive impairment, and change in appetitive measures. The results from the 1993 Teenage Attitudes and Practice Survey (CDC 1994) on withdrawal symptoms associated with discontinuation of smokeless tobacco are reported by Hatsukami and Severson (1999). Time course and symptoms of withdrawal from smokeless tobacco are similar to those of cigarette smokers with the exception of depressed mood or negative affect. Among daily users, reported withdrawal symptoms were "difficulty of concentrating" (41%), "feeling hungry more often" (39%), "feeling

more irritable" (63%), "strong need/urge to chew" (85%), "feeling restless" (55%), but only 9% reported "feeling sad, blue or depressed." The prevalence of these symptoms was similar to that of daily smokers trying to quit, with the exception of "feeling depressed" reported by 26% of cigarette smokers. In the same paper, Hatsukami and Severson (1999) refer to 3 other studies of the same laboratory reporting similar findings on depressive mood and negative symptoms in STP users.

It seems also that symptoms of withdrawal are stronger with some brands of smokeless tobacco delivering higher levels of nicotine (Tomar et al. 1995). This is in a way confirmed by NRT use which does not produce withdrawal symptoms, with the possible exception of nasal spray or nicotine gum in long-term users, if they stop abruptly.

Nonetheless, there is clear evidence that users of products with snus-like nicotine delivery profiles develop cravings and nicotine withdrawal symptoms when attempting to abstain, and find it difficult to quit (Holm et al. 1992, Fant et al. 1999). As Foulds et al. (2003) state: "While snus probably does not produce stronger nicotine dependence than smoking, it has just minimal, if any, advantages over cigarettes or other smokeless nicotine delivery products in terms of its lower potential to induce dependence. In fact, its high nicotine delivery and hence dependence potential (relative to most other nonsmoked delivery modalities) may be a critical factor enabling it to compete with the more rapidly absorbed nicotine from smoked tobacco."

3.6.1.5. Evidence of behavioural and psychological effects

Little literature exists on behavioural and psychological effects of smokeless tobacco. This is probably due to the dominant position of cigarette smoking in global tobacco consumption. The few studies dealing with these aspects have shown that the effects are similar to cigarette smoking, reflecting that nicotine is the main component that sustains the use of tobacco products (Coffey and Lombardo 1998, Holm et al. 1992).

3.6.1.6. Evidence of difficulty in quitting smokeless tobacco use

Few studies have been realised on smokeless tobacco cessation. The best source of evidence is the Cochrane review from 2004 (Ebbert et al. 2004b). In one trial with bupropion no benefit was detected after six months (Odds Ratio (OR) 1.00, 95% Confidence Interval (CI): 0.23-4.37). Four trials of nicotine patch did not detect a benefit (OR=1.16, 95% CI: 0.88-1.54), nor did two trials of nicotine gum (OR=0.98, 95% CI: 0.59-1.63). Three trials of behavioural interventions showed significant benefits of intervention. In a post-hoc analysis the trials of interventions which included an oral examination and feedback about STP-induced mucosal changes had homogeneous results and when pooled showed a significant benefit (OR=2.41, 95% CI: 1.79-3.24). A more recent pilot study gives some evidence that quit rates may be somewhat higher among STP users than cigarette smokers (Ebbert et al. 2006). In this study, 30 smokeless tobacco users received 4 mg nicotine lozenges for 12 weeks (6 weeks tapering). Although it is difficult to draw firm conclusions due to a lack of direct comparison data, the 7-day point prevalence tobacco abstinence of 47% (95% CI= 28%-66%) at 6 months is higher than abstinence reported in cigarette smokers of 13%-19% in the UK cessation guidelines (West et al. 2000).

The main conclusions are that present pharmacotherapies have not been shown to affect long-term abstinence of smokeless tobacco users, but that larger trials are needed. The main recommendation is to use at least behavioural interventions.

Novel medications recently licensed for use as smoking cessation pharmacotherapies (e.g. varenicline) or medications in development (e.g. nicotine vaccine) have not yet been tested in the context of smokeless tobacco use cessation.

3.6.1.7. Differences between smokeless tobacco products

As presented in chapter 3.3., there are considerable differences between different STP. With cigarette smoking, any brand of cigarettes can provide the user with the desired dosage, so the nicotine intake is determined by the smoking pattern of the user (Henningfield et al. 1995). In contrast, the nicotine dose obtained from a unit ("quid", "dip", "chew" or "pinch") of smokeless tobacco is primarily determined by the product itself and the size of the portion, but not by the pattern of use.

Tomar and Henningfield (1997a) report findings from the FDA's National Forensic Chemistry Center on a dialysis membrane model to study the nicotine delivery of different STP. After 2 minutes the typical dose of 1.5 g of a high-pH product known as a product for experiences users had delivered 12 times more nicotine than the standard 0.5 g pouch-contained dose of a low-pH product that is marketed for novice users. By 10 minutes post-administration, the differential was less than 3 fold.

These data enabled the identification of four levels of available nicotine across the products, with free nicotine estimates in aqueous solutions ranging from 7% to 79%.

3.6.1.8. Conclusion on the addiction potential of smokeless tobacco

When considering the addictive potential of smokeless tobacco the main influencing factors are the dose of nicotine available to the user, and the speed of delivery (depending mainly on the pH of the product). There are considerable differences between products in terms of nicotine delivery, thus the dependence potential of these products vary also widely.

In contrast with NRT, there is clear evidence that smokeless tobacco can induce dependence, since users of smokeless tobacco develop cravings and nicotine withdrawal symptoms when attempting to abstain, and find it difficult to quit. The time course and symptoms of withdrawal from smokeless tobacco are generally similar to those of cigarette smokers although depressive symptoms and negative affect do not appear to be observed among abstinent STP users. The present pharmacotherapies have not been shown to help long-term abstinence, although behavioural interventions may be more effective.

3.6.2. Cancer

3.6.2.1. Oral use of smokeless tobacco products

In the 1985 monograph published by IARC it is stated that "there is sufficient evidence that oral use of snuffs of the types commonly used in North America and Western Europe is carcinogenic to humans". Based on a subsequent re-evaluation in 2004 including more recent studies that comprised additional studies from Scandinavia, the IARC Expert Group concluded that *smokeless tobacco is carcinogenic to humans* (Cogliano et al. 2004, IARC 2007). In that report it is also stated that "there is sufficient evidence that smokeless tobacco causes oral cancer" and that exposure to NNN and NNK is "carcinogenic to humans" (Group 1).

In 2003, the Institute of Environmental Medicine of the Karolinska Institute in cooperation with National Board of Health and Welfare (National Institute of Public Health) and the Department of Medical Epidemiology and Biostatistics conducted a risk evaluation of Swedish and other snuff products based on the newest scientific findings reported and Karolinska Instititute's own research findings. The evaluation included the risk of head and neck cancers, particularly oral cancers. The overall assessment of the experimental and epidemiological evidence indicates that *Swedish snuff is carcinogenic*. (Cnattingius et al. 2005)

All of these studies that were available to IARC (2007) and the Karolinska Institute (Cnattingius et al. 2005) are reported here and commented on, together with more recent studies. These are studies based on different methodological designs, ranging from follow-up studies on Cancer Registry data to case-control studies, case series and case reports. Studies already described in the previous IARC Monograph (IARC 1985) that did not adjust for tobacco smoking are not reported here.

Head and Neck Cancers

A cohort of 10,136 men enrolled in Norway since 1966 has been followed up through 2001 (Boffetta et al. 2005). The cohort is comprised of two samples; one consists of relatives of Norwegian migrants to the United States and the other is a probability sample of the general adult population of Norway selected for the purpose of serving as a control group in a cancer case control study. Information on snuff use and smoking was collected through mailed questionnaires. This study updates a previous report from the same cohort (Heuch et al. 1983). After adjustment for age and smoking the relative risk (RR) associated with ever using snuff was 1.10 (95% CI: 0.50-2.41, 9 exposed cases) for oral/pharyngeal cancer. The relative risks for former and current users were of the same order of magnitude but based on smaller numbers.

A long-term follow-up study was published by Roosaar et al. (2006) who reported on 27-29 years register-based follow-up of 1,115 Swedish snus users with snus-induced lesions (SILs). A total of 3 cases of oral cancer were registered yielding a standardized incidence ratio of 2.3 (95% CI: 0.5-6.7). None of the cancers developed at the site of snus application or SIL. Two of the 3 individuals with cancer were concomitant daily smokers. The authors concluded that while the incidence of oral cancer in this cohort of individuals with SILs tended to be higher than expected, cancers did not occur at the site of the lesion observed in the distant past.

Luo et al. (2007) investigated the association between snus use and cancer in the Swedish construction worker cohort. From 1969 through 1992, preventive health check-ups were offered to all workers in the Swedish building industry. Because of ambiguities in the coding of smoking status for the period 1971–75, the analysis was restricted to workers with at least one visit in the 1978–92 period, when information on smoking and snus use was obtained through personal interviews by nurses. After further exclusion of women, and of men with emigration or cancer before entry, 279 897 men remained for final analysis. Population and health registers were used for follow-up for vital status and cancer incidence. Results were adjusted for smoking or restricted to never-smokers, and adjusted or not for BMI to account for a potential confounder or an intermediate. Compared to never users of any tobacco, relative risks for oral cancer in ever, current and former snus users, and by daily amount of snus consumed were below unity, e.g. ever use RR 0.8 (95% CI: 0.4-1.7).

Roosaar et al. (in press) followed-up a cohort of 9,976 men, who participated in a population-based survey in 1973-74, until January 31, 2002. Outcome was assessed through record-linkages with nationwide registers of demographics, cancer and causes of deaths. Relative risks among exposed relative to unexposed men were estimated using Cox proportional hazards regression and adjusted for smoking and alcohol drinking. A statistically significant increase in the incidence of the combined category of oral and pharyngeal cancer among ever users of snus (11 exposed cases, hazard rate ratio 3.1,

95% confidence interval 1.5-6.6) was observed. Among never smokers the relative risk was 2.3 (5 exposed cases, 95% CI 0.7-8.3).

A case–control study of squamous-cell carcinoma of the head and neck was conducted during 1988-91 in the Stockholm and southern regions of Sweden (Lewin et al. 1998). Cases included cancer in the oral cavity, pharynx, larynx and oesophagus and were identified through the hospital departments. Controls were selected as a stratified random sample from the population registries. The number of identified cases was 605 and the number of selected controls was 705; the participation rates were 90 and 85%, respectively. Of the 605 cases, 128 were oral cavity cancers. Exposure data, including snuff use, were collected by personal interviews. For head and neck cancer, the RR for the whole case group in relation to active snuff use was 1.0 (95% CI: 0.7–1.6), in relation to former snuff use it was 1.2 (95% CI: 0.8–1.9) and for use of > 50 g/week 1.6 (38 cases; 95% CI: 0.9–2.6). Simultaneous adjustment for smoking and alcohol use did not change these estimates materially. In the subgroup of never smokers, the RR in the whole case group for ever users of smokeless tobacco was 4.7 (1.6–13.8), current use was 3.3 (95% CI: 0.8–12.0), while for former use it was 10.5 (9 cases; 95% CI: 1.4–117.8). When the analysis was restricted to cancer in the oral cavity, the RR was 1.0 (0.5–2.2) among current users and 1.8 (0.9–3.7) among former users.

Another study was performed in the northern region of Sweden and comprised cases of oral cancer diagnosed during the period 1980–89 and identified through the Cancer Registry (Schildt et al. 1998). Of the 410 eligible cases, 175 were alive at the time of the study. Controls were matched on age, sex, county and vital status. For each living case, one control was selected from the population registry; for each deceased case, one deceased control was selected from the Cause of Death Registry. Exposure, including use of snuff, was assessed based on a postal questionnaire sent to the living subjects and to the next of kin for the deceased. The response rates were 96 and 91% in cases and controls, respectively. The RR was 0.7 (95% CI: 0.4–1.1) for current snuff users and 1.5 (95% CI: 0.8–2.9) for former snuff users. After restriction to never-smokers, the corresponding RR were 0.7 (95% CI: 0.4–1.2) and 1.8 (95% CI: 0.9–3.5), respectively. The odds ratio in former snuff users increased from 1.5 (95% CI: 0.8–2.9) to 3.0 (95% CI: 0.9–9.4) in an analysis restricted to alive subjects. The RR for ever smoking was 1.1 (95% CI: 0.7–1.6) in an analysis with simultaneous adjustment for snuff and alcohol use.

A further case-control study was conducted in the Southern part of Sweden during 2000-2004 (Rosenquist et al. 2005). Eligible cases of oral and oropharyngeal cancer were identified in the two university hospitals of the region, controls were selected from population registries. Exposure, including use of snuff, was assessed based on an interview administered by the principal investigator, who also performed a detailed investigation of the condition of the oral cavity. Response rate was 80% among cases and 81% among controls; the study included 132 cases and 320 controls. The RR for ever-use of snuff was 0.7 (95% CI: 0.3-1.3). The RR did not vary according to type of snuff (fermented vs. non-fermented), duration of use and time of use per day; the RR for consumption of more than 14 g/day of snuff was 1.7 (95% CI: 0.5-5.7).

From Sweden, Sundstrom et al. (1982) described the clinical features of 23 oral cancers in snuff dipping Swedish males (age range 52-93 years). Their mean age was 76 years. Seventeen of these cancers were described as clinically exophytic and 11 had histologically bulbous invading fronts consistent with verrucous carcinoma. The authors however, did not attempt to classify these 23 oral cancers as squamous or verrucous. All cancers were in the anterior vestibulum where snuff was usually deposited and retained. Nine of these patients also had second primary tumours, oral or in other sites. The 23 cases were retrieved from material collected in a 10 year register study for the years 1962-1971 and where 33 cases were found in a localisation making an association with the placement of snuff. On the other hand, another 39 cases in the same localisation were registered in which no tobacco habit was registered. These latter cases were not

analysed histopathologically. A calculated risk for the development of a snuff induced cancer was 1 case per year in 200,000 users of snuff (Axéll et al. 1978).

Hirsch et al. (2002) reported 8 oral cancer cases in Swedish snuff-dippers. Seven of this series were elderly male and had used snuff for longer than 20 years. Their cancers developed exactly at the location where the snuff was placed mostly on the upper vestibulum. All were pathologically confirmed as squamous cell carcinomas. Zatterstrom et al. (2004) described a further case of well differentiated oral carcinoma in a 90-year old Swedish man who had consumed snuff (snus).

The members of the US Veterans cohort were 293,958 US veterans who served in US Armed Forces during 1917–40, who were aged 31–84 years in 1953, and who held US government life insurance policies in 1953 (Zahm et al. 1992). Most policy holders were men (99.5%) and nearly all were white. The results regarding smokeless tobacco are based on 248,046 (84%) veterans who responded to the 1954 mailed questionnaire or the 1957 questionnaire mailed to 1954 non-respondents. The cohort was followed up for vital status from 1954 (or 1957) through 1980, and follow-up was 96% complete; death certificates were available for 97% of the deceased cohort members and identified 129 oral cancer deaths. The relative risk for oral cancer (ICD-7 140-144) was 3.0 (95% CI: 2.0–4.5) for users of chewing tobacco or snuff and relative risks for infrequent use and for frequent use were 1.9 (95% CI: 1.0–3.5) and 3.4 (95% CI: 2.1–5.6), respectively. The corresponding relative risks for the pharynx were 8.7 (95% CI: 4.1–8.3), 4.5 (95% CI: 1.7–11.7) and 11.2 (95% CI: 5.0–25.0), respectively. For early age at first use, ≤ 14 years of age, the relative risk was 20.7 (95% CI: 8.0–53.7). The results were not adjusted for tobacco smoking or alcohol drinking.

NHANES I was a national probability sample survey of the non-institutionalized US population oversampling the elderly, poor, and women of childbearing age (Accortt et al. 2002). A total of 14,407 adults aged 25–74 years underwent health examinations between 1971 and 1975. Of the participants, 13,861 persons (96%) were successfully traced in at least one of the NHANES I epidemiological follow-up studies (NHEFS) in 1982–84, 1986, 1987 or 1992. Death certificates were available for 98% of the descendents. A random sample (n=3,847) of the cohort was asked about smokeless tobacco use at baseline. In the 1982–84 follow-up information on smokeless tobacco use was obtained to infer baseline behaviour on study participants not in original random sample. Persons were considered smokeless tobacco users if they currently used smokeless tobacco at baseline or had ever used it according to the 1982-84 questionnaire. The analysis was restricted to the 6,805 black and white subjects aged 45 and older with tobacco data available. Two oral cancers were observed in ever users of smokeless tobacco and 1.9 was expected based on US rates. No oral cancers were observed among exclusive users of smokeless tobacco, but only 0.8 were expected.

The cohorts of the American Cancer Society comprised volunteers aged 30 years or older who responded to a mailed questionnaire and resided in a household in which at least one member was 35 years or older (Chao et al. 2002, Henley et al. 2005). The CPS-I cohort included 456,487 men and 594,544 women, the CPS-II included 508,351 men and 676,306 women. At enrollment in 1959 (CPS-I) or 1982 (CPS-II) cohort members were asked about use of smokeless tobacco. For CPS-I vital status was followed-up through 1972; 6.7% were lost to follow-up and follow-up was truncated for logistic reasons in 1965 for another 4.9%. Death certificates were 97% complete and were coded to ICD-7. For CPS-II vital status was followed-up through 1996 (Chao et al. 2002) or 2000 (Henley et al. 2005). Death certificates were 99.8% complete and were coded to ICD-9 (ICD-9 2007). Analyses were restricted to men without prior cancer (except non-melanoma skin cancer) at enrollment. Chao et al. (2002) further restricted the analysis to men with tobacco information (n = 467 788) and Henley et al. (2005) restricted the analysis to men who never used any other tobacco. In the CPS-I cohort the hazard ratio for oral and pharyngeal cancer (ICD-7 140-148) for current users of smokeless tobacco was 2.02 (4 deaths; 95% CI: 0.53–7.74), adjusted for potential confounders such as alcohol consumption and dietary intake. In the CPS-II cohort the multivariate adjusted hazard

ratio for oral and pharyngeal cancers (ICD-9 140-148) was 0.9 (1 death; 95% CI: 0.12–6.71) for current users of smokeless tobacco. There were no deaths among former users of smokeless tobacco.

Henley et al. (2007) also reported on the results of a follow-up of the CPS-II cohort extended to 31 December 2002, when 39.4% of the male cohort members had died. For this analysis the cohort was restricted to 116,395 men who reported being former exclusive cigarette smokers (n=111,952) or who reported currently using spit tobacco and having begun doing so at the time or after they quit exclusive cigarette smoking ("switchers", n= 4443). Further, mortality of men who never used any tobacco product was compared with those of switchers and smokers who quit using tobacco entirely. Multivariate hazard ratios were adjusted for race, educational level, alcohol consumption, level of exercise, aspirin use, body mass index, dietary factors and type of occupation. In addition, the models were adjusted for the number of cigarettes formerly smoked per day, number of years smoked, and age at which they quit smoking. Switchers had a higher death rate from cancers of the oral cavity and pharynx (ICD-9 140-149) than men who quit using tobacco entirely; the multivariate adjusted hazard ratio was 2.56 (7 deaths, 95% CI: 1.15-5.69).

Williams and Horm (1977) conducted a population-based case-control study of the aetiology of cancer at many different sites based on the interview responses of randomly selected incident cases of invasive cancer (n = 7,518; 57% of those selected) from the Third National Cancer Survey (1969-1971). Controls for smoking-related cancer case groups comprised 2102 men and 3464 women with cancers unrelated to smoking. Among men, use of chewing tobacco and snuff was strongly associated with cancer of the gum or mouth, but not with cancer of the lip and tongue or pharynx; controlling for age, race and smoking habits, relative risks were 3.9 (8 cases; $p < 0.01$) for moderate and 6.7 (3 cases; non-significant) for heavy use of chewing tobacco or snuff. Among women, the relative risk for use of chewing tobacco or snuff for cancer of the gum or mouth was 4.9 (2 cases; non-significant).

