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A Qualitative and Quantitative Risk Assessment of Snuff Dipping

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The presence of highly carcinogenic tobacco-specific nitrosamines (TSNA) in snuff has been a matter of serious concern. However, the levels of TSNA in such products may differ by orders of magnitude depending on origin and manner of processing, and the mere presence of such agents at low levels does hardly constitute a meaningful prerequisite for classifying all types of snuff as human carcinogens. Reviewing available epidemiological evidence, a wide discrepancy is found for estimated cancer risk associated with snuff dipping derived from on one hand previous investigations conducted in the United States and on the other from recent extensive Swedish epidemiological studies. In spite of the fact that approximately 20% of all grown-up Swedish males use moist snuff, it has not been possible to detect any significant increase in the incidence of cancer of the oral cavity or pharynx—the prevalence of which by international standards remains low in this country. Further, there is insufficient evidence for a causal link between the use of Swedish snuff and increased risk for cardiovascular disease. Dissimilarities in the content of TSNA in oral snuff products may represent one important reason for the different outcomes of the epidemiological surveys conducted in the United States and Sweden. Bioassays using pure TSNA in rodents appear to give exaggerated risk estimates for humans, a discrepancy that could be ascribed to species-related differences in the relation between exposure and DNA target dose and/or adduct repair rates, as well as to the presence of anticarcinogens in snuff. Although a small risk cannot be excluded, the use of smokeless tobacco products low in TSNA which now are available on the market entails a risk that at any rate is more than 10 times lower than that associated with active smoking. Nevertheless, due to the decisive role of potent TSNA in determining possible cancer risks in users of smokeless tobacco, and due to the fact that large variations in the concentrations may occur, adequate control measures should be taken to keep the levels of these nitrosamines in smokeless tobacco products as low as is technically feasible.

INTRODUCTION

The adverse health effects from tobacco smoke have been well established, and a number of national health authorities and private as well as international organizations have launched numerous campaigns in order to reduce active smoking. In some countries these efforts have been relatively successful, resulting in a decrease in total cigarette consumption among the general population. To break psychological as well as physiological dependence, many regular smokers have resorted to the use of smokeless tobacco which has increased in popularity in recent times. A recently convened United Nations expert group listed Swedish oral snuff together with other nicotine delivery systems as one feasible alternative to cigarette smoking (UN, 1998). Smokeless tobacco products can be divided into tobacco for oral use (moist or dry oral snuff, chewing tobacco, products mixed with lime, and other components) and dry finely powdered nasal snuff products (IARC, 1985). The oral snuff that is used today in northern Europe and the United States consists of finely cut or ground (Sweden) tobacco with a high moisture content (≈50%). This review is solely concerned with the types of moist snuff that are used in northern Europe and North America. In the United States the quid is generally between the lower lip, or between the gum and buccal mucosa, whereas in Sweden it is placed under the upper lip.

Since Petter Swartz about 1770 started large-scale production of snuff in Norrköping, snuff dipping has a long history in Sweden where this habit probably is more prevalent than in any other industrialized western nation. In 1919 sales figures peaked when 7000 tons of moist snuff was sold, corresponding to an average yearly consumption of about 1.5 kg per person. Until 1969 a gradual decrease occurred, which was followed by an increase to reach a sale of 4632 tons in 1990 or 0.6 kg/year and person (Axéll, 1993). Only a few tons of chewing tobacco per year are, on the other hand, currently sold on the Swedish market. In 1996 the fraction of snuff dippers reached almost 20% of the grown-up male population, a value that is one order of magnitude higher than that for the United States

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(CPS, 1997; Idris *et al.*, 1991, 1994). The tendency is typical for all strata of Swedish society. Thus, in recurrent nationwide surveys of the tobacco habits of medical doctors that have been conducted for 28 years, the prevalence of smokers decreased from 46 to 6%, while the fraction of snuff dippers reached 9% among male and 3% among female physicians (Bolinder and Himmelmann, 1996). The use of oral snuff was introduced in the United States in the middle of last century by Scandinavian lumberjacks (Hoffman and Djordjevic, 1997), and the usage became widespread especially in areas like West Virginia (Rogozinski, 1990; Winn, 1986). In the whole of the United States, snuff consumption increased by 38% during the period 1981 to 1993, and in 1995 nearly 60 million pounds was consumed (Hoffmann *et al.*, 1995; Hoffman and Djordjevic, 1997).

The main concern associated with the use of oral snuff has been induction of cancer of the oral cavity and pharynx (Winn *et al.*, 1981; IARC, 1985; Idris *et al.*, 1991, 1994), and there seems to be compelling evidence linking the nasal snuff products used by the Bantus in South Africa to an elevated risk for nasal sinus cancer (IARC, 1985; Higginson and Oettlé, 1960). The use of moist snuff also induces gingival recessions and "smokeless tobacco keratosis" that have wrongfully (Axéll, 1993; Vignesvaran *et al.*, 1995) been described as precancerous lesions. In India, the combination of smoking and the chewing of tobacco mixed with areca nut and/or betel leaf is associated with a high risk for cancers of the oral cavity, larynx, and esophagus (Jayant *et al.*, 1977).

Snuff dipping has also been incriminated as a cause of cancer in the stomach, esophagus, pancreas, urinary bladder, and kidney, but the evidence must be considered as anecdotal or inadequate (IARC, 1985). Based on incomplete data, some authors have, in addition, postulated that smokeless tobacco constitutes a risk factor for cardiovascular disease (Bolinder *et al.*, 1992, 1994, 1997; Bolinder, 1997). In this context it seems appropriate to emphasize that whereas snuff dipping is more common in Sweden than in any other western country, the population has a relatively low incidence for cancer of the oral cavity, well below, e.g., the U.S. average. Thus, the annual incidence rate of tumors of the mouth and tongue in Swedish males is 4.2 per 100,000 compared to 7.2 for the United States (IARC, 1992).

While certain physicians are engaged in a crusade to stop all uses of tobacco in a way that is reminiscent of the moods prevailing during the era of alcohol prohibition in the United States, some experts have compared the relative risks involved for active smoking with that of snuff dipping and arrived at the conclusion that turning active smokers to snuff users will drastically reduce the risk of cancer and cardiovascular disease (Kirkland, 1980; Russel *et al.*, 1980; Pindborg and Axelsen, 1980; Schievelbein, 1980; Rodu, 1994; Vignesvaran *et al.*, 1995). In their renowned review on avoidable causes of cancer commissioned by the Office of Technology Assessment of the U.S. Congress, Doll and Peto (1981) also emphasized that ". . . a change to the use of tobacco in a less dangerous way" would be expected to result in a drastic reduction in the number of cancer deaths. Another issue is the increased popularity of smokeless tobacco by youth, where there has been a concern that this habit may create a dependence on nicotine that could subsequently lead to active smoking.

When assessing the undesired effects of snuff dipping, there has often been a general lack of appreciation of the large differences in chemical composition, as well as of the divergent manners of use with respect to various kinds of smokeless tobacco. Thus, the conclusion by IARC (1985) "that there is sufficient evidence that oral use of snuff products of the types commonly used in North America and western Europe is carcinogenic to humans" does not seem to apply to the unfermented moist types of snuff marketed in Sweden and that are characterized by low levels of nitrosamines. The fact that locally grown tobacco for many "snuff products" used in Central and South East Asia are mixed with areca nuts and betel leaves (IARC, 1985)—products that contain arecoline and associated nitrosamines—further underlines that snuff by no account represents a well-defined and homogeneous group of tobacco products.

Apparently most, if not all, of the carcinogenic action of certain types of fermented snuff products can be ascribed to the presence of tobacco-specific nitrosamines (TSNA), and the high carcinogenic potency of some of these nitrosamines in the rodent has been a matter of serious concern. However, the levels of such TSNA in snuff products may differ by orders of magnitude depending on origin and manner of processing. The crucial issue from society's point of view is, clearly, whether or not the possible risk posed by exposure to TSNA in marketed brands of snuff is sufficiently high to motivate some kind of action.

