SMNSKEM

J Smoking-Related Dis 1991 2(2), 165-172

ON THE CONTROL OF TOXIC SUBSTANCES IN SMOKELESS TOBACCO*

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Abstract

Chewing of tobacco, and especially the habit of oral snuff dipping, is associated with a significantly increased risk for cancer of the oral cavity. Snuff is carcinogenic in the oral cavity of laboratory animals and, like other types of smokeless tobacco products, it contains carcinogens. Volatile nitrosamines, nitrosamino acids, and tobacco-specific N-nitrosamines (TSNA) are the most abundant carcinogens identified to date. Because of the high levels of TSNA in chewing tobacco and snuff, their proven carcinogenicity, and their ability to form DNA adducts and to activate oncogenes, the nitrosamines are considered important contributors to oral cancer in tobacco chewers and snuff dippers. Monitoring of nitrosamines in US and Swedish smokeless tobacco brands since 1980 has shown a gradual decrease of the concentrations of these carcinogens. This finding supports the concept that the formation of nitrosamines can be controlled during tobacco processing. In the US the consumption of snuff has significantly increased, especially among adolescents; therefore, upper limits should be set for permissible levels of nitrosamines in smokeless tobacco products. In addition, the use of flavour additives should be regulated.

Key words: Smokeless tobacco, snuff, oral cancer, carcinogens, regulations for nitrosamines, flavour additives

oncogenes

Introduction

In 1984 a working group of the International Agency for Research on Cancer (IARC) evaluated the carcinogenic risk of smokeless tobacco. The IARC concluded: "The oral use of snuffs of the types commonly used in North America and Western Europe is carcinogenic to humans. There is limited evidence that chewing tobacco of the types commonly used in these areas is carcinogenic" [1]. Two years later, the Surgeon General of the US Public Health Service also reviewed the toxicity of smokeless tobacco and fully supported the IARC conclusion. In addition, tobacco chewing and snuff dipping were found to be associated with an increased risk for non-cancerous and pre-cancerous lesions in the oral cavity [2]. Since then, various types of studies have substantiated the earlier findings [3–5]. Bioassays in rats and hamsters have shown that snuff is carcinogenic in the oral cavity of laboratory animals [6–8].

Carcinogens in smokeless tobacco

Natural tobacco contains at least 3,050 individual compounds [9]. Smokeless tobacco is usually modified by flavouring agents, added in the form of plant extracts, and/or as chemicals [9–12]. Among 24 tumourigenic agents that were isolated and identified in

*Presented at a "Smokeless Tobacco Workshop", National Cancer Institute and National Institute of Dental Research, Bethesda, Maryland, January 24, 1991

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Table 1. Carcinogenic agents in smokeless tobacco.

Carcinogen	Smokeless tobacco	Concentration ng/g	Reference
Volatile aldehydes			
Formaldehyde	NT, S	2,200–7,400	15
Acetaldehyde	NT, S	1,400–27,400	15
Crotonaldehyde	S	200–2,400	15
Volatile N-nitrosamines			
Nitrosodimethylamine	CT, S	ND–220	2,15
Nitrosopyrrolidine	CT, S	ND–337	2,15
Nitrosopiperidine	CT, S	ND–107	2
Nitrosomorpholine	CT, S	ND–690	2,15
Nitrosodiethanolamine	CT, S	40–6,800	15
N-Nitrosamino acids			
N-Nitrososarcosine	S	present	2
3-(Methylnitrosamino)propionic acid	CT, S	200–7,400	14,17
4-(Methylnitrosamino)butyric acid	CT, S	ND–1,770	14,17
N-Nitrosoazetidine-2-carboxylic acid	CT	4–140	16
Tobacco-specific N-nitrosamines			
N'Nitrosonornicotine	CT, S	400–154,000	14–17
4-(Methylnitrosamino)-1-(3-pyridyl) -1-butanone	CT, S	ND–13,600	14–17
4-(Methylnitrosamino)-1-(3-pyridyl) -1-butanol	S	present	2
N'-Nitrosoanabasine	CT, S	present–560	2,15
Lactones			
α-Angelica lactone	NT	present	18
β-Angelica lactone	NT	present	18
Coumarine	NT	600	19
Polynuclear aromatic hydrocarbons			
Benzo(a)pyrene	NT, S	>0.1–90	15
Metals			
Nickel	NT, CT, S	180–2,700	20,22
Cadmium	ST	700–790	20
Polonium-210	NT, S	0.3–0.64 pCi/g	15
Uranium-235 and -238	S	2.4–19.1 pCi/g	23

