# Five Leading U.S. Commercial Brands of Moist Snuff in 1994: Assessment of Carcinogenic *N*-Nitrosamines

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**_Background:_ Moist snuff is the only tