CONTROVERSY

Most health professionals recognize that smokeless tobacco (SLT) use is associated with oral cancer. However, many have an exaggerated perception of the magnitude of the risk, and there is little understanding or discussion of historical and contemporary scientific data relevant to the association. This review by Rodu and Jansson establishes that SLT use carries only modest risk for oral cancer, and it further describes the key agents in SLT that play important roles in raising—or even lowering—this risk. In other words, it challenges conventional perceptions about SLT use and provides interesting and surprising information about SLT products.

— *Olav Alvares,* Editor

SMOKELESS TOBACCO AND ORAL CANCER: A REVIEW OF THE RISKS AND DETERMINANTS

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ABSTRACT: Smokeless tobacco has been associated with oral cancer for many decades. The purpose of this article is to review research relevant to this association, including epidemiologic studies, studies of putative carcinogens such as tobacco-specific nitrosamines (TSNAs) and other contaminants, and possible cancer inhibitors. Epidemiologic studies addressing this issue primarily consist of case-control studies. They show that the use of chewing tobacco and moist snuff is associated with very low risks for cancers of the oral cavity and related structures (relative risks [RR] from 0.6 to 1.7). The use of dry snuff is associated with higher RRs, ranging from 4 to 13, while the RRs from smokeless tobacco, unspecified as to type, are intermediate (RR = 1.5 to 2.8). With regard to TSNAs, historical levels in American moist snuff products were higher than those in their Swedish counterparts, but levels in contemporary products are uniformly low. TSNA levels in chewing tobacco have always been low, but levels in dry snuff have been higher, including some very high levels in current products. In general, smokeless tobacco users are not exposed to significant levels of cadmium, lead, benzo(a)pyrene, polonium-210, and formaldehyde, when compared with concentrations of these compounds in foods. Finally, low oral cancer risk from smokeless tobacco use may be influenced by the presence of cancer inhibitors, mainly anti-oxidants, in smokeless tobacco products.

Key words. Smokeless tobacco, oral cancer, case-control studies, tobacco-specific nitrosamines, anti-oxidants.

(I) Introduction

Smokeless tobacco (SLT) has been associated with oral cancer for many decades. The purpose of this article is to review some of the evidence for this association. We review the epidemiologic studies addressing the human risks of SLT use, which primarily consist of case-control studies performed over a 40-year period starting in 1957. Then we review some of the compounds that may promote or inhibit cancer development in long-term SLT users. Potential cancer-causing agents in SLT have been studied intensively over the past 30 years. We will discuss some of the more important agents in this group, listed in Table 1, which also provides information on carcinogenic risk from the Monographs program of the International Agency for Research on Cancer (IARC), an authoritative source of information on carcinogenic risk assessment. Potential cancer inhibitors in SLT have received very little attention. But they exist, and they are found in rather impressive concentrations, mainly as anti-oxidants.

In this review, we do not include studies involving animal research, for several reasons. First, this area has been reviewed recently by others (Grasso and Mann, 1998). More importantly, animal models of SLT-induced carcinogenesis are almost entirely negative, and they provide little insight into the contribution of SLT to human oral cancer. In fact, the designation of SLT as a carcinogen by IARC is based solely on epidemiologic studies in humans. In its evaluation, IARC states that there is inadequate evidence for the carcinogenicity of SLT in animals (IARC, 1987).

This review is limited to SLT as used in Western societies, mainly in the United States and Sweden. Smokeless tobacco is indeed used by many cultures in many parts of the world, including the Middle East and the Indian subcontinent. However, SLT products in these countries are considerably different from those used in the West. For example, in India, SLT processing is performed by individual farmers and small companies with little control over fermentation and curing, which increases the production of TSNAs (Brunnemann *et al*., 1985). In

India, SLT is not homogeneous, since the tobacco is often combined with betel leaf (*Piper betle*), sliced areca nut (*Areca catechu*), and/or powdered slaked lime (Muir and Zaridze, 1986), additives that enhance the toxicity as well as the psychotropic effect of tobacco (Wary and Sharan, 1988; Thomas and MacLennan, 1992). In addition, Indian SLT users often smoke concurrently, thus confounding the effects of SLT use (Hirayama, 1966; Jayant *et al.*, 1977).

SLT is not a homogeneous category, even if we limit our review to American and Swedish products. Three distinct types of SLT—chewing tobacco, moist snuff, and dry snuff—are used in the US (in Sweden, moist snuff is practically the only type of SLT product used). All of these products are held in the mouth, usually between the buccal mucosa and gingiva, but they are distinctly different from each other with respect to the tobaccos, the procedures used in their manufacture, and the populations that consume them. Furthermore, each SLT type may have a distinct profile with regard to the agents discussed in this review, and with regard to the risks that result from their use. Thus, an understanding of SLT types is a prerequisite to an understanding of SLT-associated oral cancer.

TABLE 1

Evaluation of Carcinogenic Risk to Humans of SLT and Selected Constituents, IARC Monographs Program

Agents Discussed in This Review	Evidence in Humans	Evidence in Animals	IARC Group (Evaluation Date)	Overall Evaluation Regarding Humans
SLT[1]	Sufficient	Inadequate	1 (1987)	Carcinogenic
Cadmium	Sufficient	Sufficient	1 (1993)	Carcinogenic
Formaldehyde	Sufficient	Sufficient	1 (2004)	Carcinogenic
BaP	Inadequate	Sufficient[2]	2A (1983)	Probably carcinogenic
Lead	Limited	Sufficient	2A (2004)	Probably carcinogenic
NNN	None	Sufficient	2B (1985)	Possibly carcinogenic
NNK	None	Sufficient	2B (1985)	Possibly carcinogenic
NAB	None	Limited	3 (1985)	Not classifiable
NAT	None	Inadequate	3 (1985)	Not classifiable
Polonium-210	Inadequate	Sufficient[2]	- (2001)	None

[1] Abbreviations: SLT = Smokeless tobacco, BaP = Benzo(a)pyrene, NNN = N-nitrosonornicotine, NNK = 4-methyl-N-nitrosamino-1-(3-pyridyl)-1-butanone, NAB = N-nitrosoanabasine, NAT = N-nitrosoanatabine.

[2] Mediated by a mechanism that also operates in humans.

(II) SLT Types

Loose-leaf chewing tobacco, moist snuff, and dry snuff are the three types of SLT commonly used in the oral cavity (Wahlberg and Ringberger, 1999). Loose-leaf chewing tobacco is manufactured primarily from plants grown in Pennsylvania and Wisconsin; the leaves are air-cured, shredded into flakes, and treated with sweet flavoring solutions. It is used primarily by men in the US, commonly in conjunction with outdoor activities, where the resulting tobacco juices can be expectorated. The popularity of this product has waned, with consumption declining gradually over the past century, about 35% in just the last 15 years (Federal Trade Commission, 2001).