In the present analysis the author will (i) assess available analytical and experimental data for the most important TSNA and extrapolate the cancer risk to humans with respect to different types of snuff products and subsequently (ii) compare these estimated risks derived on the basis of rodent bioassays with available epidemiological data. A review of adverse effects other than cancer will also be included.

CHEMICAL CHARACTERISTICS OF VARIOUS TYPES OF SNUFF

In addition to the plethora of chemical substances present in any plant, snuff contains some alkaloids and derivatives thereof specific for *Nicotiana* and related

TABLE 1
Nicotine and Nitrosamine Content (Based on Dry Weight) of Snuff Products from Various Sources

Source	Nicotine (mg/g)	NNK (μg/g)	NNN (μg/g)	Total TSNA (μg/g)
		Moist oral snuff products		
Canada (Brunnemann and Hoffmann, 1991)		3.2–5.8	50–79	209–260
Sweden (Österdal and Slorach, 1984; Österdahl, 1996)				
1983 (36 samples)	8–18	1.6 (1.1–2.4)	7.6 (3.2–9.4)	18 (5–102)
1986 (34 samples)		1.5	8.6	16.0
1992 (20 samples)		1.3	3.8	8.8
Norway (Österdal and Slorach, 1984)				
1983 (2 samples)		6.6 (5.4–7.8)	42 (26–58)	77 (52–102)
Sudan (Idris *et al.*, 1991)				
Toombak		8–102	188–7870	141–3080
United States (Hoffmann *et al.*, 1991)				
Two samples of a new brand	1.8–2.0	7–16	37–57	94–156
United States (Hoffmann *et al.*, 1995)				
Copenhagen	12.0 ± 0.7	1.9 ± 0.6	8.7 ± 1.4	17.2 ± 3.0
Skoal, Original Fine cut	11.9 ± 1.3	1.3 ± 0.1	8.2 ± 1.3	14.9 ± 2.5
Skoal, Bandits Straight	10.1 ± 0.8	0.9 ± 0.3	5.1 ± 1.0	8.2 ± 1.7
Kodiak Wintergreen	10.9 ± 0.8	0.6 ± 0.2	6.3 ± 1.1	11.0 ± 2.4
Hawken Wintergreen	3.2 ± 0.2	0.2 ± 0.04	3.1 ± 0.3	4.1 ± 0.4
		Dry snuff products		
United States (Brunnemann *et al.*, 1987)				
Three brands	13 (12–14)	8 (2–15)	46 (15–84)	78 (37–135)

species of the *Solanaceae* family. The main tobacco-specific alkaloids are nicotine, nornicotine, anabasine, anatabine, and myosmine. During curing and processing, TSNA like 4-(nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), *N'*-nitrosonornicotine (NNN), *N'*-nitrosoanabasine (NAB), and *N'*-nitrosoanatabine (NAT) are formed from these tobacco constituents. Nitrate is present in fairly high levels in a number of leafy plants, including tobacco, spinach, salad, etc. The reduction of nitrate by microorganisms during processing to produce nitrite seems to constitute the mechanism of nitrosation of amines present in tobacco. The concentrations of nitrite in U.S. brands have been found to vary considerably, where the leading product contained 672 ± 297 mg/kg nitrite nitrogen, while the fourth best-selling snuff brand (2% of the market) only had 1.3 ± 0.4 mg/kg nitrite nitrogen (Hoffmann *et al.*, 1995).

It should be noted that tobacco-specific nitrosamines are apparently not absolutely specific for tobacco products. Thus, the content of nicotine in 1 kg of tomatoes or bell peppers corresponds approximately to that contained in half a cigarette (Sheen, 1988). Under conditions that favor nitrosation, low levels of TSNA are therefore bound to be formed also in food products containing various vegetables from the Solanacae family.

Sudanese "Toombak"—prepared from finely ground, locally grown *Nicotiana rustica* that is mixed with "natron powder"—contains up to 3000 μg NNN, and 7900 μg/g dry wt of NNK (Idris *et al.*, 1991). These levels should be compared to the average values with respect to total content of TSNA for Swedish moist snuff of 18 μg/g dry wt in 1983, a value that had dropped down to about 9 μg/g in 1992 (Österdahl and Slorach, 1984; Österdahl, 1996). For many U.S. products, a similar development has occurred. In Table 1 the nitrosamine and nicotine contents of moist snuff products from various sources are listed. For the sake of comparison, the TSNA levels found in three commercial U.S. brands of dry snuff have also been included.

In his review on smokeless tobacco, Rogozinski (1990) points out that until recently the production methods for many oral snuff products had not changed appreciably in the United States since the 1830s. Traditionally, snuff products intended for nasal or oral use were generally made from air- or fire-cured dark leaf. After the tobacco is cut and packed, it undergoes aging or sweating for up to 3 years. During this process, which involves fermentation, the temperature rises,

and at intervals the tobacco must be removed and returned to equalize conditions throughout the container. Some brands are flavored before packaging into the retail containers. In the production of Swedish "snus," an air-cured nonfermented tobacco is subjected to a carefully controlled heating process resulting in a practically sterile product. In this manner a moist snuff with depressed levels of TSNA as well as of polycyclic aromatic hydrocarbons is obtained. The low degree of microbial contamination of the Swedish products is probably the reason why storage for 20 weeks at temperatures ranging from −20 to +23°C did not result in significantly elevated concentrations of volatile nitrosamines in Swedish snuff (Österdahl and Slorach, 1983). In contrast, Djordjevic *et al.* (1993) found that the levels of nitrite increased drastically and those of TSNA 3-fold and of volatile nitrosamines 10-fold upon storage of U.S. snuff at 37°C for 4 weeks.

Morpholine, previously used in the manufacture and/or packaging of snuff, gave rise to the carcinogen *N*-nitrosomorpholine found to occur at levels up to 0.7 μg/g in some U.S. snuff products. Similarly, the presence of *N*-nitrosodiethanolamine at 0.3–3.3 μg/g was probably due to the agricultural use of diethanolamine as solubilizer for the growth inhibitor maleic hydrazide (Brunnemann *et al.*, 1982; Hoffmann *et al.*, 1996). The contents of volatile nitrosamines in Swedish snus have generally been low (mean for 14 samples, 0.008 μg/g, 1982), and the finding in 1981 of two Swedish products that contained *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine in levels ranging from about 0.1 to 0.2 μg/g dry wt must be regarded as an exception (Österdahl and Slorach, 1983). Today, the products found on the United States as well as on the Swedish market are practically free from these nitrosamines (Hoffmann *et al.*, 1996). As for many food products, the weakly carcinogenic non-tobacco-specific nitroso amino acids *N*-nitrososarcosine (NSAR) and 3-(methylnitrosamino)-propionic acid (MNPA) and the moderately active 4-(methylnitrosamino)-butyric acid (MNBA) are also formed in snuff upon processing and storage. However, except for the single discovery of relatively high levels of MNPA and MNBA in a U.S. brand (Hoffmann *et al.*, 1991), the contributions of these nitrosamines to total cancer risk seem to be negligible (Table 2). In snuff products containing fire-cured tobacco, elevated concentrations of polycyclic aromatic hydrocarbons (PAH) will be present. Benzo[*a*]pyrene, an indicator of PAH, may be present in such snuff products up to about 90 ng/g (Hoffmann and Djordjevic, 1997). Tobacco also contains the alpha emitter Po 210 and the activity in Swedish moist snuff has been measured and found to be in the range of 22–120 Bq/kg dry wt, corresponding to a negligible yearly dose that is equivalent to background radiation during 1 week (Samuelsson, 1989).

