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Carcinogenic Agents in Snuff [1,2,3]

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ABSTRACT—The oral use of snuff has been associated with an increased risk for cancer of the oral cavity and pharynx. The five most popular U.S. snuff brands were analyzed for alkaloids, volatile and tobacco-specific N-nitrosamines (TSNA), benzo[a]pyrene (CAS: 50-32-8), and polonium-210. The carcinogenic TSNA in the five snuff brands ranged from 9,500 to 289,000 ppb. These concentrations exceed the nitrosamine concentrations of other consumer products by at least 2 orders of magnitude. Polonium amounted to 0.16–1.22 pCi/g dry snuff. Trace amounts of benzo[a]pyrene (0.1–63 ppb) were indicative of contamination of the tobacco with thermal degradation products, probably due to fire curing or flue curing. The findings from this study, the biologic activity of snuff in animal models, and the epidemiologic studies on snuff use and oral cancer strongly suggest the need for reduction of carcinogens and especially of nitrosamines and polonium-210 in snuff.—JNCI 1986; 76:435-437.

9.6 - 289 ppm

During the last decade we have witnessed the increasing prevalence of snuff dipping in Sweden and in the United States (*1*), especially among young people (*2–4*). In 1984, about 21,600 tons of snuff were consumed in the United States by an estimated 7 million snuff dippers (*5, 6*). Snuff dipping is the practice of extracting juices from a pinch of moist fine-cut chewing tobacco, placed between the cheek and the gum.

Four case-control studies, 3 from the Southeastern United States and 1 from Sweden, have implicated snuff use in the etiology of cancer of the oral cavity and, to a lesser extent, of the pharynx (*1*). In the 1981 report of a study conducted in North Carolina by Winn et al., the relative risk of oral and pharyngeal cancer for white women who dipped snuff was four times that for women who did not use tobacco in any form (*7*). The authors observed also a strong dose-response relationship. In October 1984, the International Agency for Research on Cancer concluded: "There is sufficient evidence that oral use of snuffs of the types commonly used in North America and Western Europe is carcinogenic to humans" (*1*).

Topical administration of snuff in a surgically created oral canal in combination with herpes simplex virus type 1 infection induced squamous cell carcinoma of the oral cavity in rats (*8*). Snuff extracts induced sister chromatid exchanges in human peripheral lymphocytes (*9*).

The only type of carcinogens so far detected in snuff are VNA and TSNA (*10, 11*) (text-fig. 1). However, it has been suggested that snuff may also contain carcinogenic PAH and polonium-210 (*1*).

This study was designed to determine *Nicotiana* alkaloids, VNA and TSNA, the possible presence of BaP as an indicator for carcinogenic PAH, and that of ^{210}Po, in the five most popular U.S. brands of moist snuff.

MATERIALS AND METHODS

Sampling.—Ten boxes of each of the five snuff brands were bought on the open market in Westchester County, NY, in the winter of 1984–85 and stored in a cold room (4°C) until the time of analysis. Brands A, B, and C were packaged in waxed cardboard containers with metal lids (labeled "October-November 1984"). Brands D and E came in plastic containers. The moist snuff from 10 containers of each brand was combined (≈340 g), placed in closed containers, and mixed by a mechanical wristaction shaker. Aliquots were used for the determination of moisture, alkaloids, and carcinogens.

Analyses.—The moisture content of the snuff was determined in duplicate by a modified Dean-Stark procedure (±5%) (*12*). For the duplicate determination of VNA (±5%) and TSNA (±5–8%), we employed earlier published methods (*13–15*) using [^{14}C]NDMA (CAS: 62-75-9) (48 mCi/mmol) and NNN (CAS: 16543-55-8)-2′-^{14}C (51.7 mCi/mmol) as internal standards. BaP (CAS: 50-32-8) was determined by high-pressure liquid chromatography using a fluorescence detector and BaP-7,10-^{14}C (15.2 mCi/mmol) as the internal standard (±8%) (*16*).

The tobacco alkaloids were enriched from the snuff according to an earlier published method (*17*). The alkaloid concentrate was analyzed by GC with the use of a 25×0.25-mm (id) fused silica capillary column deactivated for basic compounds; helium (1 ml/min) served as carrier gas. GC temperatures were: for the injection port, 200°C; column, 165°C; and detector, 250°C. The average retention time for nicotine was 14.9 minutes and retention times of alkaloids relative to nicotine were for myosmine, 1.76; anabasine, 1.78; and anatabine, 2.17. By use of nicotine-2′-^{14}C (51 mCi/mmol) as the internal standard, the precision was plus or minus 5%.

ABBREVIATIONS USED: BaP=benzo[*a*]pyrene; GC=gas chromatography; NAB=*N*-nitrosoanabasine; NAT=*N*-nitrosoanatabine; NDMA=*N*-nitrosodimethylamine; NMOR=*N*-nitrosomorpholine; NNK=4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone; NNN=*N*-nitrosonornicotine; PAH=polynuclear aromatic hydrocarbons; TSNA=tobacco-specific N-nitrosamines; VNA=volatile N-nitrosamines.

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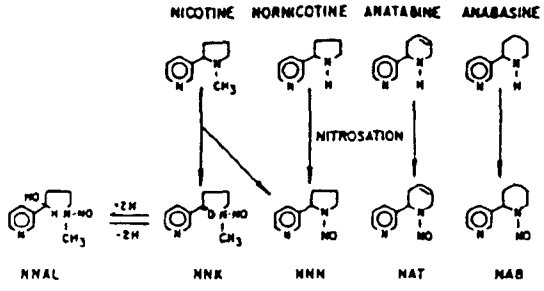

TEXT-FIGURE 1.—Formation of TSNA. NNAL=4-(methylnitrosamine)-1-(3-pyridyl)-butan-1-ol.

The concentration of ^{210}Po was determined by the method of the U.S. Department of Energy (*18*), with slight modification for the snuff analysis. Polonium was equilibrated with ^{209}Po tracer and isolated by co-precipitation with lead as the sulfide. The sulfide precipitate was dissolved in weak HCl, and Po was spontaneously deposited on a nickel disc. The Po content of the sample was determined by solid-state alpha-spectrometry. A reagent blank was analyzed with the snuff samples, and the net ^{210}Po activity was recorded. The counting error associated with all measurements was less than 10%.

RESULTS AND DISCUSSION

Table 1 presents the analytic data for moisture content, alkaloids, nitrosamines, BaP, and ^{210}Po in the five most popular U.S. brands of moist snuff in 1984–85. The moisture content of four of the snuff brands was, as expected, around 50%; but sample D had only 20%, suggesting that this snuff may have been prepared by a different process. Compared to the other brands, snuff D is also relatively low in nicotine, BaP, and ^{210}Po.

The fluctuating values for the VNA do not reveal a specific trend. This was expected because VNA are formed during aging, curing, and/or fermentation of the tobacco and are at least partially lost during processing because of their volatility. The presence of NMOR (CAS: 59-89-2) in the snuff products indicates contamination with morpholine either due to the additives and/or diffusion from packing materials (*10*). However, in contrast to an earlier analysis in which we found up to 690 ppb NMOR (*10*), levels in recent analyses have not exceeded 35 ppb. The levels of VNA are in agreement with those reported for Swedish snuffs (*19*).

The levels of total TSNA in the popular snuff brands, being 9,600–289,000 ppb, are exceptionally high and exceed by at least 2 orders of magnitude the occurrence of carcinogenic N-nitrosamines in other consumer products (*20*). These data underscore that in snuff use the tissues of the oral cavity are repeatedly exposed to high concentrations of TSNA as also indicated by the saliva analysis for nitrosamines (*21*). Furthermore, it is not unlikely that additional amounts of TSNA are formed endogenously during snuff dipping (*11*). Of the TSNA, NNN and NNK (text-fig. 1) are powerful carcinogens in mice, rats, and Syrian golden hamsters (*1, 11*). Already a single dose of 1.0 mg NNK (4.8 μmol) induces a significant number of lung tumors in hamsters (*22*).