Winn et al. (1981) conducted a case-control study of the oral cavity and pharyngeal cancers among women in North Carolina. The frequency of oral cancer had been reported to be exceptionally high in white women in South-Eastern USA where the snuff habit was prevalent at the time. A total of 232 women with oral or pharyngeal cancers were included and age-race and region of residence matched 410 controls were included in this case-control study. The relative risk for white women (5 American Indians were included in the group of 544 "whites") who used only oral snuff was 4.2 (95% CI: 2.6-6.7), while the relative risk associated with cigarette smoking among non-users of snuff was 2.9 (95% CI: 1.8-4.7). The relative risk for black woman who used oral snuff but did not smoke was 1.5 (95% CI 0.5-4.8). White women dipped snuff for longer periods and consumed more cans per week than black women. Among black and white hospital cases and controls and for cancer of gum and buccal mucosa, oral snuff-use among non-smokers was related to years of use, with relative risks ranging from 13.8 (95% CI: 1.9-98.0) for 1-24 years, 12.6 (95% CI: 2.7-58.3) for 25-49 years and 47.5 (95% CI: 9.1-249.5) for 50 or more years of use. According to later reports from different sources, the product used by many women was locally grown dry snuff as cited at the IARC report (2007).

Stockwell and Lyman (1986) ascertained cases and controls from the state of Florida, population-based cancer registry over a one year period in 1982. Cases were persons with incident cancers of the lip, tongue, salivary glands, gum, floor of mouth, other parts of mouth, oropharynx, hypopharynx, pharynx (unspecified), and nasopharynx (ICD-9 140-149). All cancers of the colon, rectum, cutaneous melanoma, endocrine neoplasias from the same source during same time period formed the control group. Data on tobacco use were obtained from clinical and registry records. For 79% of the 2,351 study subjects data on tobacco use were available (82% of cases and 78% of controls). Odds ratios for STP by anatomic site are tongue 2.3 (95% CI: 0.2–12.9), salivary gland 5.3

(95% CI: 1.2–23.4), mouth and gum 11.2 (95% CI: 4.1–30.7), pharynx 4.1 (95% CI: 0.9–18.0), nasopharynx 5.3 (95% CI: 0.7–41.6), adjusted for age, sex, race and tobacco use. A limitation of this study is that information about tobacco use was obtained from medical records. It seems unlikely that all hospitals in Florida captured this information uniformly and it is possible that clinicians may have been more careful in obtaining medical record information from persons with these head and neck cancers compared to patients with other forms of cancer.

The population-based case–control study of Blot et al. (1988) drew study subjects from cancer registries in New Jersey, Atlanta metropolitan area, Santa Clara and San Mateo counties, and Los Angeles. Cases included all black and white persons aged 18–79 years with incident, pathologically confirmed cancer (coded ICD-9 141-149), excluding cancer of the salivary gland (ICD-9 142) and cancer of the nasopharynx (ICD-9 147), from January 1, 1984 through March 31, 1985. Random digit dialling (RDD) was used to ascertain controls aged 64 years or younger, and Health Care Financing Administration (HCFA) for controls aged 65 years and older, frequency matched on age, sex and race to the case distribution. Structured questionnaires were administered by trained interviewers in homes and next-of-kin were used in 22% of cases and 2% of controls. The response rate was 75 and 76% in cases and controls, respectively and a total of 1,114 cases and 1,268 controls were included in the analysis. Among males 6% of 762 cases and 7% of 837 controls used STP, mostly chewing tobacco. Nearly all tobacco chewers were smokers. Among females 3% of 352 cases and 1% of 431 controls, used snuff, (OR=3.44). Among non-smoking women, the OR for snuff was 6.2 (95% CI: 1.9–19.8), based on 6 snuff using cases and 4 snuff using controls. Non-smoking women primarily used snuff rather than chewing tobacco. All six cases had oral cavity cancer.

Spitz et al. (1988) identified cases with histologically confirmed squamous cell carcinoma of the tongue, floor of the mouth, oral cavity, orohypopharynx and larynx in white US residents, at the MD Anderson Hospital, Houston, TX, from January 1985 through February 1987. Laryngeal cancer accounted for 38% of the 131 male cases. Controls were patients at MD Anderson Hospital from the same time period, randomly selected, and frequency matched on age (± 5 years) and sex, excluding patients with squamous-cell carcinoma of any site. There were 185 cases (131 men and 54 women) and 185 controls aged 29–95 years. Self-administered questionnaires were part of the registration procedure. The authors reported that there was 'no difference in distribution of sites of malignancy for snuff users compared to all other cases'. Among men, the crude odds ratio for chewing tobacco was 1.0. For females, the odds ratio for snuff use was 3.4 (95% CI: 1.0–10.9). There was no adjustment for smoking. All 9 snuff dipping cases drank alcohol, 7 also chewed tobacco, 8 smoked cigarettes, and 1 smoked cigars and pipes. 3 of 4 snuff dipping controls also smoked cigarettes.

Newly diagnosed cases were identified from three hospitals in Sao Paolo, Curitiba and Goiânia, Brazil, and comprised carcinomas of the tongue, gum, floor of mouth, and other oral cavity (ICD-9 141, 143-145) diagnosed from February 1, 1986–June 30, 1988 (Franco et al. 1989). Two controls per case were identified from same or neighbouring general hospitals, individually matched on sex, 5-year age group, trimester of hospital admission, and excluding neoplasms or mental disorder diagnoses. Cases were interviewed using a structured questionnaire in hospital, controls in a private place. 4% of 232 cases and 3% of 464 controls used smokeless tobacco. The authors reported that use of smokeless tobacco and oral cancer was 'not associated'. The crude odds ratio was 1.4. They noted that the relative risk estimates were independent of tobacco smoking or alcohol drinking, sex or anatomical site. The data on how adjustment was done for these factors were not shown and confidence intervals or statistical significance were not reported.

The population-based case–control study by Maden et al. (1992) drew study subjects from three urban counties of western Washington state. Cases were men aged 18–65 years with in-situ and invasive squamous cell cancers of the lip, tongue, gum, floor of mouth, unspecified mouth and oropharynx diagnosed during 1985–89. Random digit

dialling-ascertained controls were frequency matched to cases on age (5 year groups), gender and year of diagnosis. 131 cases (54.4%) and 136 controls (63%) completed in-person questionnaire interview in home or elsewhere. 15% of 131 cases used smokeless tobacco in contrast to 4% of 136 controls and the age-adjusted OR was 4.5 (95% CI: 1.5–14.3). Smoking was not controlled for.

Histologically confirmed oral and pharyngeal cancers (including cancers of the tongue, floor of the mouth, oropharynx and hypopharynx) were identified in one study (Marshall et al. 1992) from 20 hospitals in three New York counties during the period 1975–83. Cases of black ethnicity were excluded. Cases were individually matched on neighbourhood, age (± 5 years), and sex with replacement. Of 513 cases contacted, 290 (56%) participated; there were 290 controls. The authors noted that 'there was a risk associated with chewing tobacco, but it was insignificant, with very few people exposed'. The data to support this statement were not shown.

A cross-sectional study (Sterling et al. 1992) used two nationally representative surveys to examine the relationship between smokeless tobacco use and cancer of the oral cavity: the 1986 National Mortality Follow-back Survey and the 1987 NHIS. The 1986 National Mortality Follow-back Survey was based on a stratified probability sample of 18,733 decedents in 1986 who were 25 years or older at time of death. A questionnaire sent to their next of kin also included questions on use of smokeless tobacco. Information was obtained for 16,598 decedents. The NHIS annually surveys samples of the non-institutionalized civilian population using a multistage, probability sampling design. Interviewers administered a questionnaire to sample persons in the household. The 1987 NHIS obtained data on the use of smokeless tobacco. Using a reference category of less than 100 times lifetime use of smokeless tobacco, the relative risks for cancers of the oral cavity and pharynx (ICD-9 140–149) for 100–9999 and 10,000 or more lifetime use were 0.9 (95% CI, 0.3–3.4) and 1.2 (95% CI, 0.3–4.6), respectively, adjusted for sex, race, smoking, alcoholic beverage consumption and occupational group.

Mashberg et al. (1993) identified 359 cases in a Veterans hospital in New Jersey during 1972–83. Included among the cases were black or white men with in-situ or invasive squamous-cell carcinoma of the oral cavity or oropharynx. 2,280 patients from the same series of clinical examinations without cancer or dysplasia of the pharynx, larynx, lung or oesophagus served as controls and controls were recruited and interviewed in hospital between 1977 and 1982. 94% of study subjects were enrolled. Only 52 cases and 255 controls ever used smokeless tobacco. Chewing tobacco (OR=1.0, 95% CI: 0.7–1.4) and snuff (OR=0.8, 95% CI: 0.4–1.9) were not associated with oral cancer. No trend by duration of tobacco chewing was observed.

Spitz et al. (1993) identified 108 cases of white race, with histologically confirmed cancers of the oral cavity (44), pharynx (31) and larynx (33) at MD Anderson Hospital, Houston, TX from June 1987 to June 1991. Controls were ascertained from blood and platelet donors and were frequency matched to cases by age (± 5 years), race and sex, with no cancer history. Patients completed a self-administered questionnaire in the hospital. The odds ratio for chewing tobacco was 1.2. Smoking was not controlled for.

Kabat et al. (1994) ascertained cases from 28 US hospitals in 8 cities. Cases had histologically confirmed cancers of the tongue, floor of mouth, gums, gingiva, buccal mucosa, palate, retromolar area, tonsil, and other pharynx during the time period 1977–90. Controls were individually matched to cases on hospital, admission within 2 months after case's admission, age, sex and race, and excluded persons with diseases thought to be associated with tobacco or alcohol or prior history of tobacco-related cancers. The conditions among the controls were: 50% cancers (also including cancer of the stomach, endometrium and leukaemia), 7% benign neoplams, and 43% other. There were 1560 cases and 2948 controls. In hospital questionnaire interviews were conducted with the study subjects. Among men, 6.1% of 1097 cases and 5.1% of 2075 controls chewed tobacco. Among women, less than 2% of 1336 subjects chewed tobacco. Among never-smoking men, 4.9% of 82 cases were regular chewers as were 2.2% of 448 controls, yielding an odds ratio of 2.3 (0.7–7.3). Among never-smoking women, there were no

tobacco chewers. Among never smoking women, 3.5% of 113 used snuff in contrast to 0% of 470 controls, OR=34.5 (8.5–140.1).Among never smoking men, 0% of 82 cases and 0.9% of 444 controls were users. The estimate of the odds ratio of 34.5 used 0.5 snuff-using controls.

Hospitals in Illinois, Michigan, New York and Philadelphia were the source of patients aged 21–80 years diagnosed with histologically confirmed cancer of oral cavity and pharynx (ICD-9 141, 143-146, 148, 149) between 1981 and 1990 (Muscat et al. 1996). Hospital patients with conditions unrelated to tobacco use were matched to cases by sex, age (± 5 years), race, date of admission (± 3 months). Response rates were 91% of cases and 97% of controls yielding 1,009 cases (687 men, 322 women) and 923 controls (619 men, 304 women). A questionnaire interview was conducted with cases and controls. Among men, 5.5% of 687 cases used chewing tobacco at least once a week for one year or more as did 5.3% of 619 controls (crude OR=1.04). No females used chewing tobacco. Among men, 1.3% of cases and 1.6% of controls used snuff at least once a week for one or more years (crude OR=0.81). For women, the crude odds ratio for snuff use was 1.9.

Muscat et al. (1998) reported a hospital-based case-control study on salivary gland cancer. 128 patients with newly diagnosed histologically confirmed salivary gland cancer and 114 age- and gender-matched controls were interviewed. One case reported using snuff, and three cases and three controls were tobacco chewers.

Seattle area counties, WA, were the sources of study subjects for the population-based case–control study by Schwartz et al. (1998) of in-situ and invasive (92%) squamous-cell cancers of the tongue, gum, floor of mouth, unspecified mouth, tonsils, and oropharynx, in persons aged 18–65 years during 1990–95. Controls were ascertained by random digit dialling and frequency matched to the case distribution on sex and age in a 3:2 ratio controls to cases. 284 cases (165 men, 119 women) and 477 controls (302 men, 175 women) completed an in-person questionnaire interview; response rates among cases and controls were 63.3% and 60.9%, respectively. Among men, 6.7% of 165 cases and 5.6% of 302 controls used smokeless tobacco (OR=1.0; 95% CI: 0.4–2.3). Only one female control used smokeless tobacco. Smoking was not controlled for.

From the US, McGuirt (1983) described a series of 76 oral cancers who were all STP users. In this series 57 patients reported exclusive snuff use. Females were predominant (1:3). Common lesion sites were alveolar ridge (32%) and buccal cavity (47%). 80% of the tumours were located where smokeless tobacco was traditionally held — between the cheek and the gum. Only one non-squamous cell cancer was observed (Wray and McGuirt 1993).

McGuirt and Wray (1993) also described the clinical profile of 116 patients with oral cavity cancer who were exclusive users of smokeless tobacco with no exposure to smoked tobacco or alcohol. The average age of the case-series was 78.4 years and average period of consumption was 55.5 years. Females were predominant (1:23 male to female ratio). A second primary tumour developed in the oral cavity of 18% (21/116) suggesting field cancerization. 45 out of 91 who were followed up died of or with cancer.

In south Asia where oral cancer incidence is high STP use is commonly reported. Tobacco is often mixed with areca nut, considered itself a carcinogen (IARC 2004b). Only studies that have reported separate results for oral use of smokeless tobacco without betel quid are reviewed here. For slaked lime, which was used in conjunction with tobacco in some of the studies in Asia, there is evidence suggesting lack of carcinogenicity in experimental animals (IARC 2004b).

Chandra (1962) selected 450 cases of cancer of the buccal mucosa registered in a hospital in Calcutta, India, during 1955-1959, and used 500 of the friends or relatives who came to hospital with the patients as controls. Cases and controls were approximately age matched. Tobacco chewing was reported by 6.3% of 287 cases and 4.2% of 410 controls among men and 3.1% of 163 cases and 2.2% of 90 controls among

women. Relative risks for tobacco chewing compared to no chewing or smoking were 2.7 for males and 2.5 for females. The author did not clarify whether the chewing habit was tobacco only or tobacco plus lime.

A population-based prospective study was reported by Wahi (1968) from a temporary cancer-registration system established in Uttar Pradesh (Mainpuri district). Over a period of 30 months (1964–66), a total of 346 oral - and oropharyngeal cancer cases were detected and confirmed. Exposure data were obtained by questioning these patients, and a house-to-house interview survey was conducted on a 10% cluster sample of the district population. The numbers in various exposure categories were then extrapolated to the population as a whole and used as denominators for calculating oral cancer 'period prevalence rates' for different types of chewing habits. Prevalence rates among non-chewers of tobacco and chewers of Pattiwala (sun-cured tobacco leaf ± lime) were 0.36/1000 and 1.17/1000 (based on 84 exposed cases), respectively. The differences in age between cancer patients and the population sample do not seem to have been taken into account; and it is possible that the prevalence of habits within the population was age-dependent.

Jafarey et al. (1977) reported a hospital-based case-control study in Pakistan. The cases were 1192 histologically-diagnosed oral-cavity and oropharyngeal cancers. The 3,562 controls were matched for age, sex and place of birth. Among men, 4% of 683 cases and 3% of 1978 controls, and among women, 7.7% of 509 cases and 3% of 1,584 controls chewed tobacco, giving relative risks of 10.4 and 13.7, respectively, compared to those who neither chewed nor smoked. In view of other publications by the same authors, it is likely that products chewed were tobacco and lime. Eighty-four patients and 114 controls used naswar (tobacco, slaked lime and indigo) and 88 patients and 1,690 controls had no tobacco habit. The relative risk associated with naswar use was 14.2. Potential confounding due to other tobacco-related habits was not adjusted for.

Goud et al. (1990) reported a case–control study with 102 oral cancer cases from a hospital in Varanesi and an equal number of age- and sex-matched controls selected from general and surgical wards. The odds ratios were 2.1 for *khaini* use, 3.7 for *zarda* use and 2.8 for *khaini* plus *zarda*. It was not clear whether *khaini* and *zarda* were chewed by themselves or in some cases as an ingredient of betel quid. There was no mention of control for smoking.

Wasnik et al. (1998) reported a matched case–control study with 123 cases of histologically confirmed 'oropharyngeal' cancers (ICD codes not specified - probably included oral and pharyngeal cancers) selected from three hospitals in Nagpur, India. There were two control groups: one of 123 non-cancer patients and another of 123 patients with cancer of other sites (not specified). Controls were matched for age and sex. There were 24 cases which were tobacco chewers (excluding those who chewed betel quid) and 33 cases which reported using tobacco containing material for cleaning teeth. These may include betel-quid chewers. Unadjusted odds ratios for the two control groups were 11.4 (24 cases; 95% CI: 4.4–29.6) and 23.7 (95% CI: 7.7–72.4) for chewing tobacco without betel quid and 4.1 (33 cases; 95% CI: 2.0–8.7) and 8.7 (95% CI: 3.3–22.9) for using tobacco containing material for cleaning teeth. In a multivariate analysis, tobacco chewing (19.5% of cases) was combined with betel-quid chewing (63.4% of cases) and the odds ratio was 8.0 (95% CI: 4.9–14.8) when smoking, alcohol consumption, occupation and the use of tobacco containing cleaning material were included in an unconditional logistic regression model. In the same model, the odds ratio for using tobacco containing material for teeth cleaning was 5.2 (95% CI: 2.5–11.8).

Merchant et al. (2000) conducted a case–control study with 79 histologically confirmed primary oral squamous-cell carcinomas from three hospitals in Karachi, Pakistan. The 149 controls were selected from orthopaedic and general surgical wards, had no history of malignancy and were individually matched on hospital, sex and age (± 5 years). Ever

use of *naswar* was reported by 13 cases and 10 controls, giving an odds ratio (adjusted for cigarette smoking and alcohol use) of 9.5 (13 cases; 95% CI: 1.7–52.5).

Toombak dipping - a form of snuff used in the Sudan - is implicated as a toxic product causing oral cancer (Elbeshir et al. 1989, Idris et al. 1995). Idris et al. (1995) documented 646 squamous cell carcinomas of the oral cavity from the Sudan. In this series 375 neoplasms were at the primary site of toombak application (lip, buccal, floor of mouth). Toombak use was more common in people with cancers of lip, buccal or floor of mouth compared with other oral sites (58% vs 19%). 5-10% of the cases were under 30 years of age.

Using the same data, Idris et al. (1995) investigated the association between use of toombak and carcinoma of the oral cavity in a case–control study. Squamous-cell carcinomas at sites with direct contact or with less or no contact were defined as case group 1 or case group 2, respectively and the non-squamous cell cancers served as control group 1. In addition, a second control group consisting of 2,820 volunteers attending oral health education programs in various regions of Sudan was recruited. For the first case group and compared to never users of toombak, the odds ratios adjusted for age, sex, tribe and area of residence for *toombak* use were 7.3 (218 cases; 95% CI: 4.3–12.4) and 3.9 (95% CI: 2.9–5.3) for hospital and volunteer controls, respectively. Among users of toombak for >11 years, the corresponding odds ratios were 11.0 (120 cases; 95% CI: 4.8–25.1) and 4.3 (95% CI: 2.9–6.3), respectively. Corresponding odds ratios for the second case group were moderately and statistically non-significantly increased compared to hospital controls and not increased compared to the control group of volunteers.

Shammah (alshammah), sometimes known as Yemeni snuff, is a smokeless tobacco product that is usually held between the cheek and gum (gingiva). Several descriptive studies have implicated shammah as a risk factor for oral cancer (Ibrahim et al. 1986, Al-Idrissi 1990, Allard et al. 1999).

Nass use and associated oral cancers are reported in descriptive studies from Uzbekistan or Uzbecks living in Central Asia and Pakistan (Aleksandrova 1970, Nugmanov and Baimakanov 1970, Zaridze et al. 1985).

