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THE NEED FOR REGULATION OF CARCINOGENIC *N*-NITROSAMINES IN ORAL SNUFF

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Abstract—Oral snuff is carcinogenic to humans and laboratory animals. The major carcinogenic agents in snuff are the *N*-nitrosamines, especially the tobacco-specific *N*-nitrosamines. During the past decade, a gradual reduction of the levels of carcinogenic *N*-nitrosamines was observed in the leading snuff brands in the USA and in Sweden. However, in 1990 a newly introduced snuff brand in the USA contained the highest concentration of carcinogenic *N*-nitrosamines ever to be determined in a commercial tobacco product. The elevated pH and relatively high levels of nitrite in this snuff favoured the formation of *N*-nitrosamines. 2 yr after the product first appeared, it was replaced by a new preparation of snuff under the same brand name, and, according to chemical analyses, this material would be expected to have about the same carcinogenic potential as the leading snuff products. The interdependence of the formulation and manner of preparation of snuff products with their carcinogenic potential emphasizes the need for regulation and control of the harmful substances in smokeless tobacco, especially in view of the trend of increasing consumption of snuff.

INTRODUCTION

Oral snuff of the types commonly used in North America and Western Europe is carcinogenic to humans (IARC, 1985; US Department of Health and Human Services, 1986). Despite this fact, the consumption of oral snuff in the USA has steadily risen from 43.8 million lb in 1982 to 56.2 million lb in 1991 (US Department of Agriculture, 1992). One major reason for this trend is the growing prevalence of snuff dipping among male adolescents (Orlandi and Boyd, 1989); the prohibition of smoking in many public places may be another reason.

On repeated instillation of snuff into a surgically created lip canal of rats, tumours were induced in the mouths of the animals (Hecht *et al.*, 1986; Johansson *et al.*, 1989). The major known carcinogens in snuff are the *N*-nitrosamines, especially the tobacco-specific *N*-nitrosamines (TSNA, Hoffmann *et al.*, 1991c). On swabbing of the oral cavity of rats with a solution of a mixture of two major TSNA, *N'*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK; Fig. 1), oral tumours including neoplasms of the tongue were elicited (Hecht *et al.*, 1986). The reported concentrations of NNN and NNK in commercial snuff brands in North America and Western Europe ranged up to 150 and 18 ppm, respectively (Brunnemann and Hoffmann, 1991a). On the basis of epidemiological and laboratory data, it was concluded that "there is strong evidence suggesting that NNN and NNK in snuff are at least partially responsible for the excess of oral cancer among snuff dippers" (Preston-Martin, 1991). In addition to TSNA, snuff also contains the carcinogenic volatile *N*-nitrosamines, *N*-nitrosodiethanolamine (NDELA) and *N*-nitrosamino acids (NAA; Fig. 2; Djordjevic *et al.*, 1989; Brunnemann and Hoffmann, 1991a).

During the last decade, the significant reduction of the use of maleic hydrazide in diethanolamine as a sucker growth inhibitor for tobacco and eliminating morpholine from the packaging materials for snuff has led to major decreases in the contents of the carcinogens NDELA and *N*-nitrosomorpholine in smokeless tobacco products (Brunnemann and Hoffmann, 1991b). Reducing the levels of the highly carcinogenic *N*-nitrosamines NNN and NNK, for which nicotine and nornicotine are precursors (Fig. 1), appears to be feasible through changes in the formulation and manufacturing technology of snuff, especially during fermentation. In this paper, we reported the results of analyses of commercial snuff brands in the USA and in Sweden with regard to the monitoring of concentrations of *N*-nitrosamines in these products.

MATERIALS AND METHODS

Commercial snuff brands were purchased from retailers in various cities in the USA and in Sweden. Before analysis the closed snuff containers were stored at 4°C. The analytical methods for the determination of water, alkaloids, nitrite, nitrate, pH, TSNA and *N*-nitrosamino acids in snuff have been

Abbreviations: MNBA = 4-(methylnitrosamino)butyric acid; MNPA = 3-(methylnitrosamino)propionic acid; NAA = *N*-nitrosamino acids; NAB = *N'*-nitrosoanabasine; NAT = *N'*-nitrosoanatabine; NDELA = *N*-nitrosodiethanolamine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN = *N'*-nitrosonornicotine; NSAR = *N*-nitrososar-cosine; TSNA = tobacco-specific *N*-nitrosamines.