Abbreviations: CT, chewing tobacco; NT, natural tobacco; S, snuff; ND, not detected

smokeless tobacco, are volatile aldehydes and N-Nitrosamines, N-nitrosamino acids, lactones, polynuclear aromatic hydrocarbons, certain metals, as well as the α-emitters, polonium-210, uranium-235 and -238. The most abundant, potent carcinogens are the tobacco-specific N-Nitrosamines (TSNA; Table 1 [2,13–23]. The TSNA are formed by N-nitrosation of the major habituating tobacco alkaloid, nicotine, and of minor *Nicotiana* alkaloids, primarily during tobacco curing, fermentation and aging. Seven TSNA have been identified in smokeless tobacco (Figure 1 [17,24]). Among these, N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are the most prevalent, potent carcinogens in smokeless tobaccos.

In 1981, a risk assessment by the US National Research Council estimated exposures of US residents to carcinogenic nitrosamines [25]. Accordingly, a non-smoker is exposed to about 1.0μg of nitrosamines per day; a smoker of 20 cigarettes is exposed to about 11.4μg/day and, on the basis of average values for the two leading US snuff brands in 1986, a person who consumes 10g of snuff daily, ingests about 270μg of carcinogenic nitrosamines (Table 2 [15,26]).

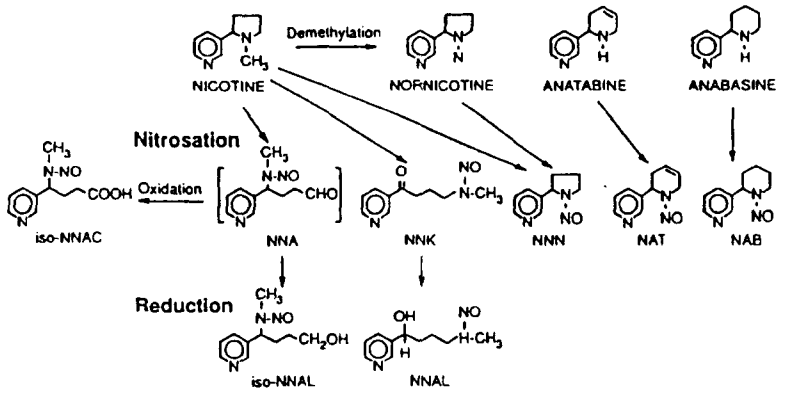

Figure 1. Formation of Tobacco-Specific N-Nitrosamines. Abbreviations:iso-NNAC, 4-(methylnitrosamino)-4-(3-pyridyl)butyricacid; NNA, 4-(methylnitrosamino)-4-(3-pyridyl)-butanal; NNK, 4-(methylnitrosamino)-1-(3-pyridyl-1-butanone; NNN, N'-nitrosonornicotine; NAT, N'-nitrosoanatabine; NAB, N'-nitrosoanabasine; iso-NNAL, 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.

Table 2. Estimated exposure of U.S. residents to carcinogenic nitrosamines*.

Mode of exposure	Nitrosamines*	Primary exposure route	Daily intake µg/person
Beer	NDMA	Ingestion	0.34
Cosmetics	NDELA	Dermal Absorption	0.41
Cured meat:			
Cooked bacon	NPYR	Ingestion	0.17
Scotch Whisky	NEMA	Ingestion	0.03
Cigarette smoking	VNA**	Inhalation	0.3
	NDELA	Inhalation	0.5
	NNN	Inhalation	6.1
	NNK	Inhalation	2.9
	NAB	Inhalation	0.7
Snuff dipping***	VNA**	Ingestion	0.45
	NDELA	Ingestion	3.2
	NNN	Ingestion	242.0
	NNK	Ingestion	12.3
	NAB	Ingestion	19.0

*From the U.S. National Research Council (25) amended by data for snuff dipping (15.)