Moist snuff consists of fire- and air-cured dark tobaccos that are finely cut. It is now the most popular form of SLT in the US; sales of this product have increased by 77% over the past 15 years (Federal Trade Commission, 2001). Moist snuff is also very popular in Sweden, where it is called 'snus'. One reason for the popularity of moist snuff in both countries is that it has become more user-friendly. Traditional moist snuff users place a 'pinch' of the finely ground tobacco between the gingiva and buccal mucosa. But the tobacco is difficult to keep in place, and the resultant migration is esthetically displeasing. Modern moist snuff products are sold in small, pre-portioned pouches similar to teabags. These products remain stationary in the mouth and generate very little juice. Thus, they can be used discreetly, with no expectoration.

There are important differences in the way that American and Swedish moist snuff products are manufactured. Traditional American products undergo fermentation, which imparts characteristic flavors but often also results in higher concentrations of unwanted bacterially mediated by-products, especially TSNAs and nitrite. In Sweden, moist snuff is exposed during manufacturing to a heat treatment akin to pasteurization, giving a virtually sterile product.

Dry snuff is a fermented, fire-cured tobacco that is pulverized into powder, and its original use was through nasal inhalation. Women in the southern US have used dry snuff as an oral form of tobacco since the early 1800s (Rogozinski, 1990; McGuirt and Wray, 1993). However, this type of use is declining, and sales have fallen almost 60% in the past 15 years (Federal Trade Commission, 2001).

(III) Epidemiologic Studies

In a recent review, one of the authors identified 21 epidemiologic studies addressing the risk of SLT use for cancers of the oral cavity and adjacent sites (Rodu and Cole, 2002). Eighteen of these are case-control studies that provided relative risk (RR) estimates. Summary RRs for cancers of several anatomic sites in SLT users according to SLT type were derived from case/control enumerations in these studies. Fig. 1 shows summary RRs for chewing tobacco, moist snuff, dry snuff, and a fourth exposure category, SLT unspecified as to type, in which the type of SLT was unclear or undetermined.

The first study evaluating the risk of chewing tobacco appeared in 1962 (Vogler *et al.*). There were two studies in 1977 (Browne *et al.*; Wynder and Stellman), two in 1988 (Blot *et al.*; Spitz *et al.*), and four studies from 1993 to 1998 (Mashberg *et al.*, 1993; Kabat *et al.*, 1994; Muscat *et al.*, 1996; Schildt *et al.*, 1998), so chewing tobacco has been studied at least once in each of four decades, spanning a total of 32 years. It is clear that chewing tobacco use is associated with low cancer risks; all RR estimates are under 2, with confidence intervals including one for all but one anatomic site.

RRs for moist snuff were reported first in 1977 (Wynder and Stellman). Another study appeared in 1988 (Spitz *et al.*), and five additional studies were published from 1993 to 1998, as this product came under intense scrutiny (Mashberg *et al.*, 1993; Kabat *et al.*, 1994; Muscat *et al.*, 1996; Lewin *et al.*, 1998;

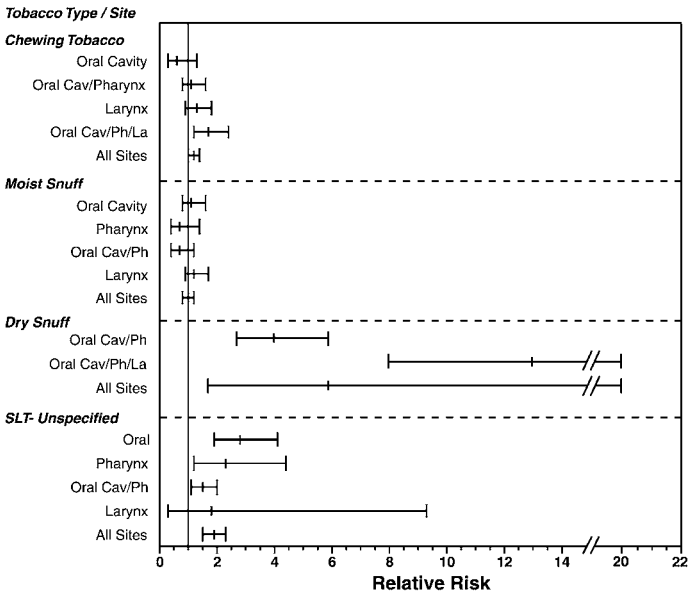

Figure 1. Summary RRs for oral cancer and related sites according to SLT product type. Adapted from Rodu and Cole (2002). Oral Cav = Oral Cavity; Ph = Pharynx; La = Larynx.

Schildt *et al.*, 1998). Similar to chewing tobacco, summary RRs are low for moist snuff, with three RRs at or below one and the highest RR at 1.2. Two of the seven studies were Swedish, both appearing in 1998. Interestingly, these studies have received considerable attention in the medical community because they are considered as showing no oral cancer risk for Swedish products. They formed the basis for the Swedish government's decision in 1999 to recommend to the EU that the warning labels be removed. In fact, the warning labels regarding oral cancer were removed in 2001 by EU directive for smokeless tobacco products (European Commission, 2001). Notably, the other five studies forming the summary RRs for moist snuff are American, and they reported RRs very similar to those from the Swedish studies.

Fewer studies evaluated RRs for dry snuff. The first appeared in 1962 (Vogler *et al.*), followed by studies in 1981 (Winn *et al.*), 1988 (Blot *et al.*), and 1994 (Kabat *et al.*), spanning a period of 32 years. In these studies, RRs for dry snuff use a range from 4 to 13, although the confidence intervals for these estimates are wide.

Eight studies provided RRs for SLT-unspecified, five of which appeared between 1957 and 1969 (Wynder *et al.*, 1957a,b; Peacock *et al.*, 1960; Vincent and Marchetta, 1963; Martinez, 1969). Additional studies appeared in 1992 (Maden *et al.*), 1993 (Mashberg *et al.*), and 1998 (Schwartz *et al.*). RRs for SLT-unspecified range from 1.5 to 2.8, and most are statistically significant. For all sites, the summary RR is 1.9 (CI = 1.5-2.3), which is intermediate between the low risks reported for chewing tobacco (1.2, 1.0-1.4) or moist snuff (1.0, 0.8-1.2) and the higher risk for dry snuff (5.9, 1.7-20). The intermediate risks for this SLT category probably reflect the use of either the lower- or higher-risk products among different groups within these studies.