Oesophageal cancer

The previously described cohort study by Boffetta et al. (2005) reported a RR of oesophageal cancer of 1.4 (95% CI: 0.61–3.24, 9 exposed cases) comparing ever snuff use to never snuff use.

Zendehdel et al (2008) linked 343,822 male construction workers identified via an ad-hoc health surveillance system which provided information on tobacco smoking and snuff use to several Swedish nationwide registers and followed them for cancer incidence from 1971 up to 2004 (see Luo et al 2007, in head and neck cancer section). Relative risks were estimated using multivariate Cox proportional regression models. Among never-smoking snuff users excess risks for esophageal squamous cell carcinoma (10 exposed cases, RR=3.5, 95% CI 1.6-7.6) and noncardia stomach cancer (68 exposed cases, RR = 1.4, 95% CI 1.1-1.9) were observed. The results are not adjusted for alcohol consumption. However, this cannot explain the elevated risks. No increase in risk was observed for adenocarcinoma of the esophagus and cardia stomach cancer.

The previously described case-control study from Stockholm and southern regions of Sweden reported results separately for oesophageal cancer (Lewin et al. 1998). The RR for ever versus never use of snuff was 1.2 (95% CI: 0.7–2.2) after adjustment for age, smoking, and alcohol intake.

All patients with a new diagnosis of adenocarcinoma of the oesophagus or gastric cardia and half of the patients with oesophageal squamous-cell carcinoma occurring in Sweden

during 1995-1997 were included in a population-based study (Lagergren et al. 2000). Cases were identified from all clinical departments in Sweden involved in the treatment of these diseases; controls were randomly selected from the study population with frequency matching for age and sex. Exposure data were collected through face-to-face interviews. For oesophageal adenocarcinoma, the participation rate was 87% and the number of cases was 189; for gastric cardia cancer, the rate was 83% and the number of cases 262; for oesophageous squamous-cell carcinoma, the participation rate was 73% and the number of participating cases was 167. The participation rate among controls was 73% and the number participating in the study was 820. For gastric cardia adenocarcinoma, no association with snuff use was seen. For oesophageal adenocarcinoma, snuff users had a relative risk of 1.2 (95% CI: 0.8–1.9) compared with never users. However, for those with more than 25 years of use, the adjusted relative risk was 1.9 (95% CI: 0.9–4.0). For oesophageal squamous-cell carcinoma, the relative risk was 1.4 (95% CI: 0.9–2.3) when ever users were compared with never users. Again for those with more than 25 years of use, the relative risk was 2.8 (95% CI: 1.4–5.4).

The case–control study by Williams and Horm (1977) (described in the section on oral cancer) also reported on oesophageal cancer. Among men, the relative risk for moderate use of chewing tobacco or snuff based on two exposed cases was 0.9, adjusted for age, race and smoking.

Oesophageal cancer cases, primarily (85%) squamous cell carcinomas, ascertained from 1982–84 in selected hospitals in South Carolina were matched with a ratio of two hospital controls per case by hospital, race and age (± 5 years). Also, oesophageal cancer deaths among men who were residents of eight coastal counties of South Carolina were identified from 1977–81 and matched by race, age, county of residence and year of death to decedents dying of other causes. Controls with diagnosis at admission or cause of death related to alcohol or diet were excluded. A total of 207 cases and 422 controls were included in the study. Users of smokeless tobacco were defined as those having used at least one pouch or plug of chewing tobacco or a small can of snuff per week for at least one year. Relative to non-tobacco users, the odds ratio for smokeless tobacco-only users was 1.7, and 1.2 (95% CI: 0.1–13.3) when adjusting for study series and alcohol (Brown et al. 1988).

A hospital-based case–control study was carried out in Assam, India, from 1997 to 1998, recruiting 502 (358 men, 144 women) histologically confirmed cases of oesophageal cancer, predominantly squamous-cell carcinomas, and two visitor controls per case group-matched for age and sex. The odds ratio for developing oesophageal cancer associated with use of dried tobacco leaf alone (locally known as *Chada*) among non-smokers compared to non-chewers (after adjusting for alcohol consumption) was 3.2 (95% CI: 1.6–9.5) and 6.2 (95% CI: 2.4–12.1), for men and women, respectively. Similarly, risk of oesophageal cancer for *Chada* users compared with non-chewer, among non-alcohol drinkers (after adjusting for smoking) was 3.8 (95% CI: 1.9–8.5) among men and 5.8 (95% CI: 2.1–12.4) among women (Phukan et al. 2001).

Stomach cancer

In the cohort study from Norway described above, the RR of stomach cancer for ever use of snuff was 1.11 (95% CI: 0.83–1.48; 74 exposed cases) (Boffetta et al. 2005).

One study on gastric cancer was conducted in five different counties in the central and northern Sweden (Hansson et al. 1994, Ye et al. 1999). Eligible cases were all patients with newly diagnosed and histologically confirmed gastric cancers during 1989–95, and were ascertained via departments of surgery and pathology supplemented by record linkages to the cancer registry. The gastric cancers were divided into gastric cardia or distal stomach cancer. About two controls per case were selected from the population registry with stratification for age and sex. Face-to-face interviews were performed by specially trained personnel. The participation rates were 62 and 76% in cases and controls, respectively; the majority of the non-participants among the cases had died

prior to the interview. For cardia cancer, the RR for current snuff use was 0.5 (95% CI: 0.2–1.1) and that for former use was 0.8 (95% CI: 0.3–1.9). For distal stomach cancer, the RR for current use were 0.8 (95% CI: 0.5–1.3) for the intestinal type and 0.6 (95% CI: 0.3–1.2) for the diffuse type. After restriction to never smokers and after combining all sites, the RR for ever using snuff was 0.5 (95% CI: 0.2–1.2).

The Lutheran Brotherhood Insurance Society (LBS) cohort consists of 17,818 (68.5%) of 26,030 white male policy holders, who responded to a mailed questionnaire in 1966. Cohort members were 30 years of age or older and lived in California, upper Midwest or Northeastern USA. After 20 years of vital status follow-up in 1986, 4,027 (23%) persons were lost to follow-up. At 11.5 years of follow-up, respondents, non-respondents and respondents lost to follow-up did not differ significantly with respect to demographic variables (Kneller et al. 1991). Relative to men who had never used tobacco, the relative risk for smokeless tobacco users was 2.3 (18 deaths; 95% CI: 0.98–5.22). Stratification by pack-years of smoking yielded relative risks of 1.6 (95% CI: 0.58–4.50). Among non-smokers who used ST, the relative risk was 3.8 (3 deaths; 95% CI: 1.00–14.32).

Among men of the CPS-II cohort, and relative to never having used any type of tobacco, the relative risk of stomach cancer among current users of only smokeless tobacco was 1.58 (8 deaths; 95% CI: 0.76–3.28) adjusting for age, race, education, family history of stomach cancer, consumption of high-fiber grain foods, vegetables, citrus fruits or juices, use of vitamin C, multivitamins, and aspirin. For former users of only ST, the relative risk was 1.11 (95% CI: 0.27–4.50) (Chao et al. 2002).

The case–control study by Williams and Horm (1977) (described in the section on oral cancer) also reported on stomach cancer. Among men, the relative risks for stomach cancer and for moderate or heavy use of chewing tobacco or snuff were 1.0 (6 cases) and 1.7 (6 cases), respectively, adjusted for age, race and smoking.

Pancreatic cancer

In the cohort study from Norway described above, the RR of pancreatic cancer for ever use of snuff was 1.67 (95% CI: 1.12–2.50, 45 exposed cases); similar results were obtained for former and current use (Boffetta et al. 2005). After stratification on smoking, it appeared that the excess risk was mainly confined to current smokers, but the never smokers were few.

In the Swedish construction worker cohort (Luo et al. 2007) and compared to never users of any tobacco, relative risks for pancreatic cancer in ever, current and former snus users were 2.0 (95% CI: 1.2-3.3), 2.1 (95% CI: 1.2-3.6), and 1.4 (95% CI: 0.4-5.9), respectively. The trend by amount of snus consumed/day was statistically significant (>10g/day RR 2.1 (95% CI: 1.1-3.8)).

In the Lutheran Brotherhood cohort, white men aged 35 years and older were followed for vital status for 20 years (Zheng et al. 1993). There were 57 deaths due to pancreatic cancer during the 20-year follow-up period. Diet was assessed by food frequency questionnaires addressing current consumption. Since dietary factors were one of the research hypotheses, 1,656 cohort members (including three pancreatic cancer deaths) who were on a special diet at the time of data collection were excluded from the analysis. The relative risk for ever users of smokeless tobacco was 1.7 (16 deaths; 95% CI: 0.9–3.1), adjusted for age, alcohol and smoking.

The case–control study by Williams and Horm (1977) (described in the section on oral cancer) also reported on pancreatic cancer. Among men, the relative risks for cancer of the pancreas and for moderate or heavy use of chewing tobacco or snuff were 0.3 (two cases) and 0.3 (one case), respectively, adjusted for age, race and smoking.

A population-based study included married men newly diagnosed with pancreatic cancer in the Seattle area and population-based controls frequency matched on age (Farrow and Davis 1990). A telephone interview with the wives was conducted between 2 and 4.5

years after diagnosis. Complete information was available for 148 cases and 188 controls. The odds ratio for chewing tobacco was 0.8 (overall prevalence, 6.9%) with a confidence interval that included 1.0 Smoking was not controlled for.

Muscat et al. (1997) conducted a hospital-based study in New York, Pennsylvania, Michigan and Illinois, USA. Interviews were conducted in the hospital. Of the 949 cases aged 20–81 years ascertained between 1985 and 1993 and the 1,526 eligible controls, 484 cases and 949 controls were interviewed. The controls did not have tobacco-related diseases, and were individually matched to cases on hospital, sex, age, race, and year of diagnosis. The major reasons for non-interviews were that the patient was too ill or unable to communicate. Relative to never smokers and long-term quitters (≥ 20 years), the odds ratio for tobacco chewers who were not current cigarette smokers was 3.6 (95% CI: 1.0-12.8).

In a large population-based case–control study in the Atlanta area, Detroit and New Jersey, USA, lifelong non-smokers of cigarettes were examined (Alguacil and Silverman 2004). Cases were incident cases of carcinoma of the exocrine pancreas. 41% of the cases died before interview, but response rates for the surviving cases and controls were 75% or better. Random digit dialling controls and HCFA controls were frequency matched to the cases on age, race, sex, and study site. Persons were considered snuff users if they ever used snuff, whereas tobacco chewers were defined as those who used one pouch or plug per week for at least 6 months. Relative to non-users of tobacco, the odds ratio for having ever used smokeless tobacco was 1.4 (95% CI: 0.5–3.6), and for having used smokeless tobacco only, 1.1 (95% CI: 0.4–3.1), adjusted for race, sex, geographic site, cigar smoking and age. In a statistical model with cigars, chewing tobacco and snuff and pancreatic cancer as the outcome the odds ratios were 1.7 (95% CI: 0.6-4.5) for chewing tobacco and 1.1 (95% CI: 0.4-3.5) for snuff. Dose–response relationships were evaluated and adjusted for age, sex, race, cigar smoking and geographical region. Users of 2.5 oz or less per week of smokeless tobacco had an odds ratio of 0.3 (95% CI: 0.04-2.5) whereas for users of more than 2.5 oz, the odds ratio was 3.5 (95% CI: 1.1–10.6; *p* for trend = 0.04). For 20 years or less of smokeless tobacco use, the odds ratio was 1.1 (95% CI: 0.1-11.0), and for more than 20 years, 1.5 (95% CI: 0.6–4.0; *p* trend = 0.42). Tobacco chewers used more ounces of tobacco per week than users of snuff (7.2 versus 2.4 oz).

Hassan et al. (2007) conducted a hospital-based study including 808 patients with pancreatic adenocarcinoma and a control group of 808 healthy individuals enrolled prospectively at the University of Texas, M. D. Anderson Cancer Center between 2000 and 2006. Cases were newly diagnosed with pathologically confirmed pancreatic adenocarcinoma. Controls were selected from visitors who accompanied cancer patients who had no past history of cancer and were genetically unrelated family members (usually spouses) of patients with cancers other than those of the pancreas, gastrointestinal system, or smoking-related cancers (lung and head and neck). Controls were frequency-matched to cases by age, race/ethnicity, and sex. Results were reported separately for chewing tobacco and snuff. There was no association (all OR statistically non-significantly below unity) between use of smokeless tobacco (ever, low or moderate, high intake) among cigarettes smokers or non-cigarette smokers, adjusted for age, sex, race/ethnicity, diabetes, alcohol consumption and other variables The response rate was not reported, a relatively weak association of tobacco smoking with pancreatic cancer was noted.

For interpretation of the studies on smokeless tobacco use and pancreatic cancer it is important to note that a recent IARC Monographs Working Group concluded that there is inadequate evidence for an association between alcohol consumption and pancreatic cancer (Baan et al. 2007). Even if there was an association between alcohol consumption and pancreatic cancer, this cannot explain the association between smokeless tobacco consumption and pancreatic cancer.

Lung Cancer

In the Norwegian cohort study, the relative risk for lung cancer was 0.80 (72 cases; 95% CI: 0.61–1.05) comparing ever users of smokeless tobacco to never users and adjusting for age and smoking. Results were similar for ever or current users of smokeless tobacco and when stratifying by smoking status (Boffetta et al. 2005).

In the Swedish construction worker cohort (Luo et al. 2007) and compared to never users of any tobacco, relative risks for lung cancer in ever, current and former snus users were 0.8 (95% CI: 0.5-1.3), 0.8 (95% CI: 0.4-1.3), and 0.9 (95% CI: 0.3-3.0), respectively.

Lung cancer deaths were examined in the NHANES I follow-up study (Accortt et al. 2002). In the multivariate analysis and relative to non-tobacco users, the hazard ratio for women using only smokeless tobacco was 9.1 (3 deaths; 95% CI: 1.1–75.4), adjusting for age, race, poverty index ratio, region of residence, alcohol, recreational physical exercise and fruit/vegetable intake. There were no deaths from lung cancer among men using smokeless tobacco only.

In the CPS-I cohort, the hazard ratio for lung cancer for current smokeless tobacco users who never used other tobacco products was 1.08 (18 deaths; 95% CI: 0.64–1.83) after adjustment for age, race, educational level, body mass index, exercise, alcohol consumption, fat consumption, fruit/vegetable intake and aspirin use (Henley et al. 2005). In the CPS-II cohort, compared with never users, the hazard ratio for men who reported current use of smokeless tobacco but never used any other tobacco products was 2.00 (18 deaths; 95% CI: 1.23–3.24) adjusted for the same variables and employment status and type. The hazard ratios were similar for those who chewed but never used snuff and those who used snuff but never chewed.

In the extended follow-up of the CPS-II cohort, Henley et al. (2007) compared lung cancer mortality of former exclusive cigarette smokers with switchers who reported currently using spit tobacco and having begun doing so at the time or after they quit exclusive cigarette smoking. Compared to those who quit entirely, the relative risks for lung cancer among all switchers, switchers to chew only, snuff only and chew and snuff combined were 1.46 (95% CI: 1.24-1.73), 1.34 (95% CI: 1.10-1.64), 1.75 (95% CI: 1.22-2.50) and 1.87 (95% CI: 1.21-2.87), respectively. Compared to men who never used any tobacco products the relative risks of lung cancer among those who quit tobacco use entirely and among switchers were 3.81 and 5.61, respectively.

The case–control study by Williams and Horm (1977) (described in the section on oral cancer) also reported on lung cancer. Among men, the relative risks for lung cancer and for moderate or heavy use of chewing tobacco or snuff were 0.7 (26 cases) and 0.8 (10 cases), respectively, adjusted for age, race and smoking.

Other cancers

Several studies have reported on the association of smokeless tobacco use and other cancers (cancers of the lip, extra-hepatic bile duct, nasal cavities, larynx, prostate, breast, brain, kidney, bladder, penis, cervix uteri, sarcoma, non-Hodgkin lymphoma and leukaemia), but no strong or consistent evidence emerged (IARC 2007).

3.6.2.2. Nasal use of smokeless tobacco products

In many regions of the world nasal use of snuff is less prevalent than oral use, and fewer studies are available on the association of nasal use of snuff with cancer.

Oral cancer

Three case–control studies from Kerala, India (Sankaranarayanan et al. 1989a, Sankaranarayanan et al. 1989b, Sankaranarayanan et al. 1990b) have reported on the association of nasal snuff use and oral cancer subsites among men.

The first part of the study (Sankaranarayanan et al. 1989a) that focused on cancer of the anterior two-thirds of tongue and floor of mouth and comprised 158 cases and 314 controls selected from a pool of 546 hospital controls with non-malignant conditions at sites other than head and neck and matched for age and religion. For cancer of the tongue and floor of the mouth the age-adjusted odds ratio was 3.0 (95% CI: 0.9–9.6) for regular snuff users and 4.3 (95% CI: 1.2–14.7) for occasional snuff users. The odds ratio for < 100 unit years was 10.0 (95% CI: 1.2–86.1) and 1.1 (95% CI: 0.2–6.2) for ≥ 100 unit years.

The second part of the study on cancer of the gingiva (Sankaranarayanan et al. 1989b), comprised 109 cases, and the third part on cancer of buccal and labial mucosa comprised 250 cases (Sankaranarayanan et al. 1990b). All 546 controls from the same pool of controls as in the first study were used for both the second and third studies. For gingival cancer the age-adjusted odds ratio for daily snuff use was 3.9 (95% CI: 1.2–12.7) and 3.8 (95% CI: 1.1–13.5) for occasional use. The odds ratio for regular snuff use was 3.0 (95% CI: 0.7–12.7) after adjustment for daily frequency of use of betel quid, bidi smoking and alcohol use.

For cancer of the buccal and labial mucosa the age-adjusted odds ratio was 4.0 (95% CI: 1.5–10.3) for regular snuff users and 2.3 (95% CI: 0.8–7.0) for occasional snuff users. After adjusting for daily frequency of use of betel quid, bidi smoking and alcohol use, the odds ratio was 2.9 (95% CI: 0.98–8.8). The odds ratio for users of < 100 unit years was 15.7 (95% CI: 2.0–125.3) and 2.0 (95% CI: 0.6–6.6) for users of ≥ 100 unit years.

Oesophagus

The series of case–control studies from Kerala, India also reported on 267 male patients with cancer of the oesophagus using the same 546 controls as in the oral cancer studies (Sankaranarayanan et al. 1991). The age-adjusted odds ratio for daily snuff use was 2.4 (95% CI: 0.8–7.0) and 3.6 (95% CI: 1.2–10.7) for occasional use. Effect estimates were not adjusted for smoking and betel quid chewing.

Paranasal sinus

Shapiro et al. (1955) studied 37 Bantu cases from radiation therapy department records from 1949–51 of a group of hospitals in Johannesburg, South Africa. Cancer of the paranasal sinuses (22 in men, five in women) accounted for a high proportion of respiratory-tract cancer (71% for men, 83% for women) in Bantu Africans. This was in sharp contrast to European cases seen in the Transvaal, where only seven (5%) of the respiratory-tract cancers occurred in the nasal sinuses. Most of the cancers were in the maxillary antrum (28/34 studied) and were described typically as well-differentiated 'squamous epitheliomata'. The authors noted that 80% of all 28 antral cancer cases reported 'prolonged and heavy' use of snuff in contrast to only 34% in Bantu men with cancer at other sites. According to Keen et al. (1955) the product snuffed by Bantus typically contained powdered tobacco leaves and an ash from aloe plants or other species, with the occasional addition of oil, lemon juice and herbs; typical use was 'one teaspoonful' per day. The authors stated that 'there was no obvious correlation' between cancer of the maxillary antrum and cigarette, pipe or *dagga* (marijuana) smoking. The source and nature of the control group is not described.

Larynx

The series of case–control studies from Kerala, India also reported on 191 male patients with biopsy-proved cancer of the larynx, using the same 546 controls as in the oral cancer studies (Sankaranarayanan et al. 1990a). The age-adjusted odds ratio for daily snuff use was 1.2 (95% CI: 0.3–4.9) and 2.8 (95% CI: 0.9–8.7) for occasional use. Effect estimates were not adjusted for smoking.