TABLE 2

Non-Tobacco-Specific Nitrosamine Contents (μg/g Based on Dry Weight) of Moist Snuff Products from Various Sources

Source	NSAR	MNPA	MNBA
United States (Hoffmann *et al.*, 1995)			
Copenhagen	0.06 ± 0.01	2.6 ± 0.6	0.3 ± 0.1
Skoal, Original Fine cut	0.04 ± 0.0	2.4 ± 0.3	0.2 ± 0.06
Skoal, Bandits Straight	0.02 ± 0.01	12.0 ± 1.8	0.1 ± 0.08
Kodiak Wintergreen	0.04 ± 0.01	2.2 ± 0.3	0.2 ± 0.04
Hawken Wintergreen	0.07 ± 0.01	5.6 ± 0.7	0.3 ± 0.06

METABOLISM AND TOXICOLOGICAL EFFECTS

Metabolic Transformations of TSNA

Most, if not all, of the carcinogenic action of snuff products can apparently be ascribed to the presence of carcinogenic TSNA, especially of NNK and NNN. Of the two nitrosamines, NNK has been most thoroughly investigated. Little or no unchanged NNK or NNN is excreted with urine, and in mammals the carbonyl group of NNK is reduced to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) that is either conjugated with glucuronic acid and excreted in urine or further metabolized by hydroxylation (for a review on metabolism, see Hecht *et al.*, 1994; Hecht, 1996). NNAL has also been found to be a potent experimental carcinogen (Hoffmann *et al.*, 1996). By one pathway α-methylhydroxylation gives α-hydroxymethyl-NNK, an intermediate that is sufficiently stable to form the corresponding glucuronide. However, by the main route of conversion, α-hydroxymethyl-NNK leads to the formation of 4-(3-pyridyl)-4-oxobutanediazohydroxide that introduces covalently bound pyridyloxobutyl (HPB) adducts at nucleophilic centers in DNA and proteins. Mild hydrolysis of these adducts releases 4-hydroxy-1-(3-pyridyl)-1-butanone, which can be derivatized and conveniently analyzed by gas chromatography–mass spectrometry (Hecht *et al.*, 1994; Hecht, 1996). The method is analogous to that previously developed by Ehrenberg's group at Stockholm University for measurement of Hb adducts derived from reaction with several directly alkylating agents (Törnqvist *et al.*, 1986). Detailed chemical analysis has revealed that the HPB adduct is a carboxylic ester of aspartate, glutamate, or a terminal carboxyl group in globin (Carmella *et al.*, 1993a). DNA pyridyloxobutylation is most probably involved in cancer initiation and induces G to A transitions as well as G to T transversions, the latter possibly due to steric hindrance caused by the bulky adducts formed (Ronai *et al.*, 1993).

A second hydroxylation pathway involves hydroxylation of the methylene group adjacent to the *N*-nitroso group producing an intermediate that apparently spontaneously decomposes to the directly methylating agent methanediazohydroxide. Upon reaction with DNA the latter will give rise mainly to 7-methylguanine and O^6-methylguanine. Although the yield of 7-methylguanine is about 10 times above that of O^6-methylguanine, the former is probably of secondary importance with respect to genotoxicological effects. O^6-Methylguanine is, on the other hand, a highly promutagenic adduct that gives rise to G to A transitions of a type found in the Ki-*ras* oncogene from mouse lung tumors induced by NNK (Belinsky *et al.*, 1989; Ronai *et al.*, 1993).

Minor metabolic pathways involve the hydroxylation of the 6-position of the pyridine ring of NNK and pyridine-N-oxidation of NNK and NNAL. Studies using human lung and liver microsomal preparations seem to indicate that the NNAL pathway is more important and direct hydroxylation less so in man than in rodents (Hecht *et al.*, 1994; Hecht, 1996).

Upon oxidative metabolism, NNN generates the same reactive diazohydroxide as is obtained upon α-hydroxylation of the terminal methyl group of NNK, thereby inducing pyridyloxobutylation of proteins and DNA. NNN gives a complex pattern of metabolites in urine with 4-hydroxy-4-(3-pyridyl)-butyric acid, *N'*-nitrosonornicotine-1-*N*-oxide, and 4-oxo-4-(3-pyridyl)-butyric acid as the main excretion products. In the exposure range 3–300 mg/kg, 86 to 91% of an ip-administered dose is excreted as metabolites in rat urine (Hecht *et al.*, 1981).

Biomarkers for Exposure to NNK and NNN

The urinary NNK metabolites NNAL and its glucuronide have been used as markers of exposure to NNK in tobacco smoke, chewing tobacco, and moist snuff (Carmella *et al.*, 1993b; Hecht *et al.*, 1994; Kresty *et al.*, 1996). In a sample of 23 snuff dippers, the urinary levels of these metabolites were on average higher than for a sample of 61 smokers. However, there was a striking individual variation in the amounts of NNK metabolites excreted (Kresty *et al.*, 1996). In the case of snuff dippers, apart from possible interindividual differences in the extent of NNK hydroxylation versus conjugation, the observed variability will certainly also reflect the frequency of intake as well as disparate contents of TSNA in the snuff consumed. In the cytochrome P450 complex of humans, several isoenzymes (CYP1A2, CYP2A6, CYP2E1, CYP2D6) exhibit catalytic activity with respect to NNK hydroxylation (Hecht, 1996). In view of the 16-fold difference in the rate of metabolic activation of nicotine to cotinine found for human subjects that seems to be related to genetic polymorphism of members of the P450 gene superfamily (Cholerton *et al.*, 1994), the observed variations with respect to NNK metabolite patterns are not at all surprising. Similarly, in a limited number of lung samples from autopsies, the variations in 7-methylguanine could not be explained by differences in tobacco exposure (Blömeke *et al.*, 1996). Obviously, several factors are involved that influence the amounts of active electrophile reacting with nucleophilic centers *in vivo*. In addition to differences in the capacity to activate NNN and NNK to the proximate electrophile, the target adduct levels may be reduced by the presence of varying levels of protective substances of endogenous or dietary origin, like phenethyl isothiocyanate derived from *Cruciferae* species mentioned below (Morse *et al.*, 1989).

The HPB adducts in Hb resulting from activation of NNK and NNN have also been utilized for monitoring purposes, and these adducts have been detected in Hb of smokers and snuff dippers (Carmella *et al.*, 1990). In comparison with 7-methylguanine and O^6-methylguanine in DNA, the HPB Hb adducts have the advantage of a greater stability as well as higher sensitivity for detection due to lower background values. Again, there were conspicuous interindividual differences with respect to the levels of adduct formation in smokers as well as in users of snuff where a significant proportion did not exhibit increased adduct levels (Carmella *et al.*, 1990; Hecht *et al.*, 1993).

Quantitative Risk Assessment of Tobacco-Specific Nitrosamines in Moist Snuff Based on Experimental Data

Among the various TSNA, NNK, NNAL, and NNN have been found to be potent experimental carcinogens, NAB displayed a weak carcinogenic activity in corresponding bioassays, whereas NNA as well as NAT were inactive. In rodents, NNK induces tumors primarily in the lung, but also in pancreas, nasal cavities, and liver (Hoffmann *et al.*, 1996). Upon application of NNK and NNN in the oral cavity of rats, tumors are induced not only at the site of application, but also in the lung (Hecht *et al.*, 1986). NNN—that induces tumors in the nasal cavity and esophagus of experimental animals—seems to be less potent than NNK. It seems reasonable to assume that the contents of NNK and NNN will be the major determinants for the overall carcinogenic potency of moist snuff products used in northern Europe and in North America.