The distinctive risk profiles of moist snuff and chewing tobacco, on the one hand, and dry snuff, on the other, have gone largely unnoticed. Prior to the review in 2002 (Rodu and Cole), the low oral cancer risk associated with chewing tobacco had been discussed briefly in only one article (Mattson and Winn, 1989). No distinction in risks had been made between dry snuff and moist snuff, even though these products are considerably different with regard to tobacco content and processing.

The majority of epidemiologic studies regarding SLT and oral cancer have limitations. Most of them did not control for confounding by two strong determinants of oral cancer, cigarette smoking and alcohol use. Only six studies partially controlled for smoking (Martinez, 1969; Winn *et al.*, 1981; Blot *et al.*, 1988; Kabat *et al.*, 1994; Schildt *et al.*, 1998; Lewin *et al.*, 1998), and those that did not may be affected by either positive or negative confounding. Positive confounding by smoking would occur if SLT users smoke more than do non-users. This would result in an artificially high RR for oral cancer among SLT users. On the other hand, negative confounding is plausible and would occur if smoking rates are lower among SLT users than among non-users. This would result in an artificially low RR for oral cancer among SLT users.

Only three studies (Winn *et al.*, 1981; Lewin *et al.*, 1998; Schildt *et al.*, 1998) controlled for alcohol use, where only positive confounding is likely. Thus, control for alcohol consumption in all studies probably would have reduced somewhat many of the RRs presented.

Many of the published studies did not define the specific anatomic sites studied. Although the oral cavity is the major site of interest in epidemiologic studies of SLT use, in many studies RRs were reported only for cancers of the oral cavity and pharynx combined, or even for the oral cavity, pharynx, and larynx combined. Nomenclature was not particularly consistent even for oral cancer, seemingly a well-defined entity. For example, although most studies used the same subsites to comprise oral cancer, four included the lips and/or major salivary glands (Vogler *et al.*, 1962; Martinez, 1969; Wynder and Stellman, 1977; Schildt *et al.*, 1998). Furthermore, four studies (Winn *et al.*, 1981; Maden *et al.*, 1992; Muscat *et al.*, 1996; Schwartz *et al.*, 1998) specified oral cancer in their titles, but the entity actually studied was cancer of the oral cavity and pharynx combined. However, even with these limitations, the results of these studies, spanning a period of 40 years, are reasonably consistent. The use of moist snuff and chewing tobacco imposes minimal risks, while the use of dry snuff is associated with higher risks.

Research related to smokeless tobacco keratosis (STK) also supports the epidemiologic evidence that SLT use has low risk for oral cancer. STK represents a thickened layer of keratin on the surface epithelium at the site of SLT use, largely a result of local irritation (Greer and Poulson, 1983; Greer *et al.*, 1986). STK has been defined by the WHO as a separate and distinct entity from other keratotic lesions—which we will designate oral leukoplakia for this discussion—frequently related to smoking. These distinctions are based on the following characteristics: location within the oral cavity, frequency of occurrence, frequency of dysplasia, and rate of malignant transformation (Axéll *et al.*, 1984; Bouquot, 1991).

STK occurs at the site of SLT placement in up to 60% of SLT users (Grady *et al.*, 1990; Sinusas *et al.*, 1992), within 6 months to 3 years of initiation (Greer and Poulson, 1983; Greene *et al.*, 1993). The frequency of its appearance is dependent on the type of ST used. Moist snuff, which is more alkaline than chewing tobacco, more often leads to STK (Greene *et al.*, 1993). However, moist snuff in pre-portioned pouches causes less-pronounced mucosal change and fewer cases of STK than does the loose form (Andersson and Axéll, 1989). In contrast, oral leukoplakia

occurs in less than 1% of the general population, primarily in long-time smokers 40 to 60 years old (Bouquot and Gorlin, 1986; Bouquot *et al.*, 1988). Smoking-related leukoplakias most commonly involve the floor of the mouth, ventral tongue, and soft palate, sites that account for 75% of oral cancer in the US (Bouquot *et al.*, 1988; Silverman and Gorsky, 1990).

There are distinct differences between STK and oral leukoplakia regarding frequency of dysplasia, which is a condition that precedes and often indicates a developing carcinoma. Dysplasia is seen infrequently in STK (less than 3% of cases) (Smith *et al.*, 1970; Roed-Petersen and Pindborg, 1973; Axéll *et al.*, 1976; Bouquot and Schroeder, 1993). Furthermore, even when dysplasia is present in STK, it usually is found in earlier stages than in oral leukoplakia (Mincer *et al.*, 1972; Kaugars *et al.*, 1989), where it is seen in about 20% of cases (Waldron and Shaffer, 1975).

With the prevalence and degree of dysplasia low, it is not surprising that malignant transformation in STK occurs only rarely. For example, one prospective study found no case of cancer in 1550 individuals with STK followed for 10 years (Smith, 1975), and a second study reported no case of oral cancer among 500 regular SLT users followed for six years (Christen *et al.*, 1991), while a retrospective study of 200,000 male snuff users in Sweden found only one case of oral cancer *per* year, an extremely low frequency (Axéll *et al.*, 1978). In comparison, a follow-up study reported that 17% of oral leukoplakias transformed to carcinoma within seven years (Silverman *et al.*, 1984).