The presence of traces of carcinogenic PAH in snuff, documented by the findings of <0.1–63 ppb BaP as an indicator for PAH, can be attributed to flue curing or fire curing and other processes during snuff preparation, which incur contamination of the tobacco with thermal decomposition products.

The presence of ^{210}Po in snuff at levels of 0.16–1.22 pCi/g dry snuff represents also a risk factor for oral cancer in snuff dippers. As snuff is often held for hours between cheek and gum, the tissues may be exposed to relatively high radiation by α-particles. In the case of cigarette smoke, about 10% of the ^{210}Po from the tobacco (0.1–1.0 pCi ^{210}Po/g cigarette tobacco) is transferred into the mainstream smoke and is thus inhaled (*23, 24*). Several studies have suggested that such inhaled traces of ^{210}Po will accumulate on the mucosal surface of the lung as hot spots and may contribute to the increased lung cancer risk of cigarette smokers (*25–27*). Similarly, it is possible that the α-radiation emitted by the ^{210}Po in snuff, concentrated in a relatively small area of the cheek and gum, may contribute to the increased risk of snuff dippers for oral cancer. This requires further investigation. However, available evidence indicates that it would be prudent to prepare snuff only from tobaccos with low levels of ^{210}Po. This approach appears feasible since certain types of fertilizers provide the major source of ^{210}Po in tobacco (*28*).

The increased risk for cancer of the oral cavity and pharynx in snuff dippers and the presence of relatively high concentrations of carcinogenic N-nitrosamines, especially of nicotine-derived nitrosamines, and of ^{210}Po

TABLE 1.—*Analytic data for five popular U.S. snuff brands*[a]

Sample	H_2O, %	Nicotine, mg/g	Anabasine, μg/g	Myosmine, μg/g	Anatabine, μg/g	VNA, ppb			TSNA, ppb				BaP, ppb	^{210}Po, pCi/g
						NDMA	NPYR	NMOR	NNN	NAT	NAB	NNK		
A	50	20.7	20	150	350	102	202	29	33,000	44,000	1,100	1,800	11.8	0.64
B	45	25.1	20	190	640	27	15	10	5,800	3,500	200	100	9.4	1.22
C	51	30.7	20	260	530	50	212	35	64,000	215,000	6,700	3,100	63.0	0.33
D	20	5.7	5	100	260	3.8	15	8.3	14,000	3,300	500	600	<0.1	0.16
E	49	14.6	20	50	150	22	19	24	16,000	14,000	1,000	1,000	0.42	0.18

[a] All values are based on dry weights. NPYR, N-nitrosopyrrolidine (CAS: 930-55-2). NAB (CAS: 1133-64-8).

strongly suggest modifications in the production of snuff. Since the TSNA are formed during tobacco processing (*11*) and since ^{210}Po originates mainly from specific fertilizers, a reduction of these carcinogens in snuff appears feasible.

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The March 1986 JNCI cover highlighted the important issue of snuff hazards and the article "Carcinogenic Agents in Snuff" by Hoffman, Harley, Fisenne, et al. on pages 435-437. In his symbolic presentation, the artist drew a formula that would be phenylcyclopentane to a chemist. The authors would have liked to have seen the formula shown at right, which is *N'*-nitrosonornicotine (NNN) and which the authors believe is one of the two most active ingredients in snuff. NNN and 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are formed from nicotine during tobacco processing to snuff. These nitrosamines are tobacco-specific agents, occur in snuff in high concentrations, and are highly carcinogenic in laboratory animals.