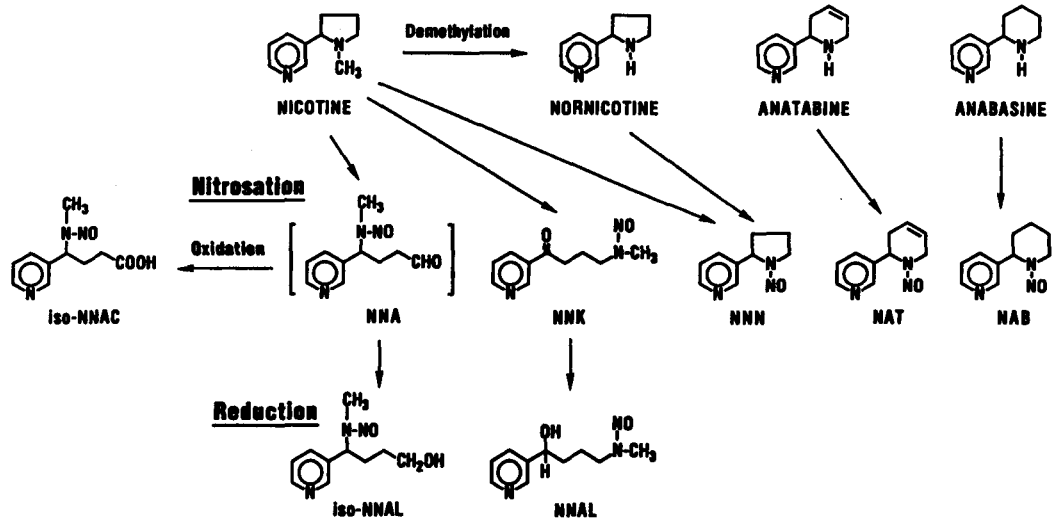

Fig. 1. Formation of tobacco-specific *N*-nitrosamines.

previously reported (Armstrong *et al.*, 1967; Crutchfield and Burton, 1989; Djordjevic *et al.*, 1989 and 1991a; von Bethmann *et al.*, 1961). The standard deviations for determinations of individual TSNA and NAA were within ±5% (Djordjevic *et al.*, 1989).

RESULTS

Table 1 lists analytical data for TSNA in two leading US snuff brands between 1980 and 1992. In 1992, these brands accounted for 84% of the snuff sales on the US market (Maxwell, 1992). The analyses demonstrated a trend towards decreasing concentrations of individual carcinogenic TSNA in these leading brands since 1980 (NNN was reduced up to 85%, and NNK up to 89%). Although a minor reduction in the nicotine content of these brands may have contributed to this trend, it appears that the significant reductions of the TSNA during the last 12 yr are due to changes in the manufacture of these smokeless tobacco products.

Table 1 also presents data on TSNA and nicotine in three popular Swedish snuff brands determined between 1980 and 1990. Snuff brands in Sweden had already in 1980 significantly lower TSNA content than that found in the two leading US snuff brands. Nevertheless, in the ensuing years further reductions of the TSNA levels in the three popular Swedish brands occurred; NNN content was decreased by 28–41%.

In 1990, two new snuff brands appeared on the US market. The analysis of brand C revealed low levels

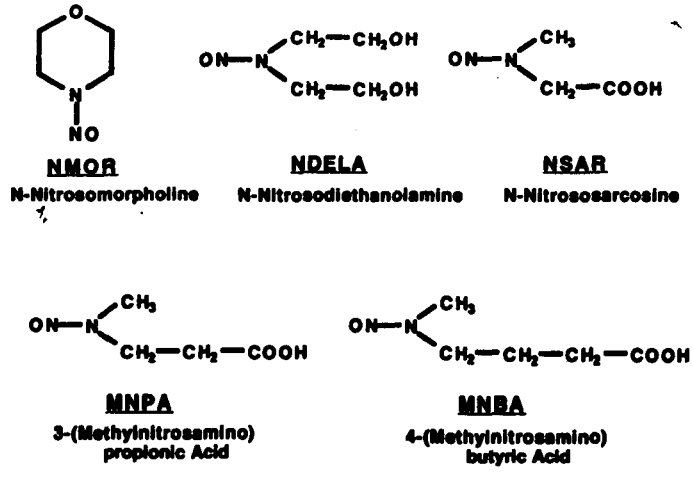

Fig. 2. Chemical structures of *N*-nitrosomorpholine and non-volatile *N*-nitrosamines in smokeless tobacco.

Table 1. Changes over the years in the contents of tobacco-specific *N*-nitrosamines in oral snuff from two US and three Swedish brands

Brand	Year	Tobacco-specific *N*-nitrosamines (μg/g) NNN	NNK	NAT*	Nicotine (%)
USA (A)	1980	26.5	4.65	22.7	2.34
	1981	19.0	2.4	19.8	2.20
	1986	33.0	1.8	44.0	2.07
	1988	13.8	0.93	10.2	1.99
	1990	10.4	2.20	9.8	2.04
	1992	6.4	0.50	3.6	1.71
Reduction 1980–92 (%)		75.8	89.0	84.1	
USA (B)	1980	39.0	2.4	44.0	2.4
	1981	33.0	4.6	41.9	2.7
	1986	64.0	3.1	215	3.07
	1988	8.5	0.76	7.8	2.61
	1990	9.6	3.1	7.9	2.17
	1991	8.0	0.8	6.0	2.1
	1992	5.7	0.7	3.9	2.2
Reduction 1980–92 (%)		85.4	70.8	91.1	
Sweden (A)	1980†	7.83	1.96	5.13	1.51
	1982	6.10	1.00	2.20	nd
	1990	5.67	2.08	3.47	1.24
Sweden (B)	1980†	7.95	1.51	4.43	1.81
	1982	4.00	0.61	1.40	nd
	1990	5.25	1.37	2.89	1.25
Sweden (C)	1980†	8.94	1.85	5.50	1.13
	1982	5.30	1.40	2.40	nd
	1990	5.24	1.44	2.58	1.13

nd = not determined

All values are based on dry weight.
*NAT contains 5–10% NAB.
†Average values for the same snuff brand bought in three different cities.