Lung

A case–control study was reported by Hsairi et al. (1993) consisting of 110 (107 men, 3 women) bronchial cancer patients and 110 controls individually matched for age, sex and number of cigarettes (± 5) smoked per day. Cases were recruited from December 1988 to May 1989 in the Ariana Hospital covering Tunis City and suburb area and controls were chosen among the same area residents. Twenty cases (18.2%) and eight controls (7.3%) had ever inhaled snuff. The crude odds ratio was 2.8 (95% CI: 1.2–6.8). Cochrane Mantel-Haenzel method was used to adjust the association for age, sex, cigarette use (0, 1–10, 11–20 ≥ 20 per day), water pipe and cannabis use. The obtained adjusted odds ratio was 2.2 (95% CI: 0.9–5.6). The authors pointed out that no quantitative analyses were appropriated as the amounts were 'relatively weak'. Nine interviewers were involved in the data collection. The control recruitment was not reported in details.

3.6.2.3. Conclusion on cancer

There is sufficient evidence that the use of a wide variety of STP causes cancer in humans. The pancreas has been identified as a main target organ in two Scandinavian cohort studies. Several studies from the USA have also provided additional support for a causal association between the use of smokeless tobacco and pancreatic cancer. It is difficult to come up with a precise risk estimate because the different STP vary considerably in form and content of toxicants, and the studies have been performed in different populations with different use patterns.

The published studies also support a causal role of STP in the etiology of esophageal cancer. Four out of six studies were from Northern Europe. Tobacco smoking and alcohol drinking was controlled in several of the studies and a causal association is further supported by positive exposure response data.

In five Swedish or Scandinavian studies, an increased risk of oral cancer has not been proven in snus users, however a recent cohort study from Sweden reported a statistically significant three-fold increase of combined oral and pharyngeal cancer, adjusted for tobacco smoking and alcohol drinking. Results among never smokers were similar. Also, in one study from Sweden among users of moist snuff, an increased overall risk of head and neck cancer was not detected. However, an increased risk was observed among a small subgroup of never-smokers.

Risks of oral cancer were strongly associated with the use of American dry snuff in a sub-group of non-black ("white") women in one large case-control study. Several studies from the US reported an increased risk for oral cancer in smokeless tobacco users, most of them among users of chewing tobacco.

Four studies in India and Pakistan (excluding subjects using areca nut) and one study from Sudan have reported large increases in the risk for oral cancers related to the use of various STP.

There is inadequate evidence that STP cause lung cancer.

There are suggestions that nasal use of STP increases the risk for certain cancers, e.g. oral cancers.

3.6.3. Cardiovascular Diseases

3.6.3.1. Epidemiology

Several Swedish studies have investigated whether use of oral tobacco (snus) may be a risk factor for myocardial infarction or for stroke. The endpoint has been mortality, morbidity, or both. Some of those studies were part of the MONICA project in Northern Sweden. The first, including 585 cases and 589 controls, all males, resulted in a relative risk estimate (odds ratio) of 0.9 (95% CI: 0.6-1.3) for acute myocardial infarction. (Huhtasaari et al. 1992). A second study from the same data base included 687 cases and 687 controls and divided the cases in fatal and non-fatal cases (Huhtasaari et al. 1999). The adjusted odds ratio for acute myocardial infarction was 0.6 (95% CI: 0.4-0.9) for all cases and 1.5 (95% CI: 0.5-5.0) among fatal cases. A nested case-control study from the MONICA project, based on 525 cases, including 93 cases of sudden cardiac death, and 1798 controls, was recent published (Wennberg et al. 2007). For current snuff use among never smokers the odds ratio for myocardial infarction was 0.8 (95% CI: 0.5-1.4). For fatal myocardial infarction the corresponding odds ratio was 1.1 (95% CI: 0.4-3.3) and for sudden cardiac death within 24 hours it was 1.1 (95% CI: 0.4-3.7). The fourth study from the MONICA project has looked exclusively at stroke as outcome in relation to use of snus (Asplund et al. 2003b). The study included 276 male cases and 551 matched controls selected from a health screening registry including a stroke registry. For snus users who never smoked the relative risk was 1.1 (95% CI: 0.4-2.9) after adjustment for established stroke risk factors.

A cohort study on snus and cardiovascular disease was based on a population of 135,036 healthy construction workers followed over 12 years (Bolinder et al. 1994). The relative risk for cardiovascular mortality was 1.4 (95% CI: 1.2-1.6) after adjustment for age and geographical region. When restricted to males under 55 years of age at the time of recruitment, and ischemic disease mortality, the relative risk was 2.0 (95% CI: 1.4-2.9). This study also reports on stroke mortality. For males below 55 years of age the relative risk was 1.9 (95% CI: 0.6-5.7) and for those above 55 it was 1.2 (95% CI: 0.7-1.8). A later follow up through 2004 of 118 395 non-smokers in this cohort yielded a relative risk of 0.9 for all myocardial infarction and 1.3 (95% CI: 1.1-1.6) for fatal myocardial infarction (Hergens et al. 2007). For users of more than 50 g snus per day the relative risk for fatal myocardial infarction was 2.0 (95% CI: 1.1-3.6).

The population based case-control study (SHEEP) uses data from the Stockholm and Västernorrland regions during 1992-1994 (Hergens et al. 2005). The study was based on males aged 45-70; the number of cases was 1432 and the number of controls 1810. Restricted to never smokers the odds ratio for all myocardial infarction was 0.7 (95% CI: 0.4-1.5). Restricted to never smokers and to fatal cases, the odds ratio was 1.7 (95% CI: 0.5-5.5).

One Swedish cohort study was based on a random sample from the general populaton conducted for the Survey of Living Conditions project. The cohort consisted of 3,120 males followed for 12 years (Johansson et al. 2005). After adjustment for established risk factors the relative risk for heart disease was 1.4 (95% CI: 0.6-3.3). A similarly designed study was based on 5002 males who were followed from 1988-89 through 2003. For ischaemic heart disease, hospitalization and mortality combined, the relative risk was 0.8 (95% CI:0.5-1.2) among non-smokers. When the endpoint was restricted to mortality from ischaemic heart disease the relative risk was 1.2 (95% CI:0.5-2.4). For stroke the relative risk was 1.1 (95% CI: 0.7-1.8) (Haglund et al. 2007).

A hospital based study from Northern Sweden on subarachnoid haemorrhage found no association with use of snus (Koskinen and Blomstedt 2006).

Interheart was a standardized case-control study of non-fatal myocardial infarction conducted in 52 countries (Teo et al. 2006). It included 12,133 cases and 14,435

controls and looked at risks related to tobacco use. All forms of tobacco combined were associated with an increased risk. For chewing tobacco alone the odds ratio was 2.2 (95% CI: 1.4-3.5). The raised odds ratio is based on data from a large number of different countries with different habits and different products. Data for snus were not reported separately because of small numbers.

An American cohort study on 6,805 males and females investigated smokeless tobacco (not distinguishing moist snuff and chewing tobacco) in relation to cardiovascular mortality (Accortt et al. 2002). After adjustment for age, ethnicity, and other potential confounders the relative risk for heart disease mortality was estimated at 0.6 (95% CI: 0.3-1.2) among males and 1.4 (95% CI: 0.8-2.3 among females. For stroke mortality the relative risks for males and females were 0.7 (95% CI: 0.2-2.2) and 1.0 (95% CI: 0.3-2.9) respectively.

Another, recently published, American prospective study was based on two large cohort studies (Cancer Prevention Study (CPS-I and CPS-II)) including 181,144 males aged 30 years and above (Henley et al. 2005). In CPS-I, in which chewing tobacco and moist snuff use were not distinguished, the relative risk for heart disease mortality was 1.1 (1.0-1.2) and for stroke mortality 1.5 (95% CI: 1.4-1.7). In CPS-II, moist snuff users were separated from chewing tobacco users; moist snuff use had a relative risk for heart disease mortality of 1.6 (95% CI: 1.1-2.4) and for stroke mortality of 0.6 (95% CI: 0.2-1.7). All these analyses were adjusted for potential confounders.

In the extended follow-up of the CPS-II cohort, Henley et al. (2007) compared mortality from coronary heart disease among former exclusive cigarette smokers and switchers who reported currently using spit tobacco and having begun doing so at the time or after they quit exclusive cigarette smoking. Compared to those who quit entirely, the relative risk for mortality from coronary heart disease of switchers, was 1.13 (95% CI: 1.00-1.29). Compared to men who never used any tobacco products the relative risks of coronary heart disease among those who quit tobacco use entirely and among switchers were statistically significantly increased (1.11 and 1.28, respectively).

3.6.3.2. Other studies

Short term effects on blood pressure and heart rate have been observed in several human studies (Benowitz et al. 1988b, Ernster et al. 1990, Fant et al. 1999, Squires et al. 1984, Westman 1995, Wolk et al. 2005). However, whether long term use of STP is a risk factor for hypertension is uncertain. Various Swedish and American studies have looked at this but the results have been contradictory (Bolinder et al. 1992, Bolinder et al. 1998, Eliasson et al. 1991, Ernster et al. 1990; Schroeder et al 1985, Siegel et al. 1992, Westman 1995). All studies on oral tobacco use and hypertension in humans have been cross-sectional making causal inference difficult. Yet, one can not exclude the possibility that oral tobacco use increases the risk of hypertension, but more appropriately designed studies are needed.

3.6.3.3. Conclusion on cardiovascular diseases

Both animal experiments and epidemiological studies indicate that oral tobacco use has short-term effects on blood pressure and heart rate. Whether long-term use increases the risk of hypertension is uncertain. It appears that the use of smokeless tobacco increases the risk of death after myocardial infarction but that it does not increase the risk of myocardial infarction.

3.6.4. Reproductive Effects

In a study of 1,217 women in India who were three to seven months pregnant and who had used a smokeless tobacco product at least once a day for the past six months, it was found that smokeless tobacco use was associated with an average reduction of 105 g in birth weight (95% CI: 30 g to 181 g) and a reduction in gestational age of 6.2 (95% CI: 3.0 to 9.4) days (Gupta and Sreevidya 2004). The odds ratio for low birth weight was 1.6 (95% CI: 1.1-2.4), adjusted by logistic regression for maternal age, education, socioeconomic status, weight, anaemia, antenatal care and gestational age. A study in South Africa has looked at birthweight and gestational age in relation to tobacco use including snuff use (Steyn et al. 2006). A non-significant association with reduced birthweight was found.

In 2003 a cohort study based on the Swedish Birth Registry and with tobacco use information collected early in the pregnancy by midwifes was presented (England et al. 2003). The study included 789 snus users and 11,495 non-users of tobacco. Several different outcomes were analyzed. For the outcome "small for gestational age" the relative risk was 1.3 (95% CI: 0.7-2.2), for prematurity it was 2.0 (95% CI: 1.5-2.7), and for preeclampsia it was 1.6 (95% CI: 1.1-2.3).

3.6.4.1. Conclusion on reproductive effects

In general the data on reproductive effects in relation to smokeless tobacco use during pregnancy are too sparse to allow conclusions.

3.6.5. Local Effects

The findings concerning oral cancer are given in section 3.6.2.1. In this chapter other reported mucosal disorders are presented and classified under the smokeless tobacco product used. Firstly we refer to oral lesions caused by snuff/snus 3.6.5.1 and then chewing tobacco 3.6.5.2. In a short section 3.6.5.3 studies on tobacco-lime user's lesions will be reported. Further, country of study will be mentioned due to differences of smokeless tobacco constituents in products consumed in different countries/parts of the world. After reviewing the clinical aspects, the pathology of these mucosal disorders are also presented.

3.6.5.1. Snuff/snus-induced lesions

Snuff is used in different settings, i.e. nasal and oral use. This chapter deals with oral use of snuff. There are different products for oral use including dry snuff, fine cut and moist snuff. Further, moist snuff products may be fermented and non-fermented (Andersson and Axéll 1989a). These products may differ concerning, among else, carcinogenic substances such as tobacco specific nitrosamines (TSNA).

Clinical changes in the oral cavity comprise changes of the non-keratinized mucosa and of the gingiva, corresponding to the site where the product is regularly placed. The primary mucosal change is a wrinkled appearance of the mucosa that appears white or yellowish brown due to surface tobacco stains, in some cases with an associated erythema.

For the mucosal changes a different terminology has been applied in various studies. Thus the term leukoplakia (white patch) (e.g. Roed-Petersen and Pindborg 1973), has been assigned for the lesions implicating a potentially malignant potential of the lesions. Later, the terms snuff dippers' lesion and snuff-induced change/lesion have been used for the purpose of differentiating the snuff-induced lesions from leukoplakia, in order to make follow-up studies feasible and also because some of the snuff-induced lesions are not white or whitish (Axéll 1976a, Andersson 1991). For a review, see further Holmstrup

and Pindborg 1988. In Scandinavia the lesions have lately been labelled snus induced lesions (SILs), in order to emphasize that they are caused by Swedish moist snuff (Roosaar et al. 2006). This use of taxonomy does not exclude the possibility that snuff-induced lesions or snus-induced lesions might carry a potentially malignant risk. In the following the terminology as used by the cited authors of relevant studies will be applied.

Scandinavian reports

In a report from Denmark leukoplakias associated with oral use of snuff were described as homogeneously white lesions with a wrinkled surface (Roed-Petersen and Pindborg 1973). They were either non-elevated or only slightly elevated and were diffusely demarcated from the surrounding mucosa. Pindborg et al. (1980) reported some morphological variations in smokeless tobacco-associated lesions in the form of discrete elevated keratinized striae particularly when involving non-keratinized mucosal sites. These striae gave the appearance to the lesion described as "pumice pattern".

A subgrouping on a four point scale of clinical snuff-induced lesion has been suggested and extensively applied in Swedish studies on snuff/snus-induced lesions (Axéll et al. 1976b):

Degree 1 - A superficial lesion with a colour 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 thickening.

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

Degree 4 - A marked yellowish to brown and heavily wrinkled lesion with intervening deep reddened furrows and/or heavy thickening.

This four grade scale has been applied in a number of studies, but in US studies a somewhat modified version has been used, where degrees 3 and 4 have been pooled together giving a three grade scale (Greer and Poulson 1983).

In Scandinavia, the snus quid is most often placed inside the upper lip except for Denmark where the quid is preferably placed inside the lower lip. Exceptionally the quid will also be placed in the vestibular mucosa in the lower jaw and under the tongue.

The severity of clinical changes seems to increase by number of hours the quid is placed in the mouth, grams of daily snus use and years with regular snus habit (Andersson and Axéll 1989a, Andersson et al. 1990). Hirsch et al. (1982) reported that the number of years of use is the most important factor for the severity of lesion. The most apparent factor for the clinically assessable severity of snus induced lesions is the type of snus used. Thus, the use of portion bag-packed snus seems to be associated with less pronounced lesions than loosely packed snus (Andersson and Axéll 1989a, Andersson et al. 1989b).

In Sweden, snuff/snus-induced changes almost invariably appear on the oral mucosa at the regular site of snuff/snus application. The prevalence of lesions among 20,333 adult individuals in the middle of Sweden was 15.9% in men and less than 1% in women 1976. Snuff dipper's lesions were registered in 94% of snuff users (Axéll 1976a). 72 (4.9%) were classified as grade 4 lesions (Mornstad et al. 1989). In another study from the middle of Sweden in 1990 the prevalence was 14.5% in 449 men (Salonen et al. 1990). Among snuff users the prevalence of snuff dipper's lesions was estimated at 79.7%.

Twenty-one snuff-induced oral mucosal lesions were described by Jungell and Malmström (1985) among 441 Finnish military recruits. All lesions were found in the upper vestibular area where the snuff quid was placed. Clinically they appeared wrinkled, greyish white and slightly elevated. The only symptom reported was slight itching.

Snuff/snus induced lesions to a great extent seem to be reversible after cessation of snuff/snus use (Jungell and Malmström 1985, Larsson et al. 1991, Roosaar et al. 2006), an observation supported by findings in animal studies (Hirsch et al. 1986). Lesions also seem to be become less pronounced after change from use of loose snus to portion bag-packed snus (Roosaar et al. 2006).

Retractions of the gingiva are prevalent at the site where snuff is placed (Offenbacher and Weathers 1985). Such retractions are far less prevalent in individuals using portion-bag packed snus than in those using loose snus (24% and 3%, respectively) (Andersson and Axéll 1989a).

Two studies were performed to compare the short-term effects on consumption and nicotine intake of switching to low-nicotine snus with those of long-term effects. In Study 1, consumption data, soft tissue changes and nicotine intake were measured in a group of 24 habitual users of Swedish portion-bag snus, both during use of their ordinary snus (Brand A) for 2 weeks and during consumption of the low-nicotine product (Brand B) for 10 weeks. In study 2, the same data were measured during 2 weeks in a reference group of 18 snus users who had been habitual users of the low-nicotine snus (Brand B) for at least one year. Although there was no increase in number of hours of daily consumption, the amount of snus consumed increased on average by 2 grams a day (+15%) when switching from Brand A to the low-nicotine Brand B (Study 1). The Brand B reference group (Study 2) consumed about 3 grams less snus a day during the same number of hours as the subjects in Study 1 who had switched to Brand B. These results indicate that snus users compensate to a small extent for the lower nicotine delivery by increasing their consumption after short-term switching but the same does not apply to long-term users (Andersson et al. 1995)

Rolandsson et al. (2005) examined 80 adolescent males between 16-25 years, 40 snuff users and 40 non-users. Out of 40 snuff users, 35 showed snuff incluced lesions. The clinical diagnosis of snuff users' mucosa showed snuff lesions of different severity clinically classified as degree 1, 2 and 3. Hours of daily snuff use and package form (portion-bag snuff versus loose snuff) had a statistically significant effect on the development of snuff lesions of degree 2 and 3. There were no statistical differences between snuff users and non-users regarding restored tooth surfaces, presence of plaque, gingival inflammation and probing pocket depth. Seventeen percent of the cases showed loss of periodontal attachment as gingival recessions. 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. However, some cases showed loss of periodontal attachment as gingival recessions.

US reports

Poulson et al. (1984) compared the use of smokeless tobacco and its effects in rural and urban teenagers. A random sample of 445 subjects from rural Colorado were examined: 82.9 percent of the total sample were Caucasian, and 94.6 percent of those who used smokeless tobacco were Caucasian. This percentage supports the findings of an earlier urban study that the habit is predominantly one of male Caucasians. The average age of the users was 16.7 years, slightly older than in the urban study. Of the rural users, 62.5 percent had lesions of the oral tissues, compared with 48.7 percent lesional incidence in urban users. In both studies, those subjects with lesions had longer daily contact with smokeless tobacco, as well as a longer history of use than those without lesions. These are numerical averages that reflect great individual variations in susceptibility. The average duration of use for rural and urban users with lesions was almost the same; the development of lesions appears to be related to the length of daily exposure, which, on the average, was greater among rural users than urban users. Additionally, more than twice as many degree 3 lesions were found among users in the rural study.

In a study by Wolfe and Carlos (1987) 226 Navajo Indians, aged 14-19, were interviewed regarding their use of smokeless tobacco, cigarettes, and alcohol. The oral mucosa was examined for evidence of leukoplakia. 64.2% (145) of the subjects (75.4% of the boys and 49.0% of the girls) were users of STP. Of these, over 95% used snuff alone or in combination with chewing tobacco. 55.9% used STP one or more days per week. 52.2% consumed alcohol, usually beer or wine, and 54.0% smoked cigarettes. 25.5% (37) of the users and 3.7% (3) of the non-users had leukoplakia. The duration (in years) and frequency of STP use (days per week) were highly significant risk factors associated with leukoplakia. However, the concomitant use of alcohol or cigarettes did not appear to increase the prevalence of these lesions. No consistent relationship was observed between the use of STP and gingival bleeding, calculus, gingival recession, or attachment loss, either when comparing users to non-users or when comparing the segment where the tobacco quid was habitually placed to a within-subject control segment. In view of these results, there is little doubt that smokeless tobacco is significantly related to the etiology of leukoplakia.