**Volatile N-Nitrosamines (VNA); N-nitrosodimethylamine (NDMA); N-nitrosodiethanolamine (NDELA); N-nitrosoethylmethylamine (NEMA); N-nitrosodiethylamine (NDEA); N-nitrosopyrrolidine (NPYR); N'nitrosonornicotine (NNN); 4-(methylnitrosamino)-1-(3-pyridyl)-1-butamone(NNK); N'-nitrosoanabasine (NAB).

***Hoffmann *et al.* [15], average values from the two leading snuff brands which accounted in 1986 for about 85% of all moist snuff consumed in the USA (10g/snuff/day).

Carcinogenicity of tobacco-specific N-nitrosamines

There are several reasons for our concern about the high concentrations of nitrosamines in smokeless tobaccos. First, during chewing, the carcinogenic N-Nitrosamines are extracted from tobacco, as has been demonstrated by saliva analysis [27–30]. Secondly, nitrosamines are strong carcinogens in mice, rats and hamsters. NNN and NNK have induced tumours of the lung, upper aerodigestive tract, and pancreas, independent of the route of application [24]. Furthermore, treatment of the oral cavity of rats with a mixture of NNN and NNK in solution elicited tumours at the site of application in addition to the tumours at distant sites [6]. Third, like N-Nitrosamines in general, the TSNA are metabolised in animals and in cultured human oral mucosa to reactive species which can bind to DNA; some of the resulting DNA adducts are known to activate oncogenes [24,31]. TSNA metabolites also bind to the protein moiety of haemoglobin. This type of adduct has been used as a biomarker for the exposure of snuff dippers to the carcinogenic NNN and NNK (Figure 2 [32]). Thus, the high degree of exposure to nitrosamines and especially that to TSNA, their carcinogenicity, and their binding to DNA, are the basis for the concept that nitrosamines are the most important contributors to the increased risk for oral cancer of tobacco chewers and snuff dippers [33,34].

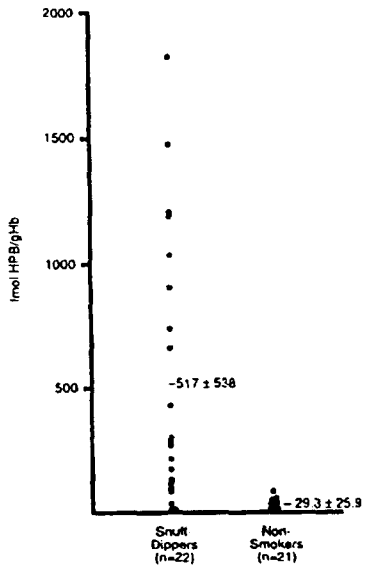

Figure 2. Levels of HPB [4-hydroxy-1-(3-pyridyl)-1-butanone] released upon Hydrolysis of Hemoglobin of Snuff-Dippers and Non-Tobacco Users.

Reduction of the carcinogenic potential of smokeless tobacco

N-Nitrosamines are formed during tobacco processing from protein and alkaloids and also from additives or contaminants. The latter group includes N-nitroso-diethanolamine (NDELA) and N-nitroso-morpholine (NMOR), two strong animal carcinogens [35]. NDELA is primarily formed from the diethanolamine salt of maleic hydrazide (MH-30), a sucker growth inhibitor, NMOR is derived from morpholine contaminations of the wax layer of the containers [36,37]. In September 1981, the US Environmental Protection Agency mandated a ban of MH-30 for use on tobacco [38]. Since then, our analyses of US smokeless tobacco products have revealed a drastic reduction of NDELA (Figure 3 [39]). The remaining NDELA residue most likely originates from diethanolamine contaminations other than MH-30 and/or from tobacco imported from countries without regulation of the use of MH-30. NMOR in snuff has practically disappeared since plastic materials have replaced wax-coated paper products as packing materials for snuff tobaccos. In 1981, NMOR amounted to 690ng/g; in 1990, we could not detect NMOR in snuff brands (≤2ng/g) [39].