Although a number of investigations have been conducted that qualitatively demonstrate the carcinogenicity of NNK and NNN in experimental animals (Hoffmann *et al.*, 1996), in most cases neither route of administration nor selection of doses has been appropriate for construction of useful dose–response relationships for the purpose of quantitative cancer risk estimation upon administration by the oral route. With

TABLE 3

Incidence of Tumors Induced by Different Doses of NNK in Fischer 344 Male Rats upon Administration with Drinking Water (Rivenson *et al.*, 1988)

	Daily intake (mg/kg/day)			
Tumor site	0	0.021	0.048	0.22
Nasal cavities, total	0/80 (0%)	1/80 (1%)	2/80 (3%)	5/30 (17%)
Liver tumors, total	6/80 (7.5%)	3/80 (4%)	11/80 (14%)	12/30 (40%)
Lung, total	6/80 (7.5%)	9/80 (11%)	20/80 (25%)	27/30 (90%)
Pancreas	1/80 (1%)	5/80 (6%)	9/80 (11%)	2/30 (6%)

respect to NNK, the study conducted by Rivenson *et al.* (1988) appears to be the most appropriate for the aforementioned purpose. Male Fischer 344 rats were administered the nitrosamine in drinking water at 0, 5, 10, or 15 ppm during the animals' lifetime. The highest oral dose corresponded to a total intake of about 120 mg/kg. Tumors were induced in lung, nasal cavities, and pancreas (Table 3). Clear dose–response relationships were evident for tumors in lung, liver, and nasal cavities, out of which the induction of lung tumors appears to be the most sensitive end point that could conveniently be used for high- to low-dose risk extrapolation. In comparison with subcutaneous injection of NNK, oral administration is considerably more effective in inducing tumors in pancreas, but similar with respect to lung and liver and much less effective with respect to tumors of the nasal cavities of Fischer F344 rats (Hoffman *et al.*, 1984). Because NNK and NNN are genotoxic carcinogens (IARC, 1985), a stochastic model like the linearized multistage model should preferably be used for high- to low-dose extrapolation (U.S. EPA, 1996). Based on the data presented in Table 3, a 95% upper-bound carcinogen potency factor for annual risk (Crump, 1982) of about 0.086 (mg/kg and day)$^{-1}$ is obtained for NNK in humans. Surface-based extrapolation from rat to man with respect to effective dose would yield an about fivefold higher risk estimate.

The administration of NNN in drinking water to Fischer 344 rats at dose levels corresponding to an intake of 3–8 mg/kg/day during 27–36 weeks resulted in a tumor incidence in nasal/oral cavities and esophagus of 100% (Hecht *et al.*, 1983; Castongay *et al.*, 1984). An oral study in BDVI rats featuring three adequately spaced dose levels has been conducted at the IARC (Gricuite *et al.*, 1986). Groups of 25 female and 25 male BDVI rats were administered 3, 1, and 0.3 mg of NNN by intragastric instillation twice a week for 78 weeks. The appearance of tumors in the nasal cavities was clearly dose related and seems to constitute the most sensitive index for cancer induction in this animal model (Table 4). Using the multistage extrapolation model, the 95% upper bound for the extrapolated carcinogen potency factor for annual risk in humans is about 0.029 (mg/kg/day)$^{-1}$ with respect to NNN for this data set.

Since the formation of promutagenic DNA adducts can be causally linked to the initiation of neoplastic processes, the adduct level in DNA may be regarded as the "effective dose," and when measured at the site of tumor development, this "target dose" provides the most relevant index of exposure that could be used for dose extrapolation. If reliable estimates of target doses are available, then these will reflect interspecies and interindividual differences more adequately than pharmacokinetic modeling. Under certain conditions the target dose may be estimated indirectly from the adduct levels in Hb as a target dose "surrogate." During the past two decades, the target dose concept has been successfully applied for estimation of cancer risk, especially several directly alkylating substances (Ehrenberg *et al.*, 1974, 1983; Wright *et al.*, 1988; Törnqvist and Ehrenberg, 1990; Segerbäck *et al.*, 1994).

Murphy *et al.* (1990) have demonstrated that the levels of HPB Hb adducts from NNK are linearly correlated with exposure over a wide dose range in the rat. Giving 21 μg/kg NNK three times per week by ip injections (9 μg/kg/day), a steady state for total Hb adduct levels of about 180 fmol/mg globin is attained after approximately 42 days (Carmella and Hecht, 1987), out of which approximately 20–40% is released as HPB after treatment with base (Murphy *et al.*, 1990). On a μg/kg basis, NNN gives about 20% lower yield of Hb adducts that only release some 5% as HPB (Carmella and Hecht, 1987; Hecht *et al.*, 1991). Twenty-two snuff dippers with an estimated daily exposure of 0.047 μg/kg body wt of NNK and 0.6 μg/kg body wt of NNN had on average a level of HPB adducts released from Hb of about 0.5 fmol/mg hemoglobin (Carmella *et al.*, 1990; Hecht *et al.*, 1991), which is in reasonable agreement with the findings from the experimental studies, thus giving no indication of a significantly higher met-

TABLE 4

Incidence of Nasal Tumors Induced in Female and Male BDVI Rats by Oral Administration of NNN at Different Dose Levels (Griciute *et al.*, 1986)

	Dose (mg/kg/day)[a]			
Site of tumor	0	0.25	0.82	2.45
Nasal cavity, malign	0/50 (0%)	1/50 (2%)	5/50 (10%)	42/50 (80%)
Nasal cavity, benign	0/50 (0%)	11/50 (22%)	30/50 (60%)	5/50 (10%)
Nasal cavity, total	0/50 (0%)	12/50 (24%)	35/50 (70%)	47/50 (94%)

[a] Based on an assumed average body weight of 350 g.

abolic activation rate in the rodent compared to humans. Theoretically, at steady state the adduct level measured in the snuff-dipping humans would translate to the daily ip administration to rats of about 0.08 μg/kg body wt of NNK or of 0.6 μg/kg body wt NNN (based on the experiments with [5-^{3}H]NNK by Carmella and Hecht, 1987). Judging from the comparability of doses in Hb, quantitative extrapolation of risk estimates from the rat to humans could, thus, be considered justified. The question then remains to what extent the expected relatively high-risk estimates in laboratory animals is supported by epidemiological evidence.

Epidemiological Data

In contrast to what has been the case for active smoking, few adequately performed epidemiological investigations have been carried out with respect to users of smokeless tobacco. Although the evidence for human carcinogenicity was considered sufficient, in its review from 1985 that was mainly based on three investigations from the south-eastern United States and one from Scandinavia, IARC pointed out a number of shortcomings characterizing most of the supporting material. Among these investigations, the basically well-conducted case–control study from North Carolina by Winn and co-workers (Winn *et al.*, 1981; Winn, 1986) that included 255 women with cancer of the oral cavity and pharynx (mostly squamous cell carcinoma) and 502 matched hospital controls provides the most convincing evidence so far obtained of a causal association between snuff dipping as practiced among women in the south-eastern United States and cancer of the oral cavity. The main weakness of the investigation was the fact that 39% of the cases had been identified from death certificates. The OR associated with the use of snuff in white nonsmoking women was 4.2 (95% CI, 2.6–6.7) which rose to 5.2 after conditional logistic regression (Winn, 1986). For cancer of the gum and buccal mucosa, tissues that come into direct contact with the tobacco, there was almost a 50-fold increase in risk. There was also a strong correlation between oral cancer and poor dental status. However, since snuff dipping causes gingival recessions, this finding may simply reflect the duration and degree of exposure to oral snuff. Although not designed as an epidemiological investigation, Wray and McGuirt (1993), similarly to earlier observations (IARC, 1985), found that a significant fraction of the cases of oral carcinoma located in the tumor registry of the University Medical Center (Winston–Salem, NC) were elderly white woman (average age, 78 years) who were or had been users of oral snuff, out of whom 20% admitted to have used cigarettes and alcohol in addition. Although it was not possible to derive reliable quantitative estimates of cancer incidences in Sudan, where about one-third of the men were said to use Toombak containing extreme levels of TSNA, clinical experience indicated a significant elevation in the incidence of cancer of the oral cavity in this population (Idris *et al.*, 1994).