In a study among adolescent male athletes almost a third of the sample had tried smokeless tobacco and 8% were current users. Differences in income strata and urban/rural settings were not significant. Peer influence was the major factor that initiated smokeless tobacco use. Abnormal mucosal findings were much more prevalent in those who had dipped smokeless tobacco than in those who had not. Most significant was a prevalence of oral leukoplakia in 5.2% of those who had ever dipped, which was 50 times that of nondippers. Using smokeless tobacco for more than 2 years or using more than three tins per week seemed to be of possible predictive value regarding the incidence of oral leukoplakia. Fifteen percent of current users had observable leukoplakia (Creath et al. 1988).

In a study on 1,094 US professional baseball players, coaches, and training staff of seven major league and their associated minor league teams Robertson et al. (1990) found that more than 50% of team members reported using smokeless tobacco, and 39% reported use during the current week. Among current week users, 46% had oral mucosal lesions, located primarily in the mandible at sites where the smokeless tobacco quid was placed. Sites adjacent to mucosal lesions in smokeless tobacco users showed significantly greater recession of the gingival and attachment loss than in sites not adjacent to lesions in users or comparable sites in non-users.

Sinusas et al. (1992) investigated in detail 88 current users of STP among 220 professional baseball players. Oral leukoplakia was found in 25 of 88 current users (28.4%). Year-round users had a significantly higher incidence rate and also higher grades of leukoplakia.

Among 565 US school children (age range 10-17 years) in whom 13.3% were STP users 9 leukoplakias were found, 8 of which were in STP users (Offenbacher and Weathers 1985).

From the US, Greer and Poulson (1983) reported on oral mucosal alterations in 117 users of STP among high school children in Denver (US) that they had identified in a school survey among a total sample of 1,119 students. Fifty had mucosal changes which appeared red or white in colour. The vast majority of lesions were white, corrugated and raised. Little et al. (1992) recorded a high prevalence of mucosal lesions (78.6%), a quarter of which were in the most clinically advanced category (grade 3). Kaugars et al. (1992) investigated oral lesions that persisted for at least 7 days after discontinuation of STP use. Among white males in this group (mean age 29.3 years) 45/347 (13%) had mucosal alterations consistent with STP use.

The risk for oral mucosal lesions associated with use of smokeless tobacco among 1,109 professional baseball players during spring training in 1988 was investigated by Grady et al. (1990). Leukoplakia was very strongly associated with use of smokeless tobacco in this population of healthy young men. Of the 423 current smokeless tobacco users, 196

had leukoplakia compared to seven of the 493 nonusers (OR = 60.0, 95% CI = 40.5-88.8). The amount of smokeless tobacco used (in hours per day that smokeless tobacco was held in the mouth), recency of smokeless tobacco use (hours since last use), type (snuff versus chewing tobacco), and brand of snuff used were significantly associated with risk for leukoplakic lesions among smokeless tobacco users. Ninety-eight leukoplakic areas in 92 subjects were biopsied and examined microscopically. All lesions were benign, but one specimen had mild epithelial dysplasia. According to the authors "The long-term significance of leukoplakia in smokeless tobacco users and their relation to oral cancer is not clear".

Creath et al. (1991) reported on the prevalence of oral leukoplakia in 1,116 teenaged American football players (567 black, 546 white) following an oral screening examination. 13% of current users had clinically evident oral leukoplakia (RR: 5.8). A significant dose response was noted. Furthermore, regular use as well as number of years of STP use were significantly associated with leukoplakia.

In the US, Tomar et al. (1997b) found among 17,027 schoolchildren degree 3 lesions to be more common among current snuff users (3%) compared with current tobacco-chewing subjects (2.6%). A quarter of all STP lesions found were on the mandibular anterior labial vestibule. A quarter of STP users examined in US also were reported with two or more lesions in the mouth (Tomar et al. 1997b). In a separate study 29% of current STP using Floridian students demonstrated oral lesions (not classified) (Stewart et al. 1989).

In a US military population two hundred fourteen soldiers completed a questionnaire-type survey regarding tobacco use and received an annual-type dental examination that included extra-oral and intra-oral examination of hard and soft tissues and counseling regarding the risks associated with the use of tobacco. More than 50% of the participants were between the ages of 18 and 24. Survey response indicated that 7.0% used smokeless tobacco, 29.0% smoked cigarettes, and 7.9% used both cigarettes and smokeless tobacco. Leukoplakia was seen in 4 of the current smokeless tobacco users (Grasser and Childers 1997).

In a report by Johnson et al (1998) a study examined clinical and inflammatory mediator parameters during the development of snuff-induced mucosal lesions. Nineteen smokeless tobacco (ST) users placed moist snuff at designated new placement sites over either a 2- or 7-day period. By day 2, the predominant clinical alteration was an erythematous reaction, and one-third of the subjects demonstrated white striations in combination with erythema or ulceration. By 7 days, 56% of the subjects displayed white striated lesions.

Martin et al. (1999) examined oral cavities of 3,051 male US Air Force trainees (mean age 19.5 years). 302/3,051 (9.9%) were current STP users. Among STP users (119/302) 39.4% had oral leukoplakia (OR=41.9, 95% CI: 28.1-62.6). The prevalence of STP associated lesions was significantly associated with length of use (months), amount used (cans or pouches per day). The authors concluded that use of STP, especially snuff, is strongly associated with development of oral leukoplakia in young adult men.

Of 3,051 male trainees examined (mean age = 19.5 years), 9.9 percent (302/3,051) were identified as current STP users. Among current STP users, 39.4 percent (119/302) had leukoplakia vs. 1.5 percent (42/2,749) of nonusers of STP (odds ratio = 41.9, 95 percent confidence interval = 28.1-62.6). At the end of the involuntary cessation of tobacco use, 97.5 percent of these leukoplakic lesions had complete clinical resolution. The type of STP used (snuff vs. chewing tobacco), amount used (cans or pouches per day), length of use (months), number of days since last use and brand of snuff used were significantly associated with the risk of developing leukoplakic lesions among STP users (Martin et al. 1999).

A study by Fisher et al (2005) indicates that those with oral leukoplakia were more likely to be older and more likely to currently use smokeless tobacco. Individuals currently using smokeless tobacco were more likely to have oral leukoplakia after simultaneously adjusting for age, gender, currently using smoked tobacco, currently using alcohol daily, and dental prostheses use.

3.6.5.2. Chewing tobacco-induced lesions

There is only one study from Sweden on the clinical and histopathological changes associated with the regular use of chewing tobacco. Axéll et al. (1992) examined such changes in 20 men who had used chewing tobacco for about 11 years as their only tobacco habit. The most common clinical finding was a leukoedema-like change of the buccal mucosa at the site where the tobacco quid was placed. Ten individuals showed changes compatible with mild snus induced ones corresponding to clinical degrees 1 and 2 on a four point scale. Histological findings corresponded well with the clinical observations. Thus, it appears that oral mucosal changes associated with chewing tobacco in Sweden are discrete.

In a study of 280 English coal miners who were tobacco chewers 10 (3.6%) were reported with leukoplakia (Tyldesley 1971).

Betel-quid chewers in India who add tobacco to the quid chew approximately 7-12 g of tobacco per day. Mehta et al. (1972) diagnosed leukoplakia in 117/3,674 (1.8%) of betel-tobacco chewers in India. These were predominantly in men over the age of 30 years. Bilateral occurrence was observed in 12-23% of 880 leukoplakias reported (Mehta et al. 1969). Gupta et al. (1980) in a ten-year follow up study reported that 15/73 new leukoplakias in males occurred in betel-tobacco chewers and all 60 new leukoplakias among females occurred in chewers (non-smokers). Although leukoplakia occurs predominantly on the tongue in Western populations, in India the buccal site is more common in tobacco chewers.

Jacob et al. (2004) in a population study in Kerala, India, stratified tobacco chewing and other risk habits of oral leukoplakia cases. Among 927 oral leukoplakia cases detected 8 reported current tobacco chewing and 3 of them had no smoking or alcohol drinking habits. OR for oral leukoplakia for tobacco chewing was reported as 30.9 (95% CI: 13.7-69.7).

Multiple oral premalignant lesions associated with leukoplakia, notably erythroplakia, and submucous fibrosis were described in a cohort of tobacco chewers in Kerala, India. The presence of multiple oral premalignant lesions suggested an effect consistent with field cancerization due to prolonged chewing of tobacco (Thomas et al. 2003).

Only one study has looked at the association of chewing tobacco with oral erythroplakia (Hashibe et al. 2000). In this study in Kerala, India, the adjusted OR for erythroplakia was 19.8 for individuals who had ever chewed tobacco. Erythroplakia was defined and characterized as a precancerous lesion by WHO but it is not clear how the authors excluded other red patches of oral mucosa (Reichart and Philpsen 2005) to diagnose erythroplakia.

3.6.5.3. Tobacco-lime user's lesions

An oral lesion in tobacco and lime users in Maharasta, India was described by Bhonsle et al. (1979). This mucosal lesion coincided with the placement of the quid and could be scrapped off leaving a raw surface. Tobacco and lime mixture also called Khaini is usually retained in the anterior part of the mouth rather than chewed (Stich et al. 1992). Among

Nepalese the habit is associated with white and red patches with a rippled/fissured surface characteristic (Shrestha et al. 1997).

Nass made with local tobacco (partly cured), ash and lime used in Central Asian Republics of the former Soviet Republic and parts of Pakistan is significantly associated with the risk of oral leukoplakia. In 118 current nass users in Uzbekistan the associated risk for oral leukoplakia (adjusted for smoking and alcohol) was 3.9 (95% CI: 2.6-5.7) (Evstifeeva and Zaridze 1992).

3.6.5.4. Pathology of leukoplakia and snuff induced/dipper's lesions

One of the basic traits to be considered when discussing premalignant potential of prevailing oral mucosal lesions, whether labelled leukoplakia or snuff/snus-induced lesions, is the concept of dysplasia. Basic traits of epithelia dysplasia have been described by Smith and Pindborg (1969). However, these traits have been challenged in trials (Pindborg et al. 1985). Further, such histopathological traits have been found to be reversible and not always implying development towards malignancy. Thus, changes with dysplastic traits have been shown to be reversible and rather markers of physical trauma. However, the finding of dysplastic traits and their potentially malignant potential in STP-induced lesions should not be overlooked and the lesions showing such traits should be carefully followed for the development of malignant changes.

The presence of dysplastic areas in the epithelium of the upper aerodigestive tract is believed to be associated with a likely progression to cancer. Dysplastic features of a stratified squamous epithelium are characterized by cellular atypia and loss of normal maturation and stratification (Pindborg et al. 1997). It is reasonable to assume that these changes are due to chromosomal, genomic and molecular alterations. Dysplastic lesions caused by smokeless tobacco do not have the same profile as mutations caused by smoking (Warnakulasuriya and Ralhan 2007). There is support for the view that in an individual lesion, the more severe the dysplasia the greater the likelihood is of progression to malignancy. However, lately this has been questioned (Holmstrup et al 2007). And thus, even non-dysplastic lesions may also transform.

Snuff-induced leukoplakia, snuff/snus-induced lesions

Histopathology of oral leukoplakia or snuff/snus-induced lesions caused by STP were reported by Roed-Petersen and Pindborg (1973), Andersson et al. (1989b) and Jungell and Malmström (1985) from Scandinavia, Daniels et al. (1992b), Greer et al. (1986) from USA, and Idris et al. (1996) from the Sudan.

Extensive studies on histopathology of snuff/snus induced lesions were conducted by Andersson (1991). Common epithelial changes noted were hyperorthokeratosis, hyperparakeratosis, chevron pattern keratinisation, pale surface staining, koilocytosis-like changes with vacuolated cells, and basal cell hyperplasia. The reversibility of histologic changes following cessation of snus habit has been reported Andersson (1991). Larsson et al. (1991) noted that dysplasia may occasionally occur in snuff dipper's lesions, although they questioned its premalignant potential.

Kaugars et al. (1989) found that women were more likely to have moderate to severe epithelial dysplasia than men ($p=0.02$) but this may be because their lesions were detected a decade or so later or were in older women. Out of all pathological studies examining oral biopsies of STP users Kaugars et al. (1989) recorded the highest prevalence of oral epithelial dysplasia (66.7% mild dysplasia; 5.4% severe dysplasia) but they noted that 91% of these biopsies with oral dysplasia were taken from the site of STP placement. However, the majority of dysplasia changes were focal in nature. In a later

study by the same group, 10 out of 45 cases with STP lesions were diagnosed with dysplasia (4 cases were focally mild; 3 mild; 1 severe).

In Sweden, loose snuff users had more increased epithelial thickening compared with portion-bag snuff users who had less pronounced morphological changes (Andersson et al. 1989b, Andersson et al. 1990, Andersson et al. 1994). Andersson et al. (1990) in a study of biopsies from mucosal lesions in Sweden noted that the daily but intermittent use of snuff caused a mixed tissue reaction of injury and repair.

From Swedish studies also the presence of eosinophilic granulocytes (Axéll et al. 1976b, Andersson et al. 1989b) and the involvement of salivary glands (Hirsch et al. 1982) were reported.

Koilocytic alterations noted in the epithelial keratinocytes in several studies (26/45 cases (Greer et al. 1986) and 22/141 cases (Idris et al. 1996)) suggest the presence of a cytopathic damage caused by a virus, possibly human papillomavirus (HPV) in STP induced lesions (Greer et al. 1986, Idris et al. 1996). However, a study using polymerase chain reaction performed on snuff-induced lesions from Scandinavia did not confirm any association with HPV or EBV (Sand et al. 2000).

Verrucous hyperplasia clinically indistinguishable from verrucous carcinoma has been described in STP users (Shear and Pindborg 1980). The surface epithelium is highly keratinised, with corrugations and sharp or blunt processes. Some progress to verrucous carcinoma or may present as a co-existing lesion with carcinomas and is therefore considered precancerous. Commonly affected site is the alveolar mucosa.

Micronuclei are considered to be markers of abnormal mitoses. This morphological change in keratinocytes involves chromosomal breaks and missegregated chromatin which result in the formation of separate smaller nuclei at the time of cell division. Micronucleus frequencies in exfoliated cells or cell scrapings have been validated as tissue-specific indicators of carcinogen exposure in humans. Several studies have shown an association of increased micronuclei and snuff use (Tolbert et al. 1991, Roberts 1997). In 48 young adults, the frequency of micronucleated cells was significantly ($p<0.01$) higher in the labial mucosa of exposed (2.22%) compared to unexposed individuals (0.27%) (Livingston et al. 1990). Ozkul et al. (1997) reported doubling of micronuclei in Turkish STP (Maras powder) users compared with controls. The possibility of reversal of the formation of micronuclei using vitamin A or β-carotene supplements has been discussed (Rosin 1992).

Proliferation and differentiation markers of oral epithelium were examined in 14 Finnish male snuff users, three of whom were also occasional smokers (Merne et al. 2002). Cell proliferation as determined by Ki67 staining was markedly reduced compared with controls. Altered CK 18 expression (but not CK19) was reported in the oral epithelium of some snuff users (5/14).

Dysplasia was uncommon in the Sudanese biopsies reported (Idris et al. 1996). Cellular atypia in buccal smears was more common in heavy toombak users (11+ quids a day) compared with cigarette smokers of similar frequency (11+ a day) but the authors remarked the method is unreliable as cells are taken from the surface while abnormalities mostly occur at the base of the epithelium in the progenitor layers (Ahmed et al. 2003).

In an electron microscopic examination widening of intercellular spaces was noted in the spinous layer (Jungell and Malmström 1985) in Finnish snuff dippers.

A reduction in Langerhans cells in smokeless tobacco-associated oral mucosal lesions was reported by Daniels et al. (1992a) suggesting an impairment of immunologic protection. Higher levels of both IL-1α and β were observed in mucosal lesions at habitual STP placement sites (Johnson et al. 1994) and this may be implicated in both the inflammatory response and epithelial proliferation.

Increased expression of keratins 13 and 14 in Sudanese snuff dippers was reported (Ibrahim et al. 1998) indicating dysregulation of keratinocyte maturation and a third of the lesions also expressed K19 a basal keratin suggesting epithelial de-differentiation. Suprabasal expression of K19 was also reported by Luomanen et al. (1997a) in oral biopsies of 11 snuff users from Sweden. Increased tenascin expression was reported in biopsies of smokeless tobacco users more conspicuous than in smokers (Luomanen et al. 1997b). This was distributed as a band under the epithelium. This suggested a marked connective tissue reaction to snuff suggesting an epithelial-mesenchymal interaction either inflammatory or preneoplastic in nature.

An amorphous deposit in the lamina propria of the oral mucosa where the snuff is habitually placed was noted from Denmark 40 years ago (Pindborg and Poulsen 1962). Several investigators subsequently commented on the presence of a similar histological appearance initially regarded as amyloid (Lyon et al. 1964) but later thought to be non amyloid (Hirsch et al. 1982, Archard and Tarpley 1972) and speculated to be collagen by Axéll et al. (1976b). Idris et al. (1998) by electronmicroscopy studies later characterised this amorphous deposit in 25 oral snuff induced lesions from the Sudan as collagen.

Tobacco chewing induced leukoplakia/lesions

In a report on chewer tobacco induced leukoplakia Tyldesley (1971) reported the lesions to show hyperorthokeratosis, acanthosis and well-marked granular layer associated with epithelial atypia in some cases. There was no evidence of incipient malignant change. At a follow-up study of 8 tobacco chewers with oral leukoplakia after five years, one case of malignant transformation was encountered at the site at which the tobacco had been held for 30 years. In 5 other men no change was found and in 2, even a regression of the lesion was seen (Tyldesley 1976).

Axéll et al. (1992) reported on 20 men using chewing tobacco in Sweden. The clinical findings showed leukoedema-like changes with vacuolated cells in the upper spinous layers, swollen cells but no evidence of keratinzed cells. In other specimens changes compatible with snuff induced lesions pf grad 1 and 2 were seen showing epithelium with a thickened and condensed structureless eosinophilic surface layer with a few pyknotic nuclei, occasionally with a slight evidence of keratinisation, with a more or less well-developed granular layer and accompanied by a slight inflammation.

Ramaesh et al. (1999) reported variations in cell and nuclear diameters in Sri Lankan tobacco chewers. While the nuclear diameter was increased the cell diameter was reduced compared with normal buccal cells, giving an increased nuclear to cytoplasmic ratio in chewers.

In the US, use of snuff was more frequently associated with development of oral mucosal lesions than was the use of chewing tobacco. Furthermore, snuff appeared to cause a greater variety of epithelial changes than chewing tobacco (Daniels et al. 1992b).

3.6.5.5. Conclusion on local effects

Oral use of smokeless tobacco almost invariably causes changes in the oral cavity (mouth), many of which show up as white and/or red patches. These are referred to as snuff dippers' lesions, snus-induced lesions (SIL) or leukoplakia. Some of these changes have been classified as potentially malignant disorders (PMD) or precancerous lesions but it is also noted that most of these lesions are reversible on quitting the habit.

Several studies from south Asia (particularly India and Pakistan) have reported oral leukoplakia associated with the use of STP available in these countries. In India a 10-year follow up study (Gupta et al. 1980) has demonstrated that oral cancers almost

always arise from pre-existing leukoplakia. Such data have strong implications for Asian migrants living in European countries who use these products imported from south Asia.

In Scandinavia only one long-time follow-up study is available. This has shown a non-statistically significant risk for subsequent cancer development.

3.6.6. Other Effects

3.6.6.1. Diabetes and metabolic disturbances

Three Swedish studies on type-II diabetes in relation to STP-use exist (Eliasson et al. 1995, Eliasson et al. 1996, Persson et al. 2000). The US intervention study mentioned above in relation to cardiovascular disease, did also look at diabetes mortality (Henley et al. 2005). These studies do find associations with diabetes. In the Persson study, for example, the relative risk was 3.9 (95% CI: 1.1-14.3) when restricted to non-smokers. The results are not consistent, however, and several methodological questions can be raised. The Persson study, for example, was a cross-sectional study which makes causal inference uncertain. A recently published study based on an intervention program in Northern Sweden has looked at the incidence of the metabolic syndrome in relation to snus use (Norbert et al. 2006). The authors found that high-dose consumption of snus at baseline was associated with ten year cumulative incidence of the metabolic syndrome (OR=1.6, 95% CI: 1.26-2.15). Snus use was also associated with components of the metabolic syndrome, including elevated levels of triglycerides and obesity. A small cross sectional study has looked at snus use in relation to cardiovascular risk factors and also found an association with triglycerides as well as with waist-hip ratio (Wallenfeldt et al. 2001). However, the study size and design limit the interpretations.

3.6.6.2. Musculoskeletal disorders

In one study on 240 older women (aged 60 – 94) in an USA multi ethnic rural community it was found that bone mineral density declined with age; the decline was greater in women who were current or former STP users than those who never use STP (Quandt et al. 2005).

A two-fold increase in the risk of musculoskeletal injuries among 480 male conscripts in the Norvegian army was found among snuff users comparing to non-users (Heir and Eide 1997).

In both studies, however, confounding factors were not properly controlled and the explanations for the observed phenomenon were not given.

3.6.6.3. Conclusion on other effects

Various studies suggest that diabetes and other components of the metabolic syndrome, as well and musculoskeletal disorders might be associated with use of snus, but findings must be interpreted with caution particularly because of study design limitations.

3.6.7. Conclusion on adverse health effects in humans

It must be recognised that marketed STP vary considerably in form and content of toxicants, including nicotine, and thereby in associated health effects which have been documented across countries. 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. Aqueous and organic extracts of American and Swedish moist snuff and Indian chewing tobacco cause mutations and chromosomal damage in bacterial and mammalian cell cultures. Increased micronuclei

formation in oral epithelial cells as evidence of chromosomal damage, has been associated with moist snuff use.

Use of American and Swedish moist snuff results in localised lesions in the oral epithelium, where the snuff is placed. These changes are reversible, whereas gingival retractions caused by moist snuff are not reversible. Moist snuff in portion-bag sachets gives less severe epithelial changes than snuff in loose form.

There is sufficient evidence that the use of a wide variety of STP causes cancer in humans. The pancreas has been identified as a main target organ in two Scandinavian cohort studies. Furthermore, several studies from the USA have provided additional support for a causal association between the use of smokeless tobacco and pancreatic cancer. There is inadequate evidence that STP cause lung cancer.

Risks of oral cancer were strongly associated with the use of American snuff in one large case-control study; however, a detailed characterisation of the product was not given but most probably it was dry snuff made by locally grown tobacco. Several other studies from the US reported an increased risk for oral cancer in smokeless tobacco users. Four studies in India and Pakistan and one study from Sudan have reported large increases in the risk for oral cancers related to the use of various STP. In Swedish studies, an increased risk of oral cancer has not been proven in snus users. However a recent cohort study from Sweden reported a statistically significant three-fold increase of combined oral and pharyngeal cancer, adjusted for tobacco smoking and alcohol drinking. In one study from Sweden among users of moist snuff, an increased overall risk of head and neck cancer was not detected. However, an increased risk of head and neck cancer has been found among the subgroup of never-smokers.

There are suggestions that nasal use of STP increases the risk for certain cancers, e.g. oral cancers.

It appears that the use of smokeless tobacco increases the risk of death after myocardial infarction, but that it does not increase the risk of myocardial infarction. Animal experiments and human studies indicate that oral tobacco use has short-term effects resulting in an increase of blood pressure and heart rate. Whether long-term use increases the risk of hypertension is uncertain. These data indicate a potential effect on the risk of cardiovascular disease.

Studies of reproductive effects in female Swedish users of moist snuff indicated an increased risk for prematurity and pre-eclampsia. Other studies indicate that the use of STP during pregnancy is associated with reduced birth weight and reduction in gestational age. However, the data on reproductive effects in relation to oral tobacco use during pregnancy are too sparse to allow conclusions.

Various studies suggest that diabetes and other components of the metabolic syndrome might be associated with the use of moist snuff, but these findings must be interpreted with caution, in particular because of study design limitations.

3.7. Smokeless Tobacco in Smoking Initiation / Cessation and Abuse of other Substances

3.7.1. Smokeless tobacco and smoking initiation

Galanti et al. (2001a, 2008) followed a cohort of 2,938 adolescents, based in the Stockholm region of Sweden, with annual follow-ups from ages of 11 to 18 years. The majority of tobacco users of both sexes (70%) started using tobacco by smoking cigarettes, 11% took up *snus* before smoking, and 19% used both tobacco types for the first time during the same year. Subjects who at baseline reported having used tobacco

already had a higher risk of being current smokers and/or smokeless tobacco users at age 18 compared to never users. The lowest excess relative risk was observed for those who only had used snus and the highest among those who had already experimented with both products. Adolescents who at any time initiated tobacco use with cigarettes or with both tobacco types, had a higher probability than "snus starters" to end up as current smokers (adjusted OR for "cigarette starters"=1.42, 95% CI=0.98-2.10; OR for "mixed starters"=2.54, 95% CI=1.68-3.91). Only "mixed starters" had a higher probability of being current users of any tobacco at age 18, compared with "*snus* starters". However, marked sex differences were observed in these associations, as initiation with cigarettes rather than with *snus* predicted current smoking and tobacco use only among females. Increasing age at initiation was associated with a decreased risk of becoming a current user of tobacco, independent of product order or sex. Intensity of tobacco consumption at end of follow-up did not vary with product order of initiation. It was concluded that at the most, 6% of the final smoking prevalence in the cohort could theoretically be attributable to a "gateway" effect of *snus*.

Order of initiation with snus or cigarettes is a predictor of progression of tobacco use among female adolescents, but not among male adolescents. Young age and initiation with both tobacco types very close in time predict escalation of use.

Haddock et al. (2001) studied 7,264 recruits enlisted in the US Air Force for one year. The mean age at recruitment was 19 years, and different sorts of STP were used daily by 403 men at the time, whereas 198 were ex-users. At follow-up 27% of the daily users of STP, and 26.3% of the ex-users reported smoking in the last week. Among men who had never used STP smoking in the last week was reported by 12.9%. In a regression model controlling for ethnicity and income, STP users (OR=2.33, 95% CI: 1.84-2.94) and ex-users (OR=2.27, 95% CI: 1.64-3.15) were significantly more prone to report smoking at follow-up than never-users. The investigators found that STP use was a stronger predictor for initiation of smoking than a row of other characteristics such as rebelliousness, use of safety belts, alcohol use and abuse, lack of exercise and eating less fruit and vegetables.

Tomar (2003a) investigated moist snuff uptake in a representative cohort of American 11-19 year-olds. The study started in 1989 and was followed up in 1993. Tobacco habits were collected from 3,996 boys on both occasions. Data were collected by self report which may have resulted in under-reporting and low estimates of prevalence and intensity of use. It was found that boys who were using STP at recruitment were more than 3 times as likely to be smokers 4 years later (23.9% versus 7.6%; controlled OR=3.45, 95% CI: 1.84-6.47) than boys who were non-users. In contrast, the investigators found that only 2.4% of those who were smoking at the onset, and only 1.5% of the non-smokers had started to use STP after 4 years. More than 80% of those who smoked at study start continued to smoke 4 years later. It was concluded that STP was a gateway to smoking and that STP had little effect on smoking cessation in that age group.

O'Connor et al. (2003) used the very same data set and the same methods as Tomar (2003a), but included a set of psycho-social risk factors in the regression analysis. In this re-analysis self-reports of school achievements, depressive symptoms and smoking in the family were included. O'Connor et al. (2003, 2005) have criticised Tomar´s (2003a) study for not having controlled for underlying variables known to be important for smoking initiation. The expanded model used by O'Connor reduced the number of observations for the different outcomes. Hence O'Connor´s positive correlation (OR=1.97; 95% CI: 0.69-5.65) did not reach significance as it was only based upon 34 observations.

Tomar has since (Tomar 2003b) used O'Connor´s analytic method restricted to boys not yet 16 at study start. Results show a significant OR of 1.67 (95% CI: 1.03-2.70) in a model including ethnicity, region, experimentation with cigarettes, school achievement,

smoking in the home, depression, and other abuse. All analyses performed on this national cohort points to a positive relation between STP and smoking initiation. However, the small numbers of STP users make results imprecise.

Two retrospective studies conducted in Sweden on Swedish snus, arrive at a different conclusion. From a cross-sectional survey of 3,125 men reporting on their tobacco histories, it was concluded that the odds of initiating daily smoking was significantly lower for men who had started using snus than for those who had not (OR: 0.28, 95% CI: 0.22-0.36). Among males who had started out as smokers, 28% switched to snus whereas 72% were persistent smokers (Ramstrom and Foulds 2006). In the study by Furberg et al. (2005) on the Swedish Twin Registry it was found that only 0.5% of men who ever smoked used snus "now and then" before they started smoking, while 1.1% of never smokers reported that they used snus "now and then". "Now and then" snus use was also inversely associated with ever smoking status (OR=0.5, 95% CI: 0.3-0.7), suggesting that men who used snus regularly or "now and then" before they began smoking were less likely to ever smoke.

3.7.1.1. Conclusion on the role of smokeless tobacco in smoking initiation

No systematic reviews have been published on the subject. The Swedish data, with its prospective and long-term follow-up do not lend much support to the theory that smokeless tobacco (i.e. Swedish snus) is a gateway to future smoking. In the USA, the interpretation of two studies is divergent. The marked social, cultural and product differences between North America and Europe, suggest caution in translating findings.

3.7.2. Smokeless tobacco and smoking cessation

3.7.2.1. Smokeless tobacco and smoking cessation trends

Rodu et al. (2003) followed 1,651 men and 1,756 women 25-64 years old in northern Sweden. New respondents were enrolled in 1986, 1990 and 1994, and they were all followed up in 1999.

In this study the investigators focused on stability of tobacco habits over 5-13 years.

It was found that smokers who had never used snus continued to smoke (57%, N=195) significantly more often than those smokers who had reported earlier experience with snus (37%, N=46).

Among men who used both products at study start (N=67), 39% continued to do so, 12% had stopped using tobacco, 43% used snus only whereas only 6% were strict cigarette smokers.

During the observation period, women more often continued to smoke (69%) than men (54%). This sex difference was interpreted as being secondary to a higher snus use among men than among women. All results were controlled for length of education, living conditions, age and time for enrolment.

At the onset of a 1-year longitudinal study of 3,550 daily smokers aged 45-69 years in 1992, Lindstrom et al. (2002) studied factors that could predict cessation and/or transition from daily to occasional smoking. At inclusion 7% of the men and 0.4% of the women used snus. At follow-up in 1994, 7.2% of the daily smokers had stopped and 6.5% had become occasional smokers. Cessation was significantly higher among men (8.4%) than among women (6.4%), but there was no difference in transition from daily to occasional smoking (6.5% men vs 6.4% women). Among male daily smokers who had

become occasional smokers (transitional smokers) 15.3% were using snus at study start. Among men who stopped smoking 12.7% were snus users at study start. The fraction of snus users at study start was only 5.6% among those men who continued to smoke daily (stable smokers). In a multiple logistic regression analysis controlling for sex and other demographic characteristics it was found that the stable daily smokers were significantly less prone (compared to the general population) to having been snus users at study start (OR=0.67, 95% CI: 0.51-0.87). Transitional smokers were significantly more often snus users at study start (OR=1.94, 95% CI: 1.07-3.51). However, at study start the fraction of snus users among successful quitters was no different than in the general population (OR=1.1, 95% CI: 0.54-2.26). It was also found that the fraction of snus users at study start among smokers who later successfully stopped smoking was no different to that of the study population at large (OR=1.1, 95% CI: 0.54-2.26).

Wetter et al. (2002) studied changing patterns of tobacco use from 1990 to 1994 in the southeastern United States among 220 blue collar working men who used both products. Compared to exclusive smokers (15.7%) and exclusive users of STP (20.1%), the mixers (11.3%) were less prone to quit smoking. The study had problems with follow up rates (52-66%) and the authors did not separate the different STP.

In the retrospective study by Ramstrom and Foulds (2006) on 3,125 Swedish men, 58% of the men who had made quit attempts had used snus (moist snuff) as a single cessation aid, compared to 38% of all other nicotine products combined. Among men who used snus as a single aid, 66% succeeded in quitting completely, as compared with 47% of those using nicotine gum (OR=2.2, 95% CI: 1.3-3.7) or 32% for those using the nicotine patch (OR=4.2, 95% CI: 2.1-8.6) (Ramstrom and Foulds 2006). In the Swedish Twin Registry study cited above, a similar conclusion was made. The OR for "regular" snus use and former smoking status was 3.7 (95% CI: 3.3-4.2), indicating that men who used snus "regularly" were over three times more likely to be former smokers than current smokers (Furberg et al. 2005). Questions arise whether the observations made in Sweden are transferable to other countries where snus is largely unknown. The fact that former smokers who have taken up snus tend to become chronic snus users could explain the relative advantage of snus as a cessation agent over pharmaceutical nicotine products which are used for shorter periods.

In a random telephone retrospective survey of Swedish smokers and ex-smokers conducted in 2000 a national sample of 1,000 former and 985 current daily smokers aged 25–55 years were interviewed (Gilljam et al. 2003). According to self-reports 33% of former smokers and 27% of current smokers had ever used snus. The difference was larger among men (55% versus 45%) (p=0.003). Current smokers who made use of snus smoked on average fewer cigarettes per day than non-users of snus. The mean duration of abstinence among former smokers was not influenced by snus use. Conditionally on age, education and use of nicotine replacement therapy there was an increased probability of being a former rather than a current smoker with ever use (OR=1.72, 95% CI: 1.30–2.28) or current use (OR=1.81, 95% CI: 1.31–2.53) of snus. Having used snus at the latest quit attempt increased the probability of being abstinent by about 50% (OR=1.54, 95% CI=1.09–2.20) but also in a 65% risk of becoming a chronic snus user. The results suggested that Swedish male smokers may increase their overall chances of abstinence. However, 71% of the men in this sample who quit smoking did so without using snus and the duration of abstinence was not affected by snus use. Snus use was very rare among women.

No systematic reviews have been published on the subject.

3.7.2.2. Use of smokeless tobacco in assisted smoking cessation

In an uncontrolled study by Helgason et al. (2004) callers to the Swedish telephone helpline were followed after 12-14 months in order to assess outcomes with reactive and

proactive counselling. At follow up 70% of reactive callers filled in a postal questionnaire (N=496). In a multiple logistic regression analysis controlling for demographic and psycho-social variables as well as nicotine consumption at first contact, stage of change and previous quit attempts, it was found that the use of snus during smoking abstinence resulted in a non-significant increase in rates of abstinence after 12-14 months (OR=1.5, 95% CI: 0.7-3.3). In the same model, 5 weeks use of nicotine replacement treatment increased abstinence rates significantly (OR=2.1, 95% CI: 1.1-4.0). It was concluded that the use of snus did not reach the smoking cessation effects as seen with nicotine replacement products, although it should be noted that these two odds ratios do not differ significantly from each other.

In an uncontrolled clinical study by Tilashalski et al. (1998) 63 smokers were offered commercially available pre-portioned oral tobacco for free and very short initial counselling. At 12 month follow-up 16 out of 63 individuals (25%) had stopped smoking and 13 were still using oral tobacco. The authors suggest that the use of smokeless tobacco merits further evaluation as a smoking cessation strategy.

No further studies have been found.

3.7.2.3. Conclusion on the role of smokeless tobacco in smoking cessation

Observational data from Sweden indicate that snus has been used more often than pharmaceutical nicotine products by some men as an aid to stop smoking. The data are consistent in demonstrating these male snus users are more likely to quit smoking than non-users. In these uncontrolled, retrospective studies, results on par with those achieved with nicotine replacement products and above, are quoted. A side effect, however, is that 60% or more smoking abstainers become chronic snus users. There are no published randomised clinical trials of use of smokeless tobacco in smoking cessation, and in the absence of such evidence it is not possible to draw reliable conclusions as to the relative effectiveness of smokeless tobacco as an aid to clinical smoking cessation in comparison with either placebo or other established therapies.

3.7.3. Smokeless tobacco and abuse of other substances

There exist relatively few data on the role of STP in the use and abuse of other substances. Those data which do exist are typically correlational in nature, and suggest that the simultaneous use of various substances, including smokeless tobacco, is very frequent (Ary et al. 1987, Galanti et al. 2001b, Kao et al. 2000). Such data, however, do not alone provide strong grounds for concluding that the association between smokeless tobacco use and the abuse of other substances is causal, although there is evidence from cigarette smoking that tobacco may act as a "gateway" drug, increasing the likelihood of subsequent use of other substances (Lai et al. 2000). In particular, there is some evidence that smokeless tobacco use may increase the likelihood of progression to subsequent cigarette smoking (Tomar 2003a, Tomar 2003b), which itself is regarded as a gateway drug to other substance use (Lai et al. 2000). Therefore, one possibility is that smokeless tobacco use may act as a gateway drug to other substance use either directly or indirectly (via effects on cigarette smoking). However, although there is some evidence for association between smokeless tobacco use and cigarette smoking initiation, this effect may be small and, at least in part, confounded by other sociodemographic factors (see chapter 3.7.1).

There is some evidence that smokeless tobacco use itself may be associated with an increased likelihood of other substance use, although not necessarily causally. This evidence indicates that the majority of smokeless tobacco users concurrently use alcohol,

marijuana and/or cigarettes (Ary et al. 1987, Galanti et al. 2001b), and that the relationship between smokeless tobacco use and other substance use is dose-dependent (Everett et al. 1998). Furthermore, there is some evidence that smokeless tobacco use is a prospective risk factor for the onset or increased use of these substances (Ary 1989, Ary et al. 1987), as well as an increased likelihood of engaging in other risky behaviours (Everett et al. 2000). Such data do not afford strong grounds for drawing conclusions regarding causation, however, and simply indicate co-occurrence. A reasonable conclusion to draw is that smokeless tobacco use is an additional activity in which adolescents experimenting with drug use are likely to engage in (Dent et al. 1987, Murray et al. 1988). One limitation to such research is that the majority has been conducted in North America.

3.7.3.1. Conclusion on the role of smokeless tobacco for the abuse of other substances

Therefore, there is some evidence that smokeless tobacco use is a risk factor for the onset or increased use of other substances, suggesting that smokeless tobacco use may operate as a "gateway" drug directly, in the same way as has been suggested for cigarette smoking, as well as indirectly via the increased likelihood of progression to cigarette smoking. This evidence is not compelling, however, and may be the result of latent (e.g. sociodemographic) variables increasing the likelihood of all substance use as part of a broader spectrum of risky and impulsive behaviours in adolescence. Further caution is also necessary, as this evidence is largely based on data from North American samples only, although the finding that smokeless tobacco use and other substance use occur simultaneously has been replicated in European samples (Galanti et al. 2001b).

3.7.4. Conclusion on the role of smokeless tobacco for the use of tobacco and other substances

In the only published prospective study on snus use among children and adolescents it was concluded that at the most, 6% of the final smoking prevalence in the cohort could theoretically be attributable to a "gateway" effect of *snus*. In the North American studies on STP the results in this respect were divergent. In Sweden, snus seems to have played a role as a cessation agent for a minority, again about 6% of men who succeeded in quitting smoking. About 2/3 of this minority ended up as chronic snus users. Snus use for cessation purposes was very rare among women. Data from other countries and products are missing. No controlled studies of STP used as smoking cessation treatment have been found. Overall, there is no compelling evidence that smokeless tobacco is a risk factor for other substances of abuse, although a clustering of drug use, including STP, has been observed.