Thus, NDELA and NMOR have at least partially disappeared from US tobacco products due to elimination of their precursors. However, this approach is not feasible for nitrosamino acids and tobacco-specific N-nitrosamines, because

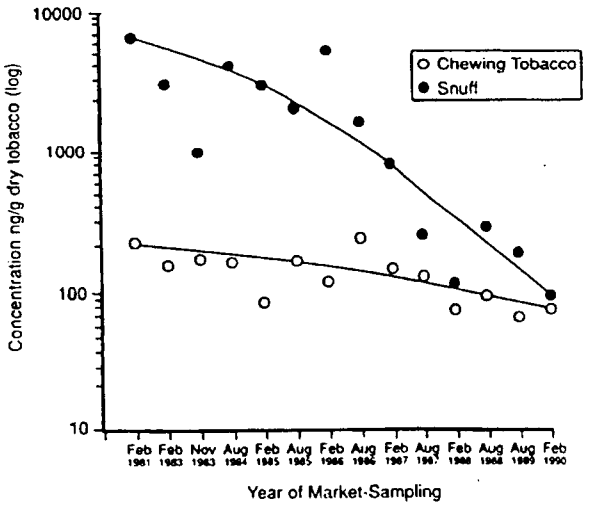

Figure 3. Decrease in the concentration of NDELA [N-Nitrosodiethanolamine] in a leading US snuff brand and a leading brand of chewing tobacco, 1981–1990.

proteins and alkaloids, the precursors for these carcinogens, are integral parts of the tobacco. Nevertheless, changes in the processing of chewing tobacco, and especially of snuff, can lead to a major reduction of these carcinogens. Between 1980 and 1990, N'-nitrosonornicotine (NNN), an indicator of the overall levels of TSNA, gradually decreased in the two leading US moist snuff brands which account for more than 85% of the US market. NNN declined from about 26.5 to 10.4µg/g in brand A, and from 39 to 9.6µg/g in brand B [40]. In Sweden, the leading five snuff brands in 1980 showed total TSNA levels between 7.0 and 17.9µg/g (average 12.6); in 1990, three leading snuff brands had total TSNA concentrations between 9.3 and 11.2µg/g (average 10.3) [27,40].

On the basis of the trends observed in US smokeless tobacco products, one may be inclined to conclude that the manufacturers have modified tobacco processing to achieve a reduction of the concentrations of carcinogenic nitrosamines. In fact, the analysis of a snuff brand which was recently imported from Sweden and test marketed in certain locations in the USA, demonstrates that levels of carcinogenic nitrosamines in smokeless tobacco products can be effectively and significantly reduced (Table 3, US. E). Therefore, it was rather surprising that another snuff brand appearing on the US market in 1989/90, contained the highest concentrations of carcinogenic nitrosamines ever reported in the literature (Table 3 [40]). Originally, this new snuff brand could only be purchased as a test market product in Texas; however, towards the end of 1990, it was also available in New York State. The increased pH of this snuff (7.7–8.2), compared to other US brands (5.6–7.3), suggests that changes in manufacturing were possibly intended to facilitate the absorption of nicotine through the oral mucosa, since a higher proportion of nicotine is present in unprotonated form at the higher pH [41].

Although snuff has elicited tumours in the oral cavity of laboratory animals [6–8], these bioassays are rather cumbersome and do not readily permit comparisons of the

Table 3. Alkaloids and N-nitroso compounds in snuff brands 1990-1991.