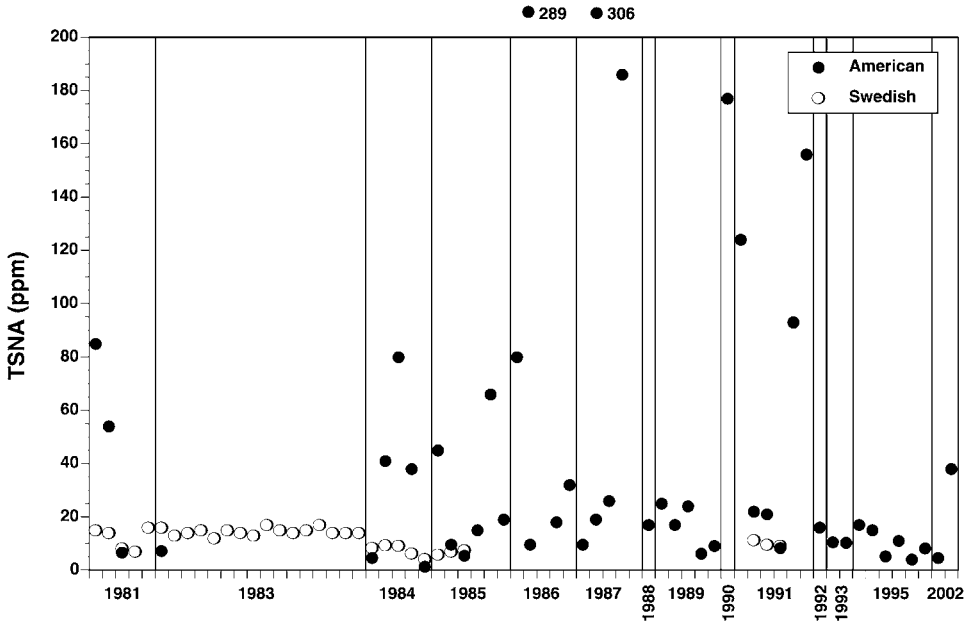

Figure 2. Historical levels of total tobacco-specific nitrosamines (TSNAs) (ppm) in American and Swedish moist snuff products, 1981-2002. Sources: Hoffmann and Adams, 1981; Österdahl and Slorach, 1984; Hoffmann *et al.*, 1984, 1986, 1988, 1991, 1995; Brunnemann *et al.*, 1985, 1987, 2002; Chamberlain *et al.*, 1988; Djordjevic *et al.*, 1989, 1993.

(IV) Tobacco-specific N'-nitrosamines (TSNAs)

The risk of oral cancer from long-term SLT use is elevated for some products, and much of this risk has been attributed to the presence of TSNAs. There are four principal compounds: N-nitrosonornicotine (NNN), 4-methyl-N-nitrosamino-1-(3-pyridyl)-1-butanone (NNK), N-nitrosoanatabine (NAT), and N-nitrosoanabasine (NAB). TSNAs are present in very low concentrations in green tobacco, but higher concentrations occur during curing when amine alkaloids in the tobacco leaf react with either nitrite, which is formed by the reduction of nitrate by bacterial activity (Bush *et al.*, 2001), or nitrous oxides, which are combustion by-products of fire-curing (Peele *et al.*, 2001). NNN can be formed directly from nicotine (a tertiary amine), while NNN, NAT, and NAB are formed from their secondary amine precursors (nornicotine, anabasine, and anatabine, respectively). In contrast, NNK is formed only from nicotine.

The conditions favoring TSNA formation have been studied intensively over the past decade (Bush *et al.*, 2001), allowing leaf growers and manufacturers to use several strategies to minimize TSNA levels in SLT products. One promising line of investigation involves the development of tobacco cultivars that produce low concentrations of alkaloids, with special emphasis on the inhibition of nornicotine and NNN formation (Shi *et al.*, 2000; Bush *et al.*, 2001). TSNA formation may be inhibited in principle by the elimination of nitrate-reducing microbial activity (*e.g.*, *Enterobacter* spp.), while maintaining activity of other flora that impart desirable flavor properties. Other reductions are possible through the careful manipulation of humidity and temperature conditions during curing. The substitution of heat exchange methods for traditional flue-cured tobacco also results in substantially lower nitrosamine levels (Peele *et al.*, 2001). Whatever proprietary methods have been used, manufacturers have substantially reduced the TSNA concentrations in finished SLT products, as we will illustrate in the following section.

Only two TSNAs, NNN and NNK, are considered to be potential carcinogens. Both of them are classified by IARC as possibly carcinogenic to humans (Group 2B) (IARC, 1985a,d), based on the fact that there is sufficient evidence of their carcinogenicity in experimental animals but no data in humans. On the other hand, both NAT and NAB are designated by IARC as not classifiable with regard to carcinogenicity (IARC, 1985b,c). For NAT, there are inadequate data in experimental animals and no data in humans. For NAB, there is limited evidence for carcinogenicity in experimental animals and no data in humans.

(A) TSNAs—HISTORICAL LEVELS

The presence of TSNAs in SLT products has been documented in the research literature since the late 1970s, with most of the relevant work performed by laboratories at the American Health Foundation. Most of their studies focus on moist snuff brands, although occasionally levels are reported for chewing tobacco and dry snuff.

Fig. 2 shows the range of TSNA levels for American and Swedish moist snuff products, as reported from 1981 to 2002 (Hoffmann *et al.*, 1979, 1984, 1986, 1988, 1991, 1995; Hoffmann

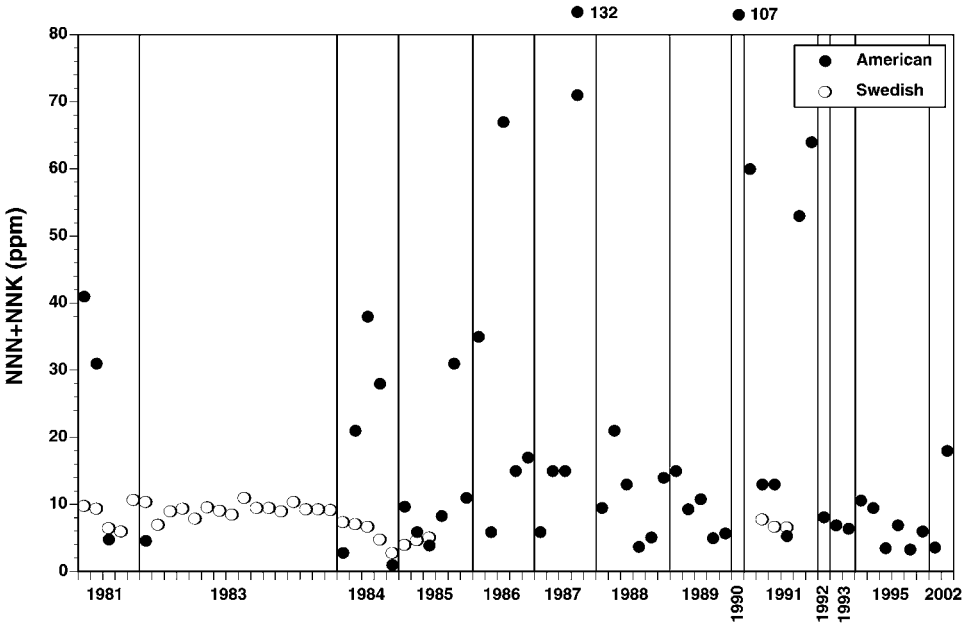

Figure 3. Historical levels of N-nitrosonornicotine (NNN) + 4-methyl-N-nitrosamino-1-(3-pyridyl)-1-butanone (NNK) (ppm) in American and Swedish moist snuff products, 1981-2002. Sources as for Fig. 2.

and Adams, 1981; Österdahl and Slorach, 1984; Brunnemann *et al.*, 1985, 1987, 2002; Chamberlain *et al.*, 1988; Djordjevic *et al.*, 1989, 1993). Swedish products had TSNA levels from 7 to 17 ppm in 1981 and 1983; thereafter, almost all products had levels under 10 ppm. American products were tested much more often. Although some products matched the low Swedish TSNA levels, others tested as high as 300 ppm, and individual products demonstrated levels above 100 ppm as late as 1993. However, studies from 1995 (Hoffmann *et al.*) and 2002 (Brunnemann *et al.*) indicated that most products in the US achieved TSNA levels below 20 ppm, which we confirmed in our extensive analysis of contemporary products (see below).