Results from U.S. studies published since 1985 have been ambiguous mainly due to limited size, but three Swedish studies that were based on much larger materials have recently been conducted that do not support an association between cancer and the use of the special brand of nonfermented moist snuff (snus) used in Sweden (Lewin *et al.*, 1994, 1998; Schildt *et al.*, 1998; Nyrén *et al.*, 1998). The creation of a central cancer registry covering the entire Swedish population offers a unique opportunity to conduct large-scale population-based epidemiological surveys in this country (Ehrenberg *et al.*, 1985). This is especially so with respect to the effects of snuff dipping, since cancers of the oral cavity and pharynx constitute relatively rare tumors, whereas a significant proportion of the male Swedish population are users of snus.

In one investigation 545 cases of cancer of the head and neck region were localized by the aid of the aforementioned registry in a population base of approximately 2.6 million inhabitants from south Sweden (Lewin *et al.*, 1994). Subsequently, this cohort was extended to comprise 605 cases out of which 128 were cancers of the oral cavity, representing more than 90% of all diagnosed head and neck cancer cases in the geographical area under study. An age- and sex-matched control group was selected from general population registries. Interviews were carried out by specially trained medical personnel. Ninety percent of the cases and 85% of the controls were interviewed in person with respect to use of tobacco, use of alcohol, occupational exposures, oral hygiene, and dietary habits (Lewin *et al.*, 1998). In contrast to active smokers, where a crude OR of 5.0 ($P < 0.001$) was obtained, no risk increase could be detected among snuff dippers. For present users of snus the OR was 1.0 (CI, 0.7–1.6) and for exusers 1.2 (CI, 0.8–2.4). Adjusting for confounding by use of alcohol and active smoking did not change these estimates. The design was such as to permit the detection of an OR of $\geq$2.0 in a population where at least 5% are exposed to oral snuff, and increases in risk similar to those reported from North Carolina by Winn and co-workers (1981)—where the estimated annual incidence rate was estimated to be 26 cases per 100,000—should easily have been detected.

In the second Swedish study, covering the northern part of Sweden during 1980–1989, 418 cases of cancer of the oral cavity were identified from the Swedish Cancer Registry (Schildt *et al.*, 1998). Controls matched with respect to sex, age, and county of residence were acquired from the general population registry, and information about exposure and other relevant factors was obtained by the use of an extensive

questionnaire complemented by contacts by telephone; the response rate was 95% for cases and 90% for controls. For active smokers an OR of 1.8 (CI, 1.1–2.7) was obtained, whereas, again, no significant increase in risk was noted for users of Swedish snuff; the OR for current snuff dippers was 0.7 (CI, 0.4–1.1) and for exusers 1.5 (CI, 0.8–2.9). The third study (Nyrén *et al.*, 1998) is a very large prospective study that was recently completed by researchers at the well-known Karolinska Institute and has provided essentially the same results as the two Swedish investigations mentioned above. In another large-population-based study on the risk of gastric cancer, no increased risk could be detected for snuff dippers (Hansson *et al.*, 1994).

It may be legitimate to raise the question about the quality of the information contained in the Swedish Cancer Registry. Differences in "diagnostic intensity" between hospitals undoubtedly contributes significantly to reported geographic differences in cancer rates for such sites as, e.g., lung, breast, and prostate. However, this does not seem to be the case for cancers like leukemia, where diagnosis is relatively straightforward (Ehrenberg *et al.*, 1985), and this would presumably also be the case for cancers of the head and neck region.

What could be the reasons for the discrepancies found between on one hand the United States and on the other the Swedish epidemiological data? In contrast to the Swedish studies, in the North Carolina (Winn *et al.*, 1981) cohort for almost half of the cases (39%) selection was based on death certificates, the reliability of which can often be questioned. Further, the difficulties in obtaining correct information by personal interviews of present and past tobacco habits have been universally recognized (Lee and Forey, 1996) and are obvious sources for misclassification bias and confounding especially when surrogate information sources are used. However, for the North Carolina cohort, relative risks were generally higher when interviews were carried out with the women themselves than for interviews with next-of-kin, and it seems most unlikely that overestimation of snuff use among deceased cases could account for the high increases in relative risk. Strong synergistic effects of alcohol and use of tobacco have been reported with respect to cancers of the oral cavity and pharynx. Although alcohol consumption by elderly North Carolina women was reported as low by Winn *et al.* (1981), this may not have been so in the past. Wray and McGuirt from Winston–Salem, North Carolina (1993), make the relevant observation that whereas snuff dipping was both acceptable and condoned for women in this area, cigarette smoking—and most probably also use of alcohol—was not considered proper in former times. It is, therefore, likely that information from these women on past smoking, and possibly also on the use of alcohol, was inadequate and could be a source of confounding, giving some overestimation of risk.

When looking for possible differences, the types of snuff used in the United States and Sweden, respectively, could be more important than differences in smoking habits and use of alcohol. According to Winn (1986), the snuff used by white women in North Carolina was mostly of a dry type and of unknown quality, and it is possible that some of the snuff consumed in the past had been produced locally. When this investigation was conducted around 1980, the average age of the cases was 65 for women with gum and buccal mucosal cancer and 69 for women with cancer in other parts of the mouth. One-third of these woman started using snuff at the age of 10. Thus, the induction period for cancer in this cohort includes long-term exposures to products that were marketed in North Carolina between the two world wars and that could have been more closely related to Sudanese Toombak with respect to levels of TSNA than Swedish snus. The presence of unidentified promoting factors in these products as well as of different amounts of anticarcinogens and antimutagens cannot be excluded. As to the latter, traditional processing and storage as previously practiced in the United States could possibly reduce the snuff content of such agents.

The manner of snuff use may also be important. The articles published by Winn and co-workers give little information on how these women actually practiced snuff dipping. However, the fact that a dry snuff had mostly been consumed leads one to suspect that the manner of snuff dipping was the traditional custom of the south, where the women applied dry snuff with a stick and rubbed it about the mouth and into the interstices of the teeth (Rogozinski, 1990). Further, there is clear evidence that the widespread use in Sweden of portion bags instead of loose snuff markedly reduces the incidence of tobacco-related oral pathological changes of the oral mucosa (Andersson and Axéll, 1989; Axéll, 1993).

Snuff-Induced Oral Lesions and Oral Cancer

It has been known for a long time that snuff dipping results in certain soft tissue changes of the oral cavity, known as "snuff dipper's lesion," snuff-induced leukoplakia, or smokeless tobacco keratosis. At the site where the quid is regularly placed, gingival recessions may also develop in about one-quarter of the users of loose snuff and in about 3% among the users of portion-bag snuff of the Swedish type (Andersson and Axéll, 1989).

As used by, e.g., IARC (1985), leukoplakia is a broad clinical entity that has been indiscriminately applied to include epithelial changes of divergent etiology, histological characteristics, and greatly varying tendency for malignant transformation (Sciubba, 1995). Consid-

erable confusion has been caused by equating these lesions with the preneoplastic leukoplakia induced by smoking and that mostly involve the floor of the mouth, ventral tongue, and soft palate (Axéll, 1993; Vignesvaran *et al.,* 1995). These sites account for a majority of all oral cancer in the United States and Sweden. Smokeless tobacco keratosis has been described as wrinkled or folded lesions, with and without change of surface color. Axéll and co-workers have introduced a graded four-point scale for classification of these tissue alterations that extends from a superficial lesion without change of color to a marked yellowish to brown, heavily wrinkled lesion with intervening deep-reddened furrows and/or heavy thickening (Axéll, 1993). Histologically, smokeless tobacco keratosis is generally characterized by a hyperplastic epithelium with vacuolization and so-called Chevron type of keratinization. Varying degrees of inflammation may also be present. In a study of 252 daily users of Swedish snus, Andersson and Axéll (1989) demonstrated by means of stepwise logistic regression the following factors to influence the severity of the lesion: package form (loose snuff vs portion-bag snuff), placement of the quid, the daily amount used, years of usage, and age of the individual. The use of portion-bag snuff seemed to cause less severe mucosal changes than loose snuff.