3.8. Smokeless tobacco, public health, and the harm reduction argument

This report has presented evidence that STP are addictive and hazardous to health. Judged only on these grounds, use of STP should clearly be discouraged and as far as possible, prevented. However, there is a further and potentially important public health consideration that arises from the trends in use of snus and smoking in Sweden, and on the relative harm associated with smokeless and smoked tobacco use that deserves consideration. It has been suggested from national data on tobacco use in Sweden (Swedish National Board of Health and Welfare 2005), and in particular, data from the MONICA cohort in northern Sweden, that snus has been used there by smokers as an alternative to smoking (either as a stage in a quitting process, or as a long-term

substitute), and by young people in place of starting smoking (Rodu et al. 2002, Stegmayr et al. 2005). However, as discussed in this report it is not clear whether or how much the availability of snus has played a role for the decreasing smoking prevalence. Whilst there is no doubt that complete abstinence from tobacco use would be the safe and preferred option for all of these snus users, the pragmatic argument is that if in practice the alternative for them would be to smoke tobacco, then if snus use is less hazardous than tobacco smoking, substitution of snus for smoking may be beneficial to individual and public health (Tobacco Advisory Group of the Royal College of Physicians, 2002, Kozlowski 2002, Bates et al. 2003, Fagerström and Schildt 2003, Foulds et al. 2003, Swedish National Board of Health and Welfare 2005, European Respiratory Society 2006, Foulds and Kozlowski 2007, Tobacco Advisory Group of the Royal College of Physicians 2007).

Cigarettes are highly addictive (Royal College of Physicians 2000), kill half of all regular users (Doll et al. 2004), and are currently used regularly by about 100 million people in the EU (TNS Opinion & Social 2006). Fifty million of these people, who are current smokers now, will die prematurely with the loss of an average of ten years of life, unless they quit smoking (Doll et al. 2004). Smoking currently causes at least 650,000 deaths in the EU each year, and serious illness in around 13 million people (The ASPECT Consortium 2004). Passive smoking kills 80,000 EU adults, predominantly from cardiovascular disease and lung cancer, every year (Smoke Free Partnership 2006). In children, passive smoking reduces lung growth and causes sudden infant death syndrome (SIDS), acute respiratory infections, middle ear disease, respiratory symptoms and more frequent and severe asthma attacks in children (US Surgeon General 2006). Smoking is thus a massive public health problem.

Conventional public health strategies to reduce the prevalence of smoking (World Bank 2003, WHO 2003) are effective in reducing incident smoking and promoting cessation (Biener et al. 2000, Chen et al. 2003, Gilpin et al. 2006, Pierce et al. 1998, White et al. 2003, Levy et al. 2004a), but the rate of the reduction they achieve in practice is slow. In the UK for example, where tobacco control policy has been relatively well advanced for some years (Joossens and Raw 2006), smoking prevalence is now falling at a rate of approximately half a percentage point per year (Jarvis 2003, Taylor et al. 2006). Although some countries, including Norway (see section 3.3.3.2) and Canada (Health Canada 2007) have achieved recent declines in prevalence of one percentage point per year, it is evident that even if the entire EU implemented all recognised population tobacco control strategies in all member states immediately, it would take years, probably decades, to reduce the prevalence of smoking even by half. Those who continue to smoke will tend to be the more heavily addicted smokers from the most disadvantaged social groups (Jarvis and Wardle 1999), thus exacerbating social inequality in health. The harm reduction argument is that if snus or other relatively low hazard STP can provide some smokers who will not otherwise quit smoking with a less hazardous source of nicotine that is acceptable to them, then the use of snus as a harm reduction option deserves consideration (Tobacco Advisory Group of the Royal College of Physicians 2002, Kozlowski 2002, Bates et al. 2003, Fagerström and Schildt 2003, Foulds et al. 2003, Swedish National Board of Health and Welfare 2005, European Respiratory Society 2006, Foulds and Kozlowski 2007, Tobacco Advisory Group of the Royal College of Physicians 2007).

If so, it is appropriate to consider the potential benefits, as well as risks, to public health if snus were to be made available elsewhere in Europe. In this context, it matters less whether snus is harmful relative to no tobacco use than how harmful snus or other STP use is in relation to cigarette smoking, both among STP users compared with smokers who never used STP, and among smokers who switch from tobacco smoking to STP use. It is also important to consider what effect wider availability of STP such as snus would have on the prevalence of smoking and all tobacco use if made available to populations that had not previously used the product.

3.8.1. How harmful are smokeless tobacco products in relation to cigarette smoking?

The harm associated with STP and smoked tobacco use varies in relation to different tobacco-related diseases, and for some outcomes differs between STP. However, since to date there is no evidence that STP use is associated with any major health hazard that does not also arise from tobacco smoking, the most important comparisons of relative hazard from a public health perspective are those relating to the major diseases associated with smoking. These are respiratory disease, cardiovascular disease, and cancer.

Respiratory disease: Respiratory diseases, predominantly lung cancer, COPD and pneumonia, account for 46% of the deaths caused by cigarette smoking in the EU (The ASPECT Consortium 2004). There is no consistent evidence that any STP cause any of these major respiratory diseases. Complete substitution of STP for tobacco smoking would thus ultimately prevent nearly all deaths from respiratory disease currently caused by smoking, which in total represent nearly half of all deaths caused by smoking.

Cardiovascular disease: Cardiovascular disease accounts for 28% of deaths caused by smoking in the EU (The ASPECT Consortium 2004). For snus, several published studies provide estimates of relative risk for both snus and smoking in the same populations, and all indicate that the risk of snus use is less. In a cohort of Swedish construction workers Bolinder et al. reported an overall relative increase in cardiovascular mortality among snus users of 1.4 in 12 years of follow-up (2.1 in those aged 35-54 at the outset), compared with 1.9 and 3.2 in smokers (Bolinder et al. 1994). A more recent follow up of the same cohort identified a significant increase in risk of fatal myocardial infarction among heavy users of snus in the cohort, but did not provide effect estimates for smokers (Hergens et al. 2007). The Swedish MONICA study found no increase in risk of myocardial infarction in regular snus users (Huhtasaari et al. 1999, Huhtasaari et al. 1992), the adjusted relative odds of myocardial infarction among snus users being 0.58 (95% CI 0.35 to 0.94) and in smokers 3.53 (95% CI 2.48 to 5.03) (Huhtasaari et al. 1999). For fatal myocardial infarction the adjusted odds ratios were respectively 1.50 (0.45 to 5.03) and 8.57 (95% CI 2.48 to 30.3) (Huhtasaari et al. 1999). More recent analysis of the MONICA cohort confirms this finding, the fully adjusted relative odds of myocardial infarction, relative to non-tobacco users, being 0.82 (0.46–1.43) in never smoking current snus users, 2.60 (1.91–3.54) in current smokers who are not snus users, and 2.14 (1.28–3.60) in current smokers who also use snus (Wennberg et al. 2007). A recent case control study estimated the odds of acute myocardial infarction among never-smoking snus users to be 0.73 (0.35–1.5) and in smokers who did not use snus 2.8 (2.3–3.4); for non-fatal myocardial infarction the respective odds ratios were 0.59 (0.25–1.4) and 2.7 (2.2–3.3); and for fatal myocardial infarction 1.7 (0.48–5.5) and 3.6 (2.4–5.2) (Hergens et al. 2005). A longitudinal analysis of 15 years of follow up of men in the Swedish Survey of Living Conditions reported incidence rate ratios for ischaemic heart disease of 0.77 (0.51–1.15) in snus users and 1.74 (1.41–2.14) in smokers; for fatal ischaemic heart disease the ratios were 1.15 (0.54–2.41) and 1.98 (1.35–2.91) respectively (Haglund et al. 2007). There was no increased risk relative to smokers among smokers in this study who also used snus. The risk of stroke was also lower among snus users (Incidence rate ratio 1.1, 95% CI 0.7–1.8) than among smokers in this study (Incidence rate ratio 1.4, 95% CI 1.0–1.9), a finding consistent with other published studies comparing these risks (Asplund et al. 2003b, Bolinder et al. 1994, Gupta et al. 2005).

The recent *INTERHEART* study findings indicate that cardiovascular risk is higher with other STP, estimating an odds ratio for myocardial infarction of 2.23 (95% CI 1.41 to 3.52) in non-smoking users of chewing tobacco (Teo et al. 2006). In this study the users of chewing tobacco were predominantly from South Asian populations (due to small numbers of users of snuff or paan (betel quid), the study did not present results for these

types of STP The odds ratio for myocardial infarction in cigarette smokers in this study was about 30% higher than that for STP, at 2.95 (95% CI 2.77 to 3.14) (Teo et al. 2006). The *INTERHEART* study also raised a concern that combined use of STP and smoked tobacco may be particularly hazardous, since the estimated odds ratio for myocardial infarction for those who combined STP use with smoking was higher than that of either product alone, at 4·09 (95% CI 2.98 to 5.61) (Teo et al. 2006). However, this finding was not confirmed in the studies of dual use of smoking and snus (Haglund et al. 2007, Wennberg et al. 2007).

Thus the evidence indicates that if snus use increases the risk of myocardial infarction it does so to a lesser extent than smoking. The reduction in risk is difficult to quantify, but for snus, using the Bolinder study of 1994 (Bolinder et al. 1994) as a conservative estimate, is around 50%. The other studies listed above indicate that the relative risk associated with snus use compared to smoking is probably substantially lower than this. It is therefore reasonable to draw a conservative conclusion that substitution of smoking by snus use would, in due course, reduce the cardiovascular mortality that currently arises from tobacco use by at least 50%.

Oral and GI cancer: Although responsible for relatively few deaths in comparison with the above causes among smokers, the combined risk of oral and pharyngeal, esophageal or pancreatic cancer is increased by smokeless tobacco use and are therefore important to consider. A study in Norwegian snus users estimated the relative risks of oral or pharyngeal cancer at 1.10 (95% CI 0.50 to 2.41), stomach cancer at 1.11 (95% CI 0.83 to 1.48), oesophageal cancer at 1.40 (95% CI 0.61 to 3.24), and of pancreatic cancer at 1.67 (95% CI 1.12 to 2.50) (Boffetta et al. 2005). This study did not provide smoking-specific risk estimates for these outcomes, but estimates are available for Swedish smokers in other studies, at 2.4 (95% CI 1.3 to 4.1) for oral cancer (Rosenquist et al. 2005) and 2.5 (95% CI 1.7 to 3.6) for pancreatic cancer (Fuchs et al. 1996). A recently reported Swedish study confirms however an increased risk of pancreatic cancer in snus users by a ratio of 2.0 (95% CI 1.2 to 3.3) for ever-users, compared to 2.8 (95% CI 2.1 to 3.7) in ever smokers (Luo et al. 2007). This study found no evidence of increased risk of oral cancer in ever-users of snus (relative risk 0.8, 95% CI 0.4 to 1.7) but a significant increase in ever-smokers (relative risk 2.0, 95% CI 1.4 to 2.7) (Luo et al. 2007). Thus it is evident that the risk of pancreatic cancer associated with snus use is less than that of smoking, and for oral cancer substantially so. Since the numbers of deaths from these diseases is relatively small, the public health impact of this reduced risk, if snus were to replace smoking, would also be modest.

Passive smoke effects: Since STP do not produce smoke they will not cause any of the health problems linked to passive smoke exposure in adults or children. Substitution of snus for smoked tobacco would therefore prevent the passive smoke-related diseases.

STP use in pregnancy: Maternal use of snus during pregnancy is associated with a reduction in birthweight of approximately 39g, compared with 190g in smokers in the same study (England et al. 2003). Use of snus was also associated with increased risks of preterm delivery (odds ratio 1.98, 95%CI 1.46 to 2.68) and pre-eclampsia (odds ratio 1.58, 95% CI 1.09 to 2.27) that were both higher than in smokers (odds ratio 1.57, 95% CI 1.38 to 1.80, and 0.63, 95% CI 0.53 to 0.75) respectively.

Other diseases caused by smoking: Evidence on the relative hazard of STP, and particularly snus, on other major smoking-related diseases is relatively sparse. However no other major areas of concern have been identified.

Overall therefore, in relation to the risks of the above 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. The magnitude of the overall reduction in hazard is difficult to estimate, but as outlined above, for cardiovascular disease is at least 50%, for oral and

GI cancer probably also at least 50%, and for respiratory disease close to 100%. A recent study using a modified Delphi approach to estimate the relative hazard of snus concluded that the product was likely to be approximately 90% less harmful than smoking (Levy et al. 2004b). An analysis based on this estimate of risk reduction applied in Australia recently concluded that current smokers who switch to using snus rather than continuing to smoke would realise substantial health gains (Gartner et al. 2007), though their precise magnitude is difficult to quantify.

2) Switching from tobacco smoking to use of smokeless products compared to continued smoking

The hazard of sustained use of STP in men who switch from smoking has been estimated in an observational study by Henley et al (2007), who compared men who switched from cigarette smoking to use of spit tobacco ("switchers") to men who quit using tobacco entirely ("quitters") in the American Cancer Society cohort. After 20 years of follow-up, the hazard ratio for overall mortality in switchers relative to those who quit completely was 1.08 (95% CI 1.01 – 1.15). Switchers had a higher mortality from cancer of the oral cavity and pharynx than quitters (RR 2·6, 95% CI 1·2, 5·8). Compared to quitters, the RR of lung cancer among all switchers, switchers to chew only, snuff only and chew and snuff combined were 1·5 (95% CI 1·2, 1·7), 1·3 (95% CI 1·1, 1·6), 1·9 (95% CI 1·2, 2·5) and 2·0 (95% CI 1·2, 3·0), respectively. Compared to men who never used any tobacco product, the RR of lung cancer among quitters and among switchers were 3·9 and 5·6, respectively.

3.8.2. Potential public health impact of the availability of moist snuff on the tobacco market

The extent and nature of the impact on public health of making moist snuff available in new markets will depend on the relative hazard of STP and smoking, and the relative uptake and use by smokers and non-smokers. Given that snus use is less hazardous than smoking, the overall effect on public health will come down to the balance between:

Beneficial effects on smoking prevalence

- Use of snus by existing smokers, who would not otherwise have quit smoking, as a complete substitute and/or cessation aid
- Use of snus but not cigarettes by new tobacco users (predominantly adolescents) who would otherwise have started to smoke

Adverse effects on overall prevalence of tobacco use

- Uptake of snus by new tobacco users who would otherwise have never smoked
- Uptake of snus and subsequent progression to regular smoking in individuals who would otherwise have never smoked
- Smokers who would otherwise have quit smoking and all tobacco use completely, instead quitting smoking but becoming regular snus users
- Smokers who would otherwise have quit smoking and all tobacco use completely, instead using snus to assist cutting down but continuing to use both snus and cigarettes

The balance of these effects will be highly dependent on the marketing of the product, the health messages delivered with it, and the extent to which switching to STP as a harm reduction strategy is endorsed by health professionals and their organisations. Levy and colleagues estimated the impact of introducing a product such as snus into the United States market, promoted with a warning label stating: *"This product is addictive and may increase your risk of disease. This product is substantially less harmful than cigarettes, but abstaining from tobacco use altogether is the safest course of action."* would reduce the prevalence of smoking by between 1.3 and 3.1 percentage points over

five years (Levy et al. 2006). That is an annual decline of between 0.25 and 0.6 percentage points per year, or approximately 0.4 percentage points per year.

Data from the MONICA cohort study in Northern Sweden on self-reported lifetime use of cigarettes and snus by men and women between 1986 and 1999, reported by authors in receipt of tobacco industry funding, provide evidence that the availability of snus and the relative cultural acceptability of the product among men may have had an impact on the prevalence of smoking in men, of an order of magnitude consistent with the above estimate (Rodu et al. 2002). Unlike the data on trends in cross-sectional prevalence of smoking and STP use reported in Section 3.3.3., these data are based on within-subject behaviour and so provide insight into patterns of migration between tobacco products within users. To our knowledge these are the only within-person longitudinal data of this kind available. The study reported that in this population in northern Sweden the overall prevalence of tobacco use in men remained relatively constant at around 40% over the duration of the study, the overall prevalence of smoking fell by 9 percentage points (from 23 to 14%), and STP use rose by 8 percentage points (from 22 to 30%), as a result of a substantial net migration from smoking to STP. In women the overall prevalence of tobacco use was also relatively stable but snus was not so extensively used. Smoking prevalence in women fell by 5 percentage points (from 27 to 22%), and STP use rose by 8 percentage points (from 0 to 8%). Migration from snus use to smoking was uncommon in both sexes. A recent follow-up of this cohort found that by 2004 the prevalence of smoking in men had fallen to 9%, and in the 25-34 age-group to 3% (Stegmayr et al. 2005). The prevalence of all tobacco use remained relatively constant at over 35%.

These reports suggest that in northern Sweden, the availability of snus and the way in which it has been used may have been beneficial to public health since the harm to health caused by any use of snus as a gateway into smoking may have been more than outweighed numerically by the numbers quitting smoking for snus. This observation is supported by evidence from Galanti (2008) that gateway progression from snus to smoking has not been a significant problem in Swedish young people. The prevalence of daily smoking in Sweden is currently the lowest in the EU. Although this undoubtedly reflects the effect of other tobacco control measures, this is not necessarily the sole explanation as Sweden ranks only 6th amongst the EU 25 countries in terms of overall tobacco control policy implementation, behind Iceland, UK, Norway, Ireland and Malta, all of which have higher smoking prevalences than Sweden (TNS Opinion & Social 2006). It is therefore possible that the particularly low smoking prevalence in northern Sweden reflects some of the estimated attributable effect of the availability of STP (Swedish National Board of Health and Welfare 2005).

Is it possible to predict the impact of the introduction of smokeless products into new markets?

The health impact of the introduction of STP to new markets will depend substantially on a number of factors, including:

- the extent to which the product is marketed and endorsed as a healthier choice than smoking
- the cultural acceptability of the product
- the extent of abuse of marketing by the tobacco industry to promote smokeless tobacco as a starter product for young people
- price and availability relative to cigarettes and medicinal nicotine products
- the extent to which the product is used as an exit rather than entry stage in tobacco use
- the extent and success of measures taken to maximise health benefits through monitoring and controlling the marketing and use of the product

- the hazard of the STP, used alone or in combination with smoking.

One recent modelling study has suggested that the adverse effects of use of snus by people who would not otherwise smoke, or would have quit tobacco use completely rather than switching to snus, would probably be substantially outweighed by the health gains realised by smokers who switch to snus or quit entirely through snus. In this study, the availability of snus was considered likely to produce a net benefit to the health at the population level (Gartner et al. 2007) The estimated years of life lost by male smokers, male smokers who quit smoking, male smokers who switch to snus, and male snus users who never smoke are represented in the figure, drawn from data tabulated in the Gartner et al. (2007) paper:

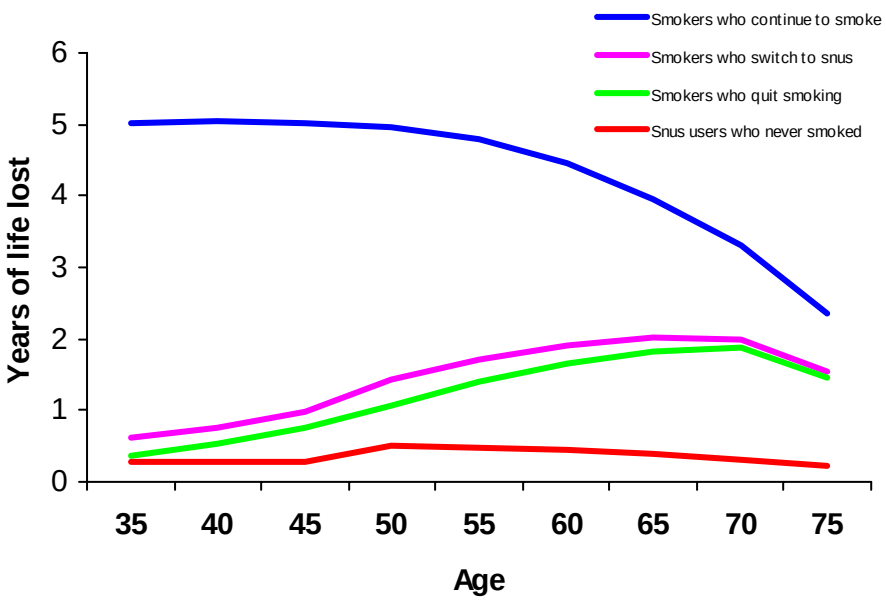

Figure 30. Estimated years of life lost by male smokers, male smokers who quit smoking, male smokers who switch to snus, and male snus users. Drawn on the basis of data from Gartner et al. (2007)

The data indicate that the health benefit experienced by a smoker who switches to snus but would not otherwise have quit smoking is substantially greater than the risk of snus use, and that whilst snus use among people who would have never otherwise have used a tobacco product will have a detrimental effect on individual and public health, this effect is relatively small. Widespread uptake of snus by young people is therefore likely to result in a modest net adverse effect on public health only if it occurs exclusively among people who would not otherwise have smoked. Thus in Sweden, where there has apparently been substantial transfer from smoking to snus, the availability of snus may have been beneficial to public health. In Norway, where to date there is little evidence of switching from smoking to snus but clear evidence of uptake by young people (see Section 3.3.3.2), the evidence points to an overall population harm from the availability of snus. However Gartner and colleagues estimate that the benefits accrued by one person not taking up smoking as a result of the availability of snus will offset the harm experienced by between 14 and 25 people who take up snus but would not otherwise have used any tobacco product (Gartner et al. 2007). According to Gartner's model, the overall effect is therefore likely to be beneficial.