The brand names of products were not revealed in most of the published studies of TSNA levels. Therefore, it is not possible for us to track longitudinal data for individual brands, which would provide much more information about manufacturers' progress in reducing TSNA levels. In preparation for this review, we contacted American Health Foundation scientists to request the codes from their published studies, so that we could describe these results more fully. They did not respond to our requests. However, in a 1993 report, AHF scientists reported that TSNA levels in the two leading US moist snuff brands (accounting together for 84% of American sales) declined 81% and 88% between 1980 and 1992 (Djordjevic *et al.*, 1993), with declines seen in all four TSNAs. The same report described continued declines of from 25 to 43% in TSNA levels in Swedish products over the period 1980-1990.

Compared with moist snuff, chewing tobacco and dry snuff have been studied far less often. In 1985, the TSNA level in chewing tobacco was 3.5 ppm (Brunnemann *et al.*), and in 1989 it was 2.3 ppm (Djordjevic *et al.*). Dry snuff products were evaluated in two studies in 1987 (Adams *et al.*; Brunnemann *et al.*) and one study in 1989 (Djordjevic *et al.*); TSNA levels in these studies ranged from 25 to 143 ppm.

NNN and NNK, classified by IARC as Category 2B carcinogens, are considered the most important of the TSNAs. Fig. 3 shows combined NNN+NNK levels in Swedish and American moist snuff products over the 1981-2002 period. Most Swedish products consistently had NNN+NNK levels under 10 ppm. Throughout the 1980s, most American moist snuff products had levels under 40 ppm, and there was a clearly declining trend. However, until the mid-1990s some individual products had NNN+NNK levels above 50 ppm.

In 1985, one chewing tobacco product contained a combined NNN+NNK level of 1.1 ppm, and a level of 1.6 ppm was reported on a single product in 1989. Four products were evaluated in 1988, with levels ranging from 1.1 to 7 ppm. Dry snuff products were evaluated in two studies in 1987 and one study in 1989; NNN+NNK levels ranged from 11 to 104 ppm.

(B) TSNAs—CURRENT LEVELS

As we have noted, there was considerable interest in TSNA levels in smokeless tobacco products during the 1980s and early 1990s, with numerous studies involving mainly moist snuff products. But we have noticed that there have been only three studies in the last ten years, and only one study since 1995, which reported levels on only two SLT products. This is particularly ironic, since there has been considerable discussion recently about smokeless tobacco, especially with regard to its potential as a 'harm reduction alternative' for inveterate smokers. In fact, during a Congressional hearing on this topic in June, 2003, there were many comments about TSNA levels in American and Swedish products. Unfortunately, the comments were not specific or particularly fact-based, because there were essentially no publicly available data on TSNA levels in currently available SLT products.

In conjunction with this review, one of the authors (CJ), from the Swedish National Food Administration (SNFA) in Uppsala, Sweden, analyzed TSNA levels in SLT products. American SLT products were purchased in Birmingham, AL, on two separate occasions and shipped by courier to Uppsala for evaluation. Two new SLT products (Revel, USST and Exalt, Swedish Match) are in limited test markets in the US, so we requested, and received, products from the manufacturers. Analyses were also performed on five popular Swedish moist snuff brands. For comparison, we also purchased two popular brands of American cigarettes.

SNFA scientists recently developed a rapid, selective, and sensitive method for routine analysis of all four TSNAs in tobacco products (Jansson *et al.*, 2003). The method involves ethyl acetate extraction of TSNAs followed by quantification by liquid chromatography and mass spectroscopy, and it has been used to analyze moist snuff products on the Swedish market. Sample preparation was modified only slightly for chewing tobacco and dry snuff samples.

Table 2 shows the levels of TSNAs in tobacco products available to American and Swedish consumers in 2003. Almost

all products exhibited TSNA levels below 10 ppm. Levels in chewing tobacco were very low, ranging from 1.5 to 4.7 ppm. The two American cigarette brands had TSNA levels of about 7. Among moist snuff products, Swedish brands were consistently lowest, with values ranging from 2.0 to 2.2 ppm. In comparison, traditional American moist snuff ranged from 7.3 to 12.3 ppm, which is higher than Swedish products but far lower than historical levels. It is of interest to note that the three American moist snuff products in pouches had TSNA levels lower than those found in loose form, from about 5 to 7 ppm.

The new products, Exalt and Revel, had levels of 5.8 ppm and 2.3 ppm, respectively, which were quite low. In addition, Ariva, which has been promoted as an SLT product with very low TSNA levels, had less than 0.1 ppm.

The most surprising results involved dry snuff products, which had TSNA levels from 41 to 1219 ppm. Two of the products, Red Seal and Bruton, had higher levels than any previously published (1096 and 1219, respectively). These levels were confirmed by independent analysis from a second laboratory.

The results of this analysis appear to confirm several trends from previous studies. First, the level of TSNAs in most contemporary smokeless tobacco products is very low. This indicates that manufacturers have achieved considerable success in controlling the conditions under which TSNAs are produced. The biggest improvement has been among products in the moist snuff category. Currently, the highest TSNA levels are around 12 ppm, with many products currently under 5.0 ppm. This is especially encouraging because the RRs for cancer among moist snuff users are historically low. Thus, it is likely that current users have even less risk than users four or five decades ago. The same holds true for chewing tobacco; all tested products have TSNA levels under 5 ppm. The high TSNA concentration in dry snuff brands is disturbing, especially since long-term use of this SLT type appears to be associated with higher risks for cancer of the oral cavity and related sites.