There seems to be some controversy concerning the appearance of dysplastic features among these lesions. Apart from differences in the chemical composition of the snuff used, reported inconsistencies seem to a considerable degree to have been caused by divergent criteria used among oral pathologists to define "dysplasia." On the basis of one case of slight epithelial dysplasia and one carcinoma among 32 oral snuff users, Roed-Petersen and Pindborg (1973) frankly stated that 6.2% of snuff-induced changes were associated with premalignancy or malignancy. Hirsch and coworkers (1982) found nine cases of mild dysplasia among 50 habitual snuff dippers, but no case of moderate or severe dysplasia. In two other limited investigations, the authors considered that such cases of mild dysplasia were probably nothing else but reactive changes due to inflammatory infiltration (Frithiof *et al.,* 1983; Jungnell and Malmström, 1985). Larger studies have indicated that whereas for smoking-induced leukoplakias—where about 20% of the oral leukoplakias demonstrate some degree of dysplasia (Waldron and Schaffer, 1975)—such changes are relatively rare in snuff dipper's lesions. Thus, upon analyzing 114 biopsies from smokeless tobacco keratoses in Swedish material, no single case of epithelial dysplasia was found (Axéll *et al.,* 1976). In one prospective study in the United States no single case of oral cancer was found during a 10-year period among 1550 persons with smokeless tobacco keratosis (Smith, 1975).

When assessing the importance of the histopathological changes caused by moist snuff, the reversibility of these changes constitutes an important factor. In a cohort of 252 habitual users of Swedish snus with no other tobacco habits, precancerous changes were absent in all biopsies. However, in 29 cases some features were present that could possibly be interpreted as components of dysplasia (e.g., elevated mitotic index, loss of cell cohesion, increased basal cell density). These individuals were subsequently induced to stop using snuff, which resulted in a complete stop in 20 and reduced usage in 9 cases. Upon reexamination at least 3 months later, the biopsies displayed normal mucosa in the 20 cases and reduced changes in the 9 cases. In other words, the dysplastic features were reversible in all 29 cases (Larsson *et al.,* 1991).

In conclusion, smokeless tobacco keratosis seems to be a separate clinical entity where the lesion has a very low probability of malignant transformation and that should be distinguished from smoking-induced leukoplakias (Axell, 1993; Ahlbom *et al.,* 1996).

Expected Human Tumor Incidences Based on Experimental Data—Discrepancies between Expected and Observed Incidences of Cancer in Swedish Snuff Dippers

In 1980–1983 it was estimated that out of a total population of 8 million, about 700,000 Swedes were snuff dippers (Österdahl and Slorach, 1984). Since the population base for the investigation conducted by Lewin and co-workers described above was 2.5 million, some 200,000 snuff dippers would presumably be included. It should be born in mind that virtually all cancer cases are reported to the central Swedish cancer registry, permitting the following general assessment to be made.

Although the present TSNA concentrations in Swedish snuff currently lie at about 9 μg/g dry wt (Österdahl, 1996), present cancer incidences will reflect historical exposures on a much higher level. Unfortunately, the earliest analytical data of Swedish snuff date from 1983, a time when technical improvements in processing may be expected to have already affected the levels of nitrosamines, providing a snuff with 1.6 μg NNK and 7.6 μg NNN per gram of dry weight. Further, 56% (portion bag) to 64% (loose snuff) of the total TSNA has been found to be extracted in the mouth (Andersson *et al.,* 1994). Based on 60% absorption and an average daily consumption of 20 g moist snuff by Swedish users (Andersson and Axell, 1989), the daily total absorbed dose will be about 10 μg NNK and 46 μg NNN. Based on the carcinogen potency factors derived above for NNK and NNN, the expected extra yearly cancer incidence in the 200,000 snuff dippers will be

$$(0.01/70) \times 0.086 \times 200{,}000 + (0.046/70) \times 0.029 \times 200{,}000 \approx 7 \text{ cases per year.}$$

This incidence is considerably higher than the background incidence of about 4 cases per 200,000 per year for cancer of the oral cavity, lips, and pharynx in the total Swedish nonsmoking population (Swedish Cancer Committee, 1984a), the majority of which do not use snuff. However, the finding in 1981 of one sample of snuff from Uppsala, Sweden, with a total content of 106 μg/g (dry weight) of TSNA out of which some 77 μg/g was identified as NNN and 4 μg/g as NNK (Hoffmann *et al.*, 1981) indicates higher nitrosamine levels in the past which are comparable with the sample of the "new brand" from 1991 analyzed by Hoffmann and co-workers (1991) in the United States containing 16 μg/g NNK and 37 μg/g NNN. The last-mentioned samples are probably more representative of historical exposures. Using the Uppsala snuff sample from 1981 just mentioned as the basis for these calculations we obtain

$$(0.024/70) \times 0.086 \times 200{,}000 + (0.46/70) \times 0.029 \times 200{,}000 \approx 44 \text{ cases per year.}$$

Thus, extrapolation of the rodent data appears to overestimate the cancer incidence in humans, and some possible reasons are presented below.

Pharmacokinetic Differences

Although the Hb adduct levels in rats and humans seem to be comparable at similar exposures to NNN and NNK, this need not be so with respect to the dose in DNA of the target organ. In addition to individual variations in metabolic activation, efficient repair processes that are saturable may complicate the picture. Thus, nonlinear relationships between degree of alkylation and exposure to NNK have been found for tissues like lung (Belinsky *et al.*, 1986, 1990; Murphy *et al.*, 1990). Especially for the methyl adducts, different cell populations will also exhibit divergent kinetics with respect to the elimination (repair) of adducts (Belinsky *et al.*, 1986, 1990). Thus, O^6-methylguanine is more slowly eliminated from Clara cells than from other cell types of the rodent lung. For this adduct there was, on the other hand, a good correlation between degree of alkylation in Clara cells determined 96 h after administration of NNK and the incidence of lung tumors in the mouse (Peterson *et al.*, 1991) as well as in the rat (Belinski *et al.*, 1990). In human cells, UV-induced cyclobutane adducts are repaired by the global as well as by the transcription-coupled excision repair pathways, but in a rodent cell line like Chinese hamster cells only transcription-coupled repair remove these adducts resulting in a lower overall repair efficiency associated with a higher genotoxic efficiency of UV light for these cells (Mullenders *et al.*, 1991). Such differences between man and rodent may also operate, e.g., with respect to repair of HPB adducts from NNN and NNK.

Modulation of the Carcinogenic Effects of TSNA

Apart from a higher sensitivity of the rodent to TSNA, the presence of antimutagenic and anticarcinogenic compounds in snuff depressing the yield of mutagenic (Jenssen and Curvall, 1996) as well as carcinogenic events (Wattenberg, 1977; Hecht *et al.*, 1986; Morse *et al.*, 1989) may provide another explanation for the observed discrepancy in carcinogenic potency.