Conclusion on the comparison of smokeless tobacco with smoking

It is possible that introducing snus in EU countries that do not presently allow the product to be marketed would eventually contribute to some or all of the following beneficial outcomes:

- Reduced initiation of cigarette smoking
- Increased cessation by switching to smokeless tobacco
- Reduced smoking-associated disease

It also must be recognised that it is possible that the overall health outcome of introducing smokeless tobacco products could be adverse due to the following possible outcomes:

- Increased overall tobacco use without substantial decline in cigarette smoking prevalence
- Impaired tobacco prevention efforts due to 'mixed messages' that attempt to advise against any tobacco use, but favour certain forms over others
- Undermining tobacco cessation efforts
- Uptake of smokeless tobacco in populations who would otherwise have not likely used any tobacco product

The balance of the benefits and risks listed above will vary according to circumstances of individuals and population groups. However, for those who substitute smoking by STPs the benefits outweigh the risks.

4. OPINION

DG SANCO has requested SCENIHR to answer the following questions:

1. What are the adverse health effects of smokeless tobacco products?
2. What is the addiction potential of smokeless tobacco products?
3. Does the available data support the claim that smokeless tobacco may constitute a smoking cessation aid comparable to pharmaceutical nicotine replacement products?
4. What is the impact of smokeless tobacco use on subsequent initiation of smoking?
5. Is it possible to extrapolate the information on the patterns of smokeless tobacco use, smoking cessation and initiation from countries where oral tobacco is available to EU-countries where oral tobacco is not available?

In this opinion the smokeless tobacco products are defined according to the EC Tobacco Products Directive (2001/37/EC): "Tobacco for oral use means all products for oral use, except those intended to be smoked or chewed, made wholly or partly of tobacco, in powder or in particulate form or in any combination of those forms, particularly those presented in sachet portions or porous sachets, or in a form resembling a food product". Synonyms for "tobacco for oral use" are moist snuff (called snus in Sweden) and oral tobacco.

The Scientific Committee has the following answers to the questions:

Question 1: What are the adverse health effects of smokeless tobacco products?

In answering this question, it must be recognised that marketed smokeless tobacco products (STP) vary considerably in form and content of toxicants, including nicotine, and thereby in associated health effects, which have been documented across countries.

All STP contain nicotine, a potent addictive substance. The major group of carcinogens in STP includes non-volatile tobacco-specific nitrosamines (TSNA) and *N*-nitroamino acids. During the last two decades the levels of TSNA in snus have been considerably lowered. One recent study documented total TSNA levels in one brand of Swedish snus to be 2.0 microgram/gram product wet weight, whereas total TNSA levels in 6 American brands varied from 1.3 to 9.2 microgram/gram. Levels of TSNA in STP from other regions such as India and Africa are higher. Some forms of STP contain polycyclic aromatic hydrocarbons depending on type of curing.

Aqueous and organic extracts of American and Swedish moist snuff and Indian chewing tobacco cause mutations and chromosomal damage in bacterial and mammalian cell cultures. Increased micronuclei formation in oral epithelial cells as evidence of chromosomal damage, has been associated with moist snuff use.

Use of American and Swedish moist snuff results in localised lesions in the oral epithelium, where the snuff is placed. These changes are reversible, whereas gingival retractions caused by moist snuff are not reversible. Moist snuff in portion-bag sachets gives less severe epithelial changes than snuff in loose form.

There is sufficient evidence that the use of a wide variety of STP causes cancer in humans. The pancreas has been identified as a main target organ in two Scandinavian cohort studies. Furthermore, several studies from the USA have provided additional support for a causal association between the use of smokeless tobacco and pancreatic cancer. There is inadequate evidence that STP cause lung cancer.

Risks of oral cancer have been found to be strongly associated with the use of American snuff in the USA. Four studies in India and Pakistan and one study from Sudan have reported large increases in the risk for oral cancers related to the use of various STP. In Sweden, the evidence for an increased risk of oral cancer in snus users is less clear. In one study from Sweden among users of moist snuff, an increased overall risk of head and neck cancer was not detected. However, an increased risk of head and neck cancer has been found among the subgroup of never-smokers. A recent cohort study from Sweden reported a statistically significant three-fold increase of oral and pharyngeal cancer taken together, adjusted for tobacco smoking and alcohol drinking.

There are suggestions that nasal use of STP increases the risk for certain cancers, e.g. oral cancers.

It appears that the use of smokeless tobacco increases the risk of death after myocardial infarction, but that it does not increase the risk of myocardial infarction. In addition, animal experiments and human studies indicate that oral tobacco use has short-term effects resulting in an increase of blood pressure and heart rate. Whether long-term use increases the risk of hypertension is uncertain. These data indicate a potential effect on the risk of cardiovascular disease.

The data on reproductive effects in relation to oral tobacco use during pregnancy are too sparse to allow conclusions. Nonetheless, studies of reproductive effects in female Swedish users of moist snuff indicated an increased risk for prematurity and pre-eclampsia. Other studies indicate that the use of STP during pregnancy is associated with reduced birth weight and reduction in gestational age.

Various studies suggest that diabetes and other components of the metabolic syndrome might be associated with the use of moist snuff, but these findings must be interpreted with caution, in particular because of study design limitations.

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.

In conclusion, all STP contain nicotine, a potent addictive substance. They also contain carcinogenic tobacco-specific nitrosamines, albeit at differing levels. STP are carcinogenic to humans and the pancreas has been identified as a main target organ in American and Scandinavian studies. All STP cause localised 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). It appears that the use of smokeless tobacco increases the risk of death after myocardial infarction, but that it does not increase the risk of myocardial infarction. Some data indicate reproductive effects of smokeless tobacco use during pregnancy but firm conclusions cannot be drawn.

Question 2: What is the addiction potential of smokeless tobacco products?

It is widely accepted that nicotine is the primary addictive constituent of tobacco, and there is a growing body of evidence that nicotine demonstrates the properties of a drug of abuse. All commercially successful tobacco products, regardless of delivery mechanism, deliver psychoactive levels of nicotine to users. Denicotinised tobacco products are typically not widely accepted by or palatable to chronic tobacco users and are of marginal commercial importance.

Smokeless tobacco contains and delivers quantities of nicotine comparable to those typically absorbed from cigarette smoking, although delivery of nicotine from STP lacks the high initial concentration that results from inhalation of tobacco smoke and may therefore have relatively less addiction potential than cigarettes. Nicotine levels obtained

from STP are generally higher than those typically obtained from nicotine replacement therapy which is considered to have a low addiction potential.

The time course and symptoms of withdrawal from smokeless tobacco are generally similar to those of cigarette smokers although depressive symptoms and negative affect do not appear to be observed among abstinent STP users. It seems also that symptoms of withdrawal are stronger with some brands of smokeless tobacco delivering higher levels of nicotine compared to other brands with lower levels.

There is a lack of evidence from animal models for the addictive potential of STP, given the conceptual difficulty in developing an animal self-administration model of smokeless tobacco. There is also a lack of evidence relating to the effects of additives introduced to tobacco in the manufacturing process on the initiation of use of STP and subsequent dependence.

In conclusion, smokeless tobacco is addictive and withdrawal symptoms are broadly similar to those seen in smokers.

<u>Question 3</u>: Does the available data support the claim that smokeless tobacco may constitute a smoking cessation aid comparable to pharmaceutical nicotine replacement products?

No randomized trial has been conducted on smokeless tobacco as an aid to smoking cessation and no randomized trial has compared smokeless tobacco to pharmaceutical nicotine replacement products in this respect.

A small number of observational studies have looked at the use of smokeless tobacco in relation to smoking habits and one of those also includes nicotine replacement products. The results of these studies are inconsistent. Due to this and methodological limitations no conclusions can be drawn.

On the available evidence it is thus not possible to draw conclusions as to the effectiveness of smokeless tobacco as an aid to smoking cessation. Nor it is possible to draw conclusions on its relative effectiveness in comparison with established therapies.

<u>Question 4</u>: What is the impact of smokeless tobacco use on subsequent initiation of smoking?

The association between smokeless tobacco use and cigarette smoking initiation is likely to be confounded by socio-demographic factors. In addition, across countries there are possible differences in risk for which the determinants are not fully understood. The associations observed may be due to an increased likelihood of all substance use (including STP and cigarettes) as part of a broader spectrum of risky and impulsive behaviours in adolescence.

There is some evidence from the USA that smokeless tobacco use may lead to subsequent cigarette smoking. On the other hand the Swedish data do not support the hypothesis that smokeless tobacco (i.e. Swedish snus) is a gateway to future smoking. The marked social, cultural and product differences between North America and Europe suggest caution in translating findings across countries, also within Europe.

<u>Question 5</u>: Is it possible to extrapolate the information on the patterns of smokeless tobacco use, smoking cessation and initiation from countries where oral tobacco is available to EU-countries where oral tobacco is not available?

Presently, Sweden is the only EU-country in which it is legal to supply oral tobacco as defined in the Tobacco Products Directive (2001/37/EC)23. All other smokeless tobacco products (chewing tobacco, nasal snuff) can be sold in all EU-countries. Aggregate data on smokeless tobacco product use and cigarette smoking show that particularly in Swedish men, there is a clear trend over recent decades for smoking prevalence to decrease and for use of oral tobacco (snus) to increase. The prevalence of smoking has also decreased markedly in Swedish women during this period, but to a lesser extent than in men, and in conjunction with a lesser increase in snus use. It has been suggested that the greater decline in smoking prevalence in men compared to women in Sweden is explained by the availability of snus, and this interpretation is supported by trends in longitudinal, within-person data from a population cohort in northern Sweden (report partly funded by the tobacco industry). However, these trends could also be due to successful smoking reduction programs or other socio-cultural factors, and it is therefore not clear whether or by how much the availability of snus has influenced smoking prevalence. In Norway, smoking cessation rates in young Norwegians have been similar in both genders during the last decade, however, increased prevalence of smokeless tobacco use is observed only in young males. In California both the prevalence of smoking and smokeless tobacco use have decreased concurrently. These data imply that the association between patterns of smokeless tobacco use and smoking cessation differs from one population to the other and is likely to be affected by cultural and societal factors.

In conclusion, it is not possible to extrapolate future patterns of tobacco use across countries. In particular, it is not possible to extrapolate the trends in prevalence of smoking and use of oral tobacco if it were made available in an EU-country where it is now unavailable.

[23] tobacco for oral use' means all products for oral use, except those intended to be smoked or chewed, made wholly or partly of tobacco, in powder or in particulate form or in any combination of those forms, particularly those presented in sachet portions or porous sachets, or in a form resembling a food product.

5. COMMENTS RECEIVED DURING THE PUBLIC CONSULTATION

Information about the public consultation has been broadly communicated to national authorities, international organisations, and other stakeholders. The web site opened for comments the 5th of July 2007 and the deadline for submission was the 28th of September 2007. The number of responses submitted by the website was 52; a few additional comments were received by mail or fax. Thirty contributions were from organisations, and 22 from individuals. In three cases the same contribution was received from an individual and an organisation. Of the organisations, 14 were non governmental, 5 business, 4 public authorities and 7 other institutes.

In evaluating the responses from the consultation, submitted material has only been considered for revision of the opinion if

1. it is directly referring to the content of the report and relating to the issues that the report addresses,
2. it contains specific comments and suggestions on the scientific basis of the opinion,
3. it refers to peer-reviewed literature published in English, the working language of the SCENIHR and the working group,
4. it has the potential to add to the preliminary opinion of SCENIHR.

Each submission which meets these criteria has been carefully considered by the Working Group. Overall, many of the comments were of good quality and the opinion has been partly revised based on these comments. The literature has been updated with relevant publications up to the end of 2007.

In the following the comments and revisions to each of the 5 questions to the committee are considered:

1. What are the adverse health effects of smokeless tobacco products?

The majority of the responses agreed or mostly agreed with the response given by the committee. Modifications of the opinion have been done in several places to be more precise on the action on different organs. Some diseases without strong evidence (osteoporosis, musculoskeletal disorders) have been included. Tables 1 and 3 have been revised. Also specifications on types of studies and products used (snus versus other STP) have been introduced. Some new studies have been addressed including one on biomarkers. There is also added some text on the subject of comparison with smoking and possible harm reduction in the relevant sections.

2. What is the addiction potential of smokeless tobacco products?

The majority of the responses agreed or mostly agreed with the opinion. Several comments asked for a more explicit comparison with smoking and the text has been changed accordingly.

3. Does the available data support the claim that smokeless tobacco may constitute a smoking cessation aid comparable to pharmaceutical nicotine replacement products?

Most of the comments agreed or mostly agreed with the opinion. Several of the comments that disagree consider the Swedish experience stronger than the WG has done. The text has been modified accordingly and it has also been stressed that lack of randomised trials make definite conclusions difficult. A report about the situation in Canada was considered to suffer from qualitative limitations.

4. What is the impact of smokeless tobacco use on subsequent initiation of smoking?

The majority of the comments agreed or mostly agreed with the opinion. A frequent comment was that the (negative) Swedish results should be given more weight than the (positive) US data, as the Swedish product is more relevant for the European market. However, the group recommends no change of the report.

5. Is it possible to extrapolate the information on the patterns of smokeless tobacco use, smoking cessation and initiation from countries where oral tobacco is available to EU-countries where oral tobacco is not available?

Most of the submitted contributions agreed or mostly agreed with the response given. It was clarified that at present, Sweden is the only EU-country where oral tobacco as defined by the EC (see above) is legally supplied and that all other smokeless tobacco products (chewing tobacco, nasal snuff) can be sold in all EU-countries. Some comments concerned the importance of age and socioeconomic differences and additional data on trends in Sweden according to age and educational level have been included. Relative trends in progression from STP into and from smoking have been found to differ between countries and it is thus very difficult to extrapolate the patterns of tobacco use from one country where oral tobacco is available to other countries.

6. MINORITY OPINION

None

7. LIST OF ABBREVIATIONS

AUC	Area-under-the-curve
B(a)P	Benzo(a)pyrene
BMI	Body mass index
bw	Bodyweight
CAN	Swedish Council for Information on Alcohol and other Drugs
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
Cmax	Maximum concentration
CPS	Cancer Prevention Study
DA	Dopamine
DMBA	7,12-dimethylbenz(a)anthracene
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders (4th edition) Text Revision
EBV	Epstein-Barr Virus
FDA	Food and Drug Administration
GI cancer	Gastrointestinal Cancer
HCFA	Health Care Financing Administration
HDL	High-density lipoprotein (cholesterol level)
HPV	Human papillomavirus
HPB	4-hydroxy-1-(3-pyridyl)-1-butanone
HSV	Herpes Simplex Virus
IARC	International Agency for Research in Cancer
ICD-7	International Classification of Diseases (7th edition)
ICD-9	International Classification of Diseases (9th edition)
ICD-10	International Classification of Diseases (10th edition)
i.p.	intraperitoneal
L	Litre
LBS	The Lutheran Brotherhood Insurance Society
LDL	Low-density lipoprotein (choresterol level)
LOEL	lowest-observed-effect-level
MAO	Monoamine Oxidase
MDPH	Massachusetts Department of Public Health
MTD	Maximum tolerated dose
NAB	N'-nitrososanabasine
NAB-N-Gluc	pyridine-N-glucuronide of NAB
NAcc	Nucleus Accumbens
NAT	N'-nitrosoanatabine
NAT-N-Gluc	pyridine-N-glucuronide of NAT
ND	not detected
NDELA	N-nitrosodiethanolamine

NDMA	N-nitrosodimethylamine
NHANES	National Health And Nutrition Examination Survey
NHEFS	NHANES I epidemiological follow-up studies
NMBA	4-(N-methylnitrosamino)butyric acids
NMDA	N-nitrosodimethylamine
NMOR	N-nitrosomorpholine
NMPA	3-(N-methylnitrosamino)propionic acids
NNK	4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone
NNK-Gluc	NNK-N-glucuronides
NNN	N'-nitrosonornicotine
NNN-Gluc	NNN-N-glucuronides
NNS	Nicotine Nasal Spray
NNAL	4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butan-1-ol
NNAL-Gluc	NNAL-N- glucuronides
NOEL	No-observed-effect-level
NPIP	N-nitroso-piperidine
NPRO	N-nitrosoproline
NPYR	N-nitrosopyrrolidine
NRT	Nicotine Replacement Therapy
NQO	4-nitroquinoline-N-oxide
NSAR	N-nitrososarcosine
OR	Odds Ratio
oz	ounce
PAH	Polycyclic Aromatic Hydrocarbons
pH	Potential of Hydrogen
pKa	-log(Ka) with Ka being the acid-ionization constant
PMD	Potentially Malignant Disorder
POB-DNA	Pyridyloxobutyl-DNA
RDD	Random digit dialling
RR	Relative risk
s.c.	subcutaneous
SIDS	Sudden infant death syndrome
SIL	Snus-Induced Lesion
STP	Smokeless Tobacco Products
TSNA	Tobacco-Specific Nitrosamines
USEPA	United States Environmental Protection Agency

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9. GLOSSARY

Betel quid	Usually prepared by smearing a betel (*Piper betle*) leaf with slaked lime, to which pieces of areca (*Areca catechu*) nut are added. Catechu (resin from *Acacia catechu*) may be added. Crushed leaves of cured tobacco and flavouring agents are added.
DA	Dopamine; A monoamine neurotransmitter formed in the brain by the decarboxylation of dopa. It is implicated in the formation of dependence to virtually all drugs of abuse.
Delphi method	A systematic interactive forecasting method based on independent inputs of selected experts. Key elements are: structuring of information flow, regular feedback and anonymity of the participants. Despite shortcomings the Delphi method is a widely accepted forecasting tool and has been used successfully for thousands of studies in many areas.
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders (4th edition) Text Revision; A publication of the American Psychiatric Association that classifies and defines psychiatric diagnoses and lists the criteria for them.
Gutkha	Commercial preparation of powdered areca nut and tobacco.
ICD-10	International Classification of Diseases (10th edition); An internationally accepted classification of death and disease published by the World Health Organisation.
MAO	Monoamine Oxidase; A family of enzymes involved in the breakdown of certain neurotransmitters via the catalyzation of the oxidation of monoamines (e.g. dopamine).
Moist snuff, oral tobacco	Finely ground dry tobacco mixed with aromatic substances, salts, water, and humidifying agents. The product is heated and kept cool to avoid fermentation. Moist snuff used in Sweden is called snus.
NAcc	Nucleus Accumbens; A part of the brain reward system, located in the limbic system that processes information related to motivation and reward. It is the key brain site where virtually all drugs of abuse act to reinforce drug taking.
pH	Potential of Hydrogen; A measure of the acidity or alkalinity of a solution, numerically equal to 7 for neutral solutions, increasing with increasing alkalinity and decreasing with increasing acidity.