TABLE 2
TSNA Levels[a] in American and Swedish Tobacco Products, 2003

Tobacco Type/ Brand Name	Dry Matter (%)	NNN	NNK	NAT	NAB	Total TSNAs
Cigarettes						
Camel	91	3.4	0.8	2.2	0.1	6.5
Marlboro	91	3.5	1.5	1.9	0.1	7.0
Chewing Tobacco						
Beech Nut	78	3.0	0.8	0.8	0.1	4.7
Red Man	76	1.0	0.3	0.5	0.0	1.8
Oliver Twist-Tropical	81	0.9	0.1	0.5	0.0	1.5
Oliver Twist-Senior	80	1.7	0.3	1.3	0.1	3.4
Moist Snuff, US						
Skoal Straight Long Cut	46	5.2	1.6	3.8	0.3	10.9
Skoal Bandits Straight	51	4.2	0.7	1.8	0.1	6.8
Skoal Wintergreen	44	2.7	0.6	1.4	0.1	4.8
Copenhagen	46	5.5	1.3	5.0	0.3	12.1
Copenhagen pouches	46	2.4	0.4	1.5	0.1	4.5
Hawken Wintergreen	74	4.8	1.1	1.1	0.3	7.3
Kodiak Wintergreen	48	6.4	0.7	4.8	0.4	12.3
Moist Snuff, Sweden						
General	45	1.1	0.4	0.6	0.1	2.1
Ettan	47	1.1	0.3	0.6	0.1	2.0
Catch Licorice	52	1.0	0.4	0.6	0.0	2.0
Goteborgs Rape	44	1.1	0.4	0.6	0.0	2.2
Grovsnus	45	1.1	0.5	0.6	0.1	2.2
Dry Snuff						
Bruton	94	287	922	77	31	1219
Red Seal	94	210	280	210	32	1096
Dental Sweet	93	19	6.5	14	1.2	41
Scotch	93	21	22	20	2.1	65
New Products						
Revel	95	1.3	0.2	0.7	0.1	2.3
Ariva	97	0.0	< 0.1	0.0	0.0	< 0.1
Exalt	91	3.1	1.1	1.5	0.2	5.8

[a] All concentrations in parts *per* million based on dry weight.

[NB: Two dry snuff products with atypically high TSNA levels were re-tested in June, 2004. TSNA levels were as follows (parts *per* million, dry weight):

Red Seal: NNN- 3.7; NNK-0.8; NAT- 1.3; NAB- 0.2; Total TSNAs- 6.0
Bruton: NNN- 5.6; NNK-2.2; NAT- 1.9; NAB- 0.3; Total TSNAs-10.0]

(V) Other Putative Carcinogens

Research studies have determined that there are other possible carcinogenic agents in SLT products, among the most important of which are cadmium, lead, polonium-210, benzo(a)pyrene, and formaldehyde. Although these compounds are not frequently the subject of medical and public health discussions of SLT use, disparaging references to them appear regularly in literature prepared for public consumption. For example, Web-based 'fact' sheets from Texas A&M University (TAMU, 2003) and from the National Institute of Dental and Craniofacial Research and National Cancer Institute (NIDCR-NCI, 2003), present the following descriptions: cadmium—used in car batteries; lead—a poison (TAMU only); polonium-210—nuclear waste; benzo(a)pyrene—cancer-causing agent or chemical; formaldehyde—embalming fluid. These descriptions are intended to provide maximum shock value for anti-tobacco propaganda, but they are not even factual (*e.g.*, cadmium is used in rechargeable nickel-cadmium batteries but not in automobile batteries). More importantly, these descriptions beg more important questions: What concentrations of these agents are present in SLT products? To what extent do SLT products contribute to the overall exposure of SLT users to these toxic agents, compared with other environmental sources such as

TABLE 3
Other Contaminants in Moist Snuff and in Foods

Agent	Presence in Moist Snuff[a]	Presence in Foods (Reference)	
Cadmium μg/day[d]	3.6 to 8.1[b]	General diet-typical	23 (WHO, 1998b)
		General diet-maximum	52 (WHO, 1998b)
Polonium-210 piC/day	0.9 to 6.7[b]	General diet	1 to 10 (Watson, 1985)
Formaldehyde[c] μg/g	4.5 to 6.8	Meat and poultry	0.5 to 6 (Möhler and Denbsky, 1970)
		Fish	6 to 14 (Möhler and Denbsky, 1970)
		Fruits	2 to 8 (Möhler and Denbsky, 1970)
		Smoked meat	3 to 30 (Möhler and Denbsky, 1970)
		Apples	17 to 22 (Tsuchiya *et al.*, 1975)
		Green onion	13 to 26 (Tsuchiya *et al.*, 1975)
		Carrots	7 to 10 (Tsuchiya *et al.*, 1975)
Benzo(a)pyrene[c] ng/g	< 0.1 to 63	Charcoal-broiled meats	1 to 50 (Lijinsky, 1991)
		Lettuce	1 to 13 (Verschueren, 1983)
		Leek	13 to 25 (Verschueren, 1983)
		Spinach	3 to 50 (Verschueren, 1983)
		Tea	4 to 21 (Zedeck, 1980)
		Cereals	0.2 to 4 (Zedeck, 1980)
Lead μg/day	2.6 to 16[b]	General diet-typical	48 (WHO, 1998a)
		General diet-maximum	146 (WHO, 1998a)

[a] The reference for all agents in moist snuff is Hoffmann *et al.* (1987).
[b] Based on a 10-gram daily consumption (Hoffmann *et al.*, 1987).
[c] Calculation based on dry weight (Hoffmann *et al.*, 1987).
[d] Abbreviations: μg = micrograms, g = grams, piC = picoCuries, ng = nanograms.

foods? We reviewed studies examining these agents in moist snuff and in foods, to provide some perspective on exposure contributions by SLT use. The results are summarized in Table 3.

Cadmium is found in low concentrations in most soils (IARC, 1993), and it has the most widespread distribution of all heavy metals in foods (Mahaffey *et al.*, 1975). Cadmium is considered by IARC as a Group 1 human carcinogen, primarily based on increased lung cancer risk among workers exposed *via* inhalation to high concentrations in industrial settings, as well as animal research (IARC, 1993). Cadmium is present in the general diet. Cereals and grains provide the highest percentage of total intake, but cadmium is also present in shellfish and some vegetables, such as spinach (Mahaffey *et al.*, 1975). A typical general diet supplies about 25 μg of cadmium daily, and the Codex Alimentarius Commission of the WHO set a maximum recommended intake of 52 μg *per* day (World Health Organization, 1998a). In 1987, an American consuming 10 g of moist snuff *per* day was exposed to approximately 4 to 8 μg of cadmium daily (Hoffmann *et al.*, 1987).