In an experiment where NNN and NNK, with and without an extract derived from snuff, were swabbed in the oral cavity (Hecht *et al.*, 1986), the yield of oral tumors was reduced from 8/30 in the absence of extract to 3/30 in the presence of the extract. These observations are consistent with the previously demonstrated presence of potent anticarcinogens in several plants. For instance, in the mouse, phenetylisothiocyanate—a hydrolytic product from gluconasturtin present in a number of *Cruciferae* species—will markedly inhibit the induction of tumors in breast, forestomach, and lung by 7,12-dimethylbenzanthracene (Wattenberg, 1977). Pretreatment *in vivo* by isothiocyanates was found to inhibit NNN hydroxylation *in vitro* by liver microsomes and cultured by esophagus cell cultures isolated from the treated animals. Likewise, cytochrome P450 inhibitors as well as pretreatment with phenethylisothiocyanate *in vivo* caused inhibition of NNK-induced DNA methylation catalyzed by isolated microsomes *in vitro* (Chung *et al.*, 1984; Guo *et al.*, 1991). Moreover, oral administration to rats of phenethylisothiocyanate reduced the incidence of lung tumors from NNK, while the tumor incidences in liver and nasal cavity remained unaffected. In concordance with these results, the levels of methyl- as well as pyridyloxobutyl adducts in lung DNA were reduced by 50% in the presence of the thiocyanate, while the methyl adduct levels in liver and nasal tissue remained approximately the same (Morse *et al.*, 1989). Investigations of the genotoxicity of extracts from Swedish snuff have, likewise, provided support for the presence of antimutagenic compounds in Swedish snuff (Ahlbom *et al.*, 1996; Jensen and Curvall, 1996).

The concentrations of TSNA used in the above-mentioned study by Hecht and co-workers (1986)—and where approximately a 60% protection was observed in the presence of the snuff extract—were much higher than those to which snuff dippers are exposed. It is therefore possible that at more realistic exposure levels, and where the relation between the levels of protective agents and TSNA is more favorable, a still higher degree of protection will occur.

As mentioned above, available information seems to indicate that for U.S. snuff dippers the intake of TSNA is comparable or even higher than for cigarette smok-

ers (Kresty *et al.,* 1996). NNK and NNN are systemic carcinogens for which lung tumors can be induced in rodents upon various routes of intakes. Although no study seems to have been specifically designed to assess the relation between snuff dipping and lung cancer, this author is not aware of any adequate data suggesting such an association.. However, in contrast to active smokers, snuff dippers do not seem to exhibit any significant elevation of risk for cancer of the lung, which seems inconsistent with the claim (Hecht, 1996) that TSNA play a major role in the etiology of human lung cancer in active smokers. This brings into focus the role of promotive effects for induction of tobacco-related cancers in humans. The Swedish Cancer Committee (1984a,b) underlined that most of the carcinogenic action associated with tobacco smoke can be ascribed to unspecific promotive action that is reversible. Thus, 10 years after cessation of smoking the risk has been reduced by about 75%, and with increasing time the risk for exsmokers approaches a value that is approximately double that for a never-smoker reflecting initiator-induced, nonrepaired DNA damage remaining in lung tissue. This is in contrast, e.g., to radiation-induced cancer where the integrated total dose is the major determinant of risk. Nonsmoking snuff dippers are not exposed to a similar high degree of chronic pulmonary irritation, and in spite of aforementioned facts TSNA could still be important for cancer initiation in the lung caused by tobacco smoke. Although its implications for man are not known, an interesting observation made by Park *et al.* (1986) deserves special mentioning in this context: Snuff introduced in the buccal pouch of hamsters induced tumors of the oral cavity only when the animals were infected with herpes simplex virus type 1 (HSV-1). Similarly, upon exposure to high doses of the initiator 4-nitroquinoline-*N*-oxide, snuff introduced in a surgically crated canal of the lower lip of rats was found to act as a promoter (Johansson *et al.,* 1989).

Other Adverse Effects—Cardiovascular Disease

At lower doses, nicotine stimulates the heart rate, elevates the blood pressure, and constricts peripheral blood vessels, providing a theoretical basis for a possible relation between snuff dipping and cardiovascular disease. As to the chronic effects of low-dose nicotine administration from smoking, it has been difficult to distinguish the effects of nicotine from the complex actions of tobacco smoke. However, given the constrictive properties of nicotine on the peripheral blood vessels, it would seem logical to implicate nicotine—at least as a contributory factor—in the development of, e.g., Buerger's disease characterized by obstruction of medium and small arteries mainly of the extremities in heavy smokers. Nicotine also has some decidedly positive pharmacological effects. Although the mechanism is still unknown, it has now been demonstrated beyond doubt that this alkaloid (and smoking) reduces the risk of ulcerous colitis (Benoni and Nilsson, 1987; Lindberg *et al.,* 1988; Osborne and Stansby, 1992; Tysk and Järnerot, 1992).

Active smoking is associated with an increased risk of cardiovascular disease, and some studies have also implicated snuff dipping as a risk factor for such adverse effects (Bolinder *et al.,* 1992, 1994, 1997; Bolinder, 1997). In other large studies no such association has been found (Huhtasaari *et al.,* 1988, 1992). Further, whereas in the WHO Northern Sweden MONICA study the number of cigarettes smoked was found to be quantitatively related with higher plasma fibrinogen levels, no such relation was found for snuff dippers nor were several key fibrinolytic variables affected in the latter group (Eliasson *et al.,* 1995). Thromboxane A2 is a powerful platelet aggregatory and adhesive agent as well as a strong vasoconstrictor. In an investigation covering 577 randomly selected Swedish men aged 18–19, out of which 7.5% were cigarette smokers and 22% snuff dippers, cigarette smoking—but not the use of snuff—significantly promoted the production of thromboxane A2 (Wennmalm *et al.,* 1991). Further, the use of snuff does not decrease the plasma levels of antioxidants in a manner similar to active smoking (Stegmayr *et al.,* 1993).

Cardiovascular disease normally affects a large proportion of the non-tobacco-using population, and a number of risk factors other than tobacco smoke have been implicated as risk factors. In a representative study of relative risks with respect to coronary heart disease and cardiovascular death conducted by Pfaffenberger *et al.* (1986), hypertension (RR = 2.2; CI, 1.94–2.42) carried the highest risk followed by cigarette smoking (RR = 1.84; CI, 1.64–2.04), history of parental coronary heart disease (RR = 1.33; CI, 1.13–1.53), and sedentary life-style (RR = 1.31; CI, 1.09–1.53). Other important factors are abnormal serum lipid patterns, diabetes, overweight, history of cardiovascular disease, as well as a high-fat diet coupled with a low intake of fruits and vegetables. Considering the level of increase in odds ratios for ischemic heart disease reported by Bolinder and co-workers (1994), the impact on the reliability of derived risk estimates caused by a lack of control for the aforementioned confounding factors can readily be appreciated.

The cohort studied by Bolinder and co-workers (1992, 1994) consisted of workers registered in the Swedish Construction Industry's Organization for Working Environment Safety and Health which covers the entire country. Within this group consisting of almost 100,000 construction workers, blood pressure and incidence of disability pensions due to cardiovascular disease were assessed for active smokers, snuff dippers, and nonusers of tobacco. The two published reports were based on medical examinations carried out

from 1971 to 1974, covering some 75% of these workers who did report for health examination. In comparison with nonusers, disability pensions due to cardiovascular disease were nearly 50% more frequent in smokers as well as among snuff dippers. Both smokeless tobacco users and smokers showed higher prevalence of circulatory and respiratory disorders, and the incidence of hypertension was higher among snuff dippers than in nontobacco users (Bolinder *et al.*, 1992). In a follow-up study during 1974–1985, where the original cohort of 37,700 construction workers was diluted with some 47,000 white-collar workers, based on the occurrence of 44 cases among snuff dippers a higher death rate in cardiovascular disease was claimed to exist for this group. There were no differences with respect to gastrointestinal symptoms or cancer incidence between users of smokeless tobacco and never-users. Body weight and height were determined, but a number of other important confounders, like dietary habits, alcohol abuse, and blood lipid pattern, family history of cardiovascular disease, etc., were not recorded. In a summary it was stated (Bolinder, 1997) that the relative risks were adjusted for confounding with respect to "area of domicile, blood pressure, blood pressure medication, previous cardiac symptoms, and diabetes and body mass index," but except for body mass index no data were presented. Snuff dipping is much more common in the northern part of Sweden, where the general population is characterized by a 40% higher mortality than the southern parts with respect to cardiovascular disorders and that is unrelated to the use of tobacco (Huhtasaari *et al.*, 1988). Consequently, it is of utmost importance that cases and controls are matched with respect to area of residence. The fact that 25% of the registered workers did not show up for medical examination is a possible source of selection bias. Further, the snuff dippers tended to be more obese than controls, and although part of the elevated blood pressure could be ascribed to the temporary action of nicotine, the existence of a background of persistent elevated blood pressure among snuff dippers may as well be related to life-style-related confounding factors in this group instead of being a result of exposure to snuff. Subsequently, Bolinder and co-workers—in contrast to the findings in smokers—failed to detect any significant differences between snuff dippers and controls with respect to factors associated with atherosclerosis in a more homogenous group of 269 firemen, 35–60 years of age, that had been more carefully checked with respect to confounding. Daytime blood pressures at rest were higher in snuff dippers, but not at maximal exercise, and nighttime measurements showed no significant differences. Also, snuff dipping was found to have no negative effects on physical performance (Bolinder *et al.*, 1994; Bolinder, 1997).