of TSNA (4.14 ppm NNN, 1.24 ppm NNK and 2.97 ppm NAT) despite an average nicotine content of 2.2% in tobacco. However, brand D contained disturbingly high amounts of TSNA. On the basis of this finding, we monitored brand D by purchasing the product during 1990 and 1991 in several US cities and by analysing the material for alkaloids, pH, nitrite, nitrate, TSNA and *N*-nitrosamino acids (Table 2). The levels of carcinogenic TSNA and *N*-nitrosamino acids in this product were exceptionally high and exceeded the TSNA levels measured in the US snuff brands A and B five- to 10-fold. The high nitrite content (2.0 and 1.7 mg/g for 1990 and 1991, respectively) and the alkaline pH (8.01 and 7.6 for 1990 and 1991, respectively) strongly suggest that a different formulation and/or technology had been used in the manufacture of this new snuff brand. Because of the high pH, snuff D contains about 50% of the nicotine in unprotonated form, while conventional snuff contains about 10% unprotonated nicotine (Brunnemann and Hoffmann, 1974). Nicotine is more rapidly absorbed through the mucous membranes of the oral cavity in the unprotonated state than in the protonated state; thus, the acute effect of nicotine on the central nervous system is increased in the case of snuff D (Armitage and Turner, 1970; US Department of Health and Human Services, 1988). By 1992, the product marketed as snuff D no longer showed such high levels of carcinogenic nitrosamines and nitrite. The pH of snuff D as well as all other analytically determined parameters have, since then, been within the range of values found in the popular US snuff brands A and B.

DISCUSSION

The risk for diseases that are related to tobacco use can only be eliminated by abstaining from smoking cigarettes, cigars, pipe tobaccos, and by avoiding oral and nasal use of tobaccos (US Department of Health and Human Services, 1989). However, as long as millions of people continue to smoke, chew or snuff-dip tobacco, great care must be taken to ensure the least possible concentration of toxic and carcinogenic agents in these consumer products. In the case of the US cigarette, significant changes have occurred since 1960 towards smoke with lower carcinogenic potential than that of smoke of the earlier decades (Hoffmann *et al.*, 1991b). In oral snuff we have also observed a gradual reduction of the carcinogenic potential of leading US brands since 1980 (Table 1). Therefore, it was most surprising that a new snuff brand (D), first appearing on the US market in 1990, contained the highest concentrations of carcinogenic nitrosamines ever to be reported (Brunnemann and Hoffmann, 1991a; Table 2). Since this snuff product was a new threat to oral health, we felt obliged to report our findings at scientific meetings (Djordjevic *et al.*, 1991b; Hoffmann *et al.*, 1991a). Interestingly, the 1992 samples of snuff brand D contained only about one-fifth to one-tenth of the levels of the carcinogenic TSNA and *N*-nitrosamino acids that were measured in samples of the same product when it was sold in 1990 and in 1991 (Table 2).

The fact that a potentially highly carcinogenic product can at any time appear on the market and pose a considerable cancer risk for the consumer

Table 2. Changes from 1990 to 1992 in the chemical composition of the US snuff brand D

Year	No. of samples	pH	NO_2-N (mg/g)	NO_3-N (mg/g)	Nicotine (mg/g)	TSNA			NAA		
						NNN	NNK (μg/g)	NAT*	NSAR	MNPA (μg/g)	MNBA
1990	9	8.01 ± 0.15	2.0 ± 0.5	0.92 ± 0.6	18.7 ± 3.1	92 ± 61	14.9 ± 9.2	69.8 ± 32.7	2.5 ± 2.1	36.9 ± 25.3	6.9 ± 6.1
1991	4	7.60 ± 0.39	1.7 ± 1.1	1.41 ± 0.83	18.9 ± 0.5	51 ± 9	8.9 ± 2.9	63.8 ± 1.7	3.2 ± 1.7	24.0 ± 10.8	5.0 ± 3.0
1992	7	7.18 ± 0.22	0.09 ± 0.12	2.39 ± 0.83	20.6 ± 4.7	5.9 ± 1.8	2.2 ± 1.5	8.3 ± 2.3	0.2 ± 0.5	6.4 ± 2.3	0.7 ± 0.4
Brand A											
1992		7.06	0.075	5.46	17.1	6.39	0.51	3.55	0.08	2.63	0.18
Brand B											
1992		6.96	0.058	5.68	17.4	5.70	0.73	3.93	0.16	2.84	0.29
Brand C											
1990		5.61	nd	nd	21.5	4.14	1.24	2.97	0.00	2.72	0.09

nd = not determined

Values are based on dry weight.
*NAT contains 5–10% NAB.

underscores the need for implementation of a proposal issued by the World Health Organization in 1988, stating that "the analysis of smokeless tobacco products and the regulation of harmful substances should be subject to government control".

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