Polonium-210 is a radiodecay product of Radium-226, which is itself a product of Uranium-238 decay (Watson, 1985). Although Radium-226 is located primarily in uranium-bearing ores and associated soils, Polonium-210 is disbursed widely in the environment by rain and other weather events, because an intermediate decay product, Radon-222, is an extremely mobile gas. IARC has not definitively classified polonium-210 (IARC, 2001). The dominant mechanism of plant deposition is from surface absorption (rather than from root uptake) and transfer to edible foliage and seeds, berries, or fruits. Food ingestion is the major source of polonium-210, and the diet supplies from 1 to 10 picoCuries (piC) *per* day. Polonium-210 in tobacco is derived both from fertilizers and from airborne particles that are trapped by leaf trichromes (Hoffmann *et al.*, 1987). In 1987, an American consuming 10 g of moist snuff *per* day was exposed to about 1 to 7 piC daily from polonium-210 (Hoffmann *et al.*, 1987). In 1989, Swedish moist snuff users were estimated to have a polonium-210 exposure consistent with that from three dental radiographs, and it was concluded that the risk to 'snus' users was so small that no special measures or other actions were necessary (Samuelsson, 1989).

Formaldehyde is primarily produced as an industrial chemical and has widespread use in manufacturing (IARC, 2004b). It has been classified by IARC as carcinogenic to humans (Group 1), based primarily on exposure among workers in certain industrial settings, as well as on animal research. Formaldehyde is also present as a natural product in the environment and in most plant and animal systems. For example, endogenous formaldehyde is present in human blood in concentrations of 2 to 3 mg/L, which is unaltered by exposure to the substance in ambient air (IARC, 1995). It is present in various concentrations in a wide variety of foods, from meat and poultry (up to 6 μg/g) to fish (6 to 14 μg/g) to fruits and vegetables (2 to 26 μg/g) (Möhler and Denbsky, 1970; Tsuchiya *et al.*, 1975). In 1987, the concentration of formaldehyde in moist snuff was about 5 to 7 μg/g (Hoffmann *et al.*, 1987).

Polycyclic aromatic hydrocarbons (PAHs) are produced in the incomplete combustion of organic compounds, so they have been present in the environment since the advent of fire (Lijinsky, 1991). More specifically, they have been present in human diets since man began cooking meat. One of the most common, and intensely studied, PAHs is benzo(a)pyrene (BaP), which has been classified by IARC in Group 2A (probably carcinogenic to humans) (IARC, 1983). The major dietary source of BaP is charbroiled meats, but plants also contain measurable amounts of BaP due to direct surface contamination of plants from environmental sources (Zedeck, 1980). Thus, in addition to charcoal-broiled steaks (1 to 50 ng/g), dietary sources include leafy vegetables (1 to 50 ng/g), tea (4 to 21 ng/g), and cereals (up to 4 ng/g) (Zedeck, 1980; Verschueren, 1983;

Lijinsky, 1991). In 1987, some moist snuff brands contained low levels of BaP (< 0.1 to 4 ng/g), while others contained concentrations up to 63 ng/g (Hoffmann *et al.*, 1987). BaP in SLT is largely from fire-curing (Hoffmann *et al.*, 1987), so the concentration of BaP in any brand of moist snuff probably reflects the proportion of fire-cured tobacco used in that blend.

Lead and lead compounds are considered by IARC as probably carcinogenic to humans (Group 2A), primarily based on evidence from animal research (IARC, 2004a). Throughout much of the 20th century, the major source of lead in the environment was from atmospheric contamination from combustion of lead-containing fuels (Sanstead *et al.*, 1974). Absorption from dietary sources is limited, because lead in the soil is not readily taken up by most plants. In addition, lead in livestock is largely deposited in bone tissue, which limits its transfer to meat consumers. Fruits and vegetables remain the largest sources of dietary lead, and the average diet supplies about 50 μg *per* day, with a maximum daily intake of 150 μg set by the Codex Alimentarius Commission (WHO, 1998a). In 1987, an American consuming 10 g of moist snuff *per* day was exposed to about 3 to 16 μg of lead daily (Hoffmann *et al.*, 1987).

In summary, it would appear that moist snuff consumption does not result in exposure to levels of lead, cadmium, polonium-210, benzo(a)pyrene, or formaldehyde that are out of proportion to levels of these compounds found in the general diet or common foods. However, this conclusion must be made with caution for several reasons. First, only one major study of these compounds in moist snuff has been performed, and this was published in 1987. It is possible that the concentrations of these compounds in moist snuff have changed since that time, just as processing refinements by manufacturers have resulted in lower TSNA levels. Second, although we have compared concentrations of these compounds in moist snuff with those in foods, the route of exposure and putative absorption will vary greatly. For example, foods are ingested, providing the opportunity for prolonged contact of these contaminants with mucous membranes designed to maximize absorption (*i.e.*, stomach and small intestine). On the other hand, trace levels of compounds in foods are diluted by the volume of fluids and solids that are consumed, with the overall effect being low exposure over a large number of tissues and organs. There is very little information about the absorption and metabolism of contaminants resulting from SLT use. It is possible that SLT use results in higher exposures of very limited mucosal surfaces, because users habitually place these products in the same location for years or decades. However, pre-packaged products may modify the SLT user's mucosal and systemic exposure to contaminants.

(VI) Cancer-inhibiting Properties of Tobacco

The presence of TSNAs and other potentially carcinogenic compounds in SLT products has been a major issue in the scientific literature and in the general press. But earlier we showed that epidemiologic studies conducted over the past 50 years have consistently documented that oral cancer risk in SLT users is elevated only minimally. Furthermore, experimental evidence is inconsistent with regard to how great a factor these agents are in the production of tumors among SLT users. For example, in animal models, the chronic application of purified TSNAs to oral mucosa results in tumor formation (Hecht *et al.*, 1986). However, the application of TSNAs together with water-soluble SLT-extracts results in fewer tumors, and water-soluble extracts of SLT alone produce no tumors. One possible explanation for minimal cancer risk among human users and inconsistent experimental results among animals is that SLT contains agents that inhibit carcinogenesis *in vivo*.