In the WHO Northern Sweden MONICA study covering all 35- to 64-year-old men who had a first myocardial infarction and an age-matched population based control sample from the same geographical area (total population 510,000) who had not had an infarction, the results obtained by Bolinder and co-workers on an increased risk for cardiovascular disease in snuff dippers could not be confirmed. For 585 cases, the age-adjusted OR in comparison with nontobacco users for myocardial infarction was 0.89 (95% CI, 0.62–1.29) for exposure to snuff and 1.87 (95% CI, 1.40–2.48) for cigarette smokers (Huhtasaari *et al.*, 1992).

CONCLUSIONS AND DISCUSSION

The presence of highly carcinogenic TSNA in smokeless tobacco deserves serious consideration. However, the levels of TSNA in such products may differ by orders of magnitude depending on origin and manner of processing. Reviewing available epidemiological evidence, a wide discrepancy in estimated cancer risk associated with snuff dipping is found between on one hand previous studies on snuff dipping in Sudan as well as among women from North Carolina and on the other the results from three recent extensive epidemiological studies conducted in Sweden where it has not been possible to detect any increase in risk for cancers of the head and neck region. In Sweden snuff dipping has a long history, and in spite of the fact that about 20% of all grown-up Swedish males use moist snuff, the incidence of cancer of the oral cavity or pharynx remains low by international standards. There is also insufficient evidence for a causal link between the use of Swedish snuff and increased risk for cardiovascular disease. Dissimilarities in the content of TSNA in oral snuff products probably may represent the most important reason for the different outcomes of the epidemiological surveys conducted in the United States and Sweden.

Bioassays in rodents appear to give exaggerated risk estimates for humans, a discrepancy that could be ascribed to species-related differences in the relation between exposure and DNA target dose and/or adduct repair rates, as well as due to the presence of anticarcinogens in moist snuff. The lower limit of detection in the Lewin *et al.* (1998) study on snuff dipping in Sweden and risk for cancer of the oral cavity and pharynx—where no increase in risk was observed—was estimated at double the background incidence of about 2 cases per 100,000. The risk from consuming Swedish snuff should, therefore, not be larger than this risk level. Frankfurter sausages grilled over a camp fire may contain up to 1600 μg/kg of polycyclic aromatic hydrocarbons out of which some 212 μg/kg may consist of the potent carcinogen benzo[*a*]pyrene (BaP; Larsson *et al.*, 1983). The carcinogenic potency factor on an annual basis for this polycyclic compound was estimated by U.S. EPA to be 0.1 $(\text{mg/kg and day})^{-1}$ (IRIS, 1995), i.e., slightly higher than for NNK. Based on 90%

absorption in the gut, we obtain an uptake for BaP of 15 μg from a consumption of 0.3 kg grilled sausage containing 54 μg/kg of BaP, the mean value for grilled sausages found by Larsson *et al.* (1983). Since BaP is not the only carcinogenic PAH present in grilled meats, contributions from compounds like dibenz[*a,h*]anthracene, benzo[*a*]anthracene, benzofluoranthenes [*b,j,k*], and indeno[1,2,3-*c,d*]pyrene (Larsson *et al.*, 1983) would be expected to contribute some two additional BaP equivalents, giving an effective BaP equivalent dose of 45 μg or 0.28 μg/kg/day for a person weighing 70 kg and who consumes 0.3 kg grilled sausage three times per week. For a population of 100,000 consumers of grilled sausages we obtain

$$0.00028 \text{ mg/kg} \times 0.1 \text{ (mg/kg)}^{-1} \times 100{,}000 \approx 3 \text{ cases per year.}$$

Allowing for an overestimation of cancer potency based on rodent data for carcinogens giving rise to bulky DNA adducts repaired by excision repair, this risk could be said to be comparable to the maximum risk associated with the consumption of Swedish snuff.

The UN expert group on tobacco (UN, 1998) that was mentioned initially concluded "However, it is now evident that the risk of death and disease is related to not only the amount but also the nature of tobacco exposure; for example, daily cigarette smoking is far more dangerous than occasional use of Swedish snuff." Since many individuals resort to the use of smokeless tobacco in order to quit the habit of smoking, it is of interest to calculate the gain in terms of the number of cancer cases that can be avoided in, e.g., a population of 100,000 active smokers who turn snuff dippers. In Sweden the background lung cancer incidence in nonsmokers is about 6 per 100,000. On the average, active smoking increases this risk by a factor of 10, a risk that upon quitting smoking rapidly decreases and eventually reaches a level that is about twice that for nonsmokers (Swedish Cancer Committee, 1984a). To this comes smoking-induced cancers of the oral cavity, pharynx, larynx, and esophagus that can be estimated at about 5 additional avoidable cases. The number of cases avoided in 100,000 smokers turned snuff dippers (in time) would then be approximately 65 − 6 (remaining risk) − 2 (maximum risk caused by snuff) = 57 cases per year. Translated into a smoking U.S. population of 46 million, some 26,000 cases of cancer per year could be prevented. In addition, more than 50% of all deaths from chronic obstructive disease as well as some 20% of the deaths from cardiovascular disease could be avoided, which would add another 80,000 lives saved.

Against a background of very low levels of consumption of oral snuff on the European continent, IARC's conclusion to classify snuff as a human carcinogen no doubt nurtured the decision of the Commission of the European Communities (CEC) to introduce a general ban on sales of oral snuff products within the European Union (EU), and that for some inexplicable reason was not to apply to nasal snuff products or to any other tobacco product (EU Council Directive 92/41/EEC). When joining EU in 1995, a general exemption from these restrictions was granted for use of snus in Sweden, provided that the packages carried a cancer warning. Upon reviewing the two recent Swedish epidemiological studies mentioned above, the Swedish Government Agency for Health and Welfare noted that "although these studies cannot exclude such a relationship, no significant association between snuff dipping and cancer in humans can be established" and further that these findings "have raised doubt as to the scientific justification for the currently used hazard labeling, '*Causes cancer.*" ' In light of new evidence, the agency also suggested that the Swedish government renegotiate the issue with the commission (Örtendahl, 1997).

The total ban on oral snuff imposed by the Commission of the European Communities—but not on nasal snuff products or on any other tobacco product—does not seem to be based on rational scientific considerations. Nevertheless, due to the decisive role of potent TSNAs in determining potential cancer risks in users of smokeless tobacco, and due to the fact that large variations in the concentrations of marketed products may occur, adequate measures should be taken to keep the levels of these nitrosamines in such products as low as is technically feasible. This author agrees fully with the opinion of Hoffmann and co-workers (1981) at the American Health Foundation: "Although we concur with other scientists" . . . "that snuff use may be a feasible alternative to cigarette smoking, we feel that no efforts should be spared to reduce the concentration of alkaloid derived N-nitrosamines in snuff, since the use of this type of tobacco product has been associated with an increased risk for cancer of the oral cavity."

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