	US A	US B	US C	US D[a]	US E[b]	Sweden 3 brands
Moisture (%)	56.0	57.8	51.8	50.0–57.8	51.9	46.6–54.2
pH	7.11	7.30	5.61	7.72–8.17	7.36	7.67–7.9
Nicotine (%)	2.04	2.17	2.15	1.22–2.21	1.47	1.13–1.25
Total Alkaloids (%)	2.18	2.32	2.32	1.32–2.38	1.59	1.24–1.41
Volatile Nitrosamines (ng/g)						
NDMA	n.d.[c]	n.d.[c]	n.d.[c]	147–265	n.d.[c]	51–63
NPYR	44	59	120	245–757	n.d.[c]	n.d.[c]–155
Nitrosamino acids (µg/g)						
NSAR	0.06	0.06	n.d.[d]	0.4–2.5	0.1	0.03–0.68
MNPA	5.13	3.62	2.72	8.9–65.7	2.2	3.10–3.28
MNBA	0.47	0.26	0.09	1.9–9.1	0.2	0.19–0.23
Total	5.7	3.9	2.8	11.2–77.3	2.5	3.3–4.2
TSNA (µg/g)						
NNN	10.4	9.57	4.14	21–147	3.20	5.24–5.67
NNK	2.19	3.14	1.24	6–18	0.70	1.37–2.08
NAT + NAB	9.76	7.90	2.97	22–115	2.00	2.58–3.47
Total	22.3	20.6	8.3	48–280	5.9	9.2–11.2

All values are based on dry weight; [a]range of 5 samples bought in different stores in Texas; [b]snuff in sachets imported from Sweden.
Abbreviations: NDMA, N-nitrosodimethylamine; NPYR, N-nitrosopyrrolidine; NSAR, N-nitrososarcosine; MNPA, 3-(methylnitrosoamino)propionic acid; MNBA, 4-(methylnitrosoamino)butyric acid; NNN, N'-nitrosonornicotine; NNK, 4-(methylnitosamino)-1-(3-pyridyl)-l-butanone; NAT, N'-nitrosoanatabine; NAB, N'-nitrosoanabasine; TSNA, tobacco-specific N-nitrosamines; [c]n.d. not detected <0.005µg/g; [d]n.d. not detected <0.01µg/g. Total alkaloids includes nicotine, nornicotine, mysomine, anatabine, anabasine, 2.3'-dipyridyl and cotinine.

tumourigenic potential of different snuff brands. It appears to be more realistic to evaluate differences in genotoxicity by means of *in vitro* assays with tester strains [42], or with SV-79 cells [43]. However, thus far such assays have not been applied to comparative studies of smokeless tobacco brands. Independent of these bioassay data, the chemical-analytical data in Table 3 clearly indicate different carcinogenic potentials for the snuff brands.

Control of harmful substances

In 1987, a study group of the World Health Organisation (WHO) made a number of recommendations with regard to the control of smokeless tobacco [44]. One of these recommendations reads: "The analysis of smokeless tobacco products and the regulation of harmful substances should be subject to government control." On the basis of our present knowledge we offer the following recommendations for the US market.

1. The total concentration of carcinogenic tobacco-specific N-nitrosamines (TSNA) should not exceed 10µg/g smokeless tobacco.

2. A study group should determine which of the tobacco additives are tumourigenic. Use of such agents should then be suspended.

In respect to the first recommendation, it has been affirmed that it is feasible to produce chewing tobacco and snuff with TSNA levels no higher than 10µg/g (concentration should be based on dry weight of the tobacco product). Setting an upper limit for permissible TSNA levels will also reduce exposure to carcinogenic volatile nitrosamines and nitrosamino acids since these agents are formed during the tobacco processing by reactions similar to those that yield TSNA.

With regard to aspect 2, a number of publications and government lists have revealed the major flavour components and other chemical additives which are used in

the manufacture of smokeless tobacco products [10,12,45]. These agents should be evaluated for their tumourigenic potential, and if considered active, they should be banned.

In agreement with the WHO, we consider the regulation of harmful substances as urgent measures. This applies especially to the situation in the United States where the manufacture of moist snuff has steadily risen by more than 13% over the past three years [46,47], and where this smokeless tobacco product is increasingly being used by adolescents [48]. Furthermore, the nicotine habituation induced by the onset of smokeless tobacco use, is strongly related to increases in the onset of cigarette smoking [49].

Acknowledgement

Our studies in tobacco carcinogenesis are supported by Grant No. CA-29580 from the US National Cancer Institute.

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