TABLE 4
ORAC and Total Phenolic Content of Tobacco Products[c]

Tobacco Type/ Brand	Dry Matter (%)	ORAC Activity[a]	Phenolic Content[b]
Cigarettes			
Camel	89	230	30
Marlboro Reds	89	190	27
Chewing Tobacco			
Oliver Twist-Senior	82	196	26
Oliver Twist-Tropical	86	193	28
Red Man	76	68	9.3
Beech-Nut	78	68	9.9
Moist Snuff, US			
Kodiak-Wintergreen	47	132	17
Skoal-Bandits-Straight	51	97	18
Rooster-Bold Wintergreen	46	95	18
Copenhagen	46	92	15
Skoal-Straight	45	82	16
Skoal-Wintergreen	46	80	17
Hawken-Wintergreen	74	74	12
Skoal-Bandits-Wintergreen	50	70	15
Skoal-Spearmint	46	70	15
Skoal-Bandits-Mint	52	66	10.8

[a] Expressed as μmol Trolox equivalent *per* gram.
[b] Expressed as mg gallic acid equivalent *per* gram.
[c] Adapted from Rodu and Ou, 2000.

Of all the chemical constituents in the tobacco leaf, there are two classes of compounds that may inhibit carcinogenesis. The first is the carotenoids, of which lutein, β-carotene, neoxanthin, and violaxanthin are found in greatest concentrations in tobacco (Leffingwell, 1999). There is experimental evidence that β-carotene inhibits formation of cancerous lesions (Garewal, 1995). It can also produce clinical regression in about half of oral leukoplakias, but few lesions resolve completely (Garewal *et al.*, 1999). The second category is the phenolic compounds, which probably comprise the major anti-oxidants in tobacco (Wahlberg and Ringberger, 1999). Polyphenols from black and green teas have been studied extensively in animal and cell culture models of carcinogenesis. Interestingly, polyphenols exert an inhibitory effect on lung tumorigenesis by NNK at both the initiation and promotion stages (Chung *et al.*, 1993; Yang *et al.*, 1998). As we noted earlier, NNK is an important TSNA.

Recently, one of the authors collaborated with an independent laboratory on a study evaluating the anti-oxidant properties of tobacco products available in the US (Rodu and Ou, 2000. The investigation involved the oxygen radical absorbance capacity (ORAC) assay, which uses fluorescence to measure inhibition of damage to β-phycoerythrin, a protein isolated from *Porphyridium cruentum*, by the peroxide radical, one of the most common reactive oxygen species *in vivo*. The ORAC assay has been used to measure the anti-oxidant capacity of a wide range of biological samples, from pure compounds to fruits, vegetables, and animal tissues (Cao *et al.*, 1995; Ou *et al.*, 2002).

Table 4 contains information on the ORAC activity and phenolic content of the tobacco products tested. The anti-oxidant activity of SLT products, measured as ORAC, varied from 66 to 196 μmole TE/g on a dry weight basis. Chewing tobacco products had among the highest levels (Oliver Twist), while moist snuff products had a broad range. There was a strong correlation between ORAC level and total phenolic content (R^2 = 0.96). The general range of ORAC activity in the tobacco products was similar to that reported in fruits and vegetables (Prior and Cao, 2000).

There are several possible explanations for the considerable variation in anti-oxidant activity among tobacco products. Tobacco is grown in geographically diverse locations, resulting in qualitative differences in leaf constituents (Leffingwell, 1999; Li *et al.*, 2003). In addition, methods of curing (*e.g.*, smoke-, air-, or flue-curing) and processing (fermentation, temperature variations) play an important role in the characteristics of the products (Li *et al.*, 2003). Finally, the anti-oxidant activity of tobacco products may be influenced by the addition of flavoring agents and preservatives.

Further studies will be needed to determine the extent to which anti-oxidants in tobacco products are biologically available to tobacco users, or whether their presence plays any role in risk modification. It is known that tobacco combustion produces a chemical mix rich in reactive oxygen species, and that smokers have low plasma levels of anti-oxidant vitamins such as ascorbate, tocopherols, and carotenoids (Stegmayr *et al.*, 1993; Traber *et al.*, 2000). On the other hand, SLT users have plasma levels of these vitamins that are similar to those of non-users of tobacco (Stegmayr *et al.*, 1993). Certainly, a potential benefit of anti-oxidants in SLT is at the site of placement in the oral cavity, where they may inhibit the actions of carcinogens.

(VII) Conclusions and Policy Implications

The available epidemiologic studies indicate that the use of chewing tobacco and American moist snuff is associated with minimal risk for oral cancer, while the use of Swedish moist snuff is associated with no demonstrable risk. In comparison, some studies have reported elevated risks for dry snuff use, and these studies may have influenced the perception of the entire SLT category in past evaluations. For example, in 1986 the US Congress passed the Comprehensive Smokeless Tobacco Education Act, which mandated placement of an oral cancer warning on all SLT products. One year later, IARC classified SLT products as carcinogenic to humans, based almost entirely on epidemiologic studies. In the 17 years since these two actions occurred, the number of relevant epidemiologic studies has nearly doubled. The new body of epidemiologic evidence suggests that these actions may now be seen as too broad. It may be time for these agencies to re-examine the issue of the risks of specific forms of SLT use.

In the 1970s, developments in analytical chemistry allowed for the identification and quantification of TSNAs in tobacco products, and in the 1980s and 1990s several studies reported TSNA levels in SLT products. During this period, TSNA levels in chewing tobacco and Swedish moist snuff were reported to be very low. American moist snuff products had higher levels in the early 1980s but showed substantial declines by the mid-1990s. Remarkably, as TSNA levels declined, the number of published reports also declined, resulting in little recent data. As we report in this review, TSNA levels in current products, with the exception of dry snuff, are very low.

Swedish Match, the principal manufacturer of Swedish moist snuff, has adopted a voluntary standard for TSNAs and other contaminants discussed in this review. It is called the Gothiatek standard, and in February, 2003, a group of European tobacco research and policy experts recommended its adoption by the European Union as the standard for all products (Bates *et al.*, 2003). The standard is exacting and sets low levels for contaminants. There are some questions concerning the adoption of a standard for SLT products. First, there is virtually no information correlating levels of TSNAs (and other contaminants) with risk for oral cancer or any other disease. Second, because American consumers are accustomed to much different tobacco flavors than are Swedish consumers, modification of some Gothiatek targets may be required for American products. For example, all Swedish moist snuff products that we tested have TSNA levels at about 2 ppm, well below the Gothiatek limit of 10 ppm (dry weight). But another Swedish Match product (Exalt) on the American market has higher TSNA levels (5.8 ppm), suggesting the important influence of American taste expectations in product manufacturing.

But there are reasons to endorse the adoption of toxicity standards for SLT products. Tobacco is an agricultural product, and the quality from year to year varies with environmental conditions. The adoption of standards would promote further research and development into means for achieving further contaminant reductions. Most importantly, standards may encourage the communication of factual information to consumers. They have a fundamental right to information about SLT products, just as they have access to information about nutritional and toxic aspects of the foods they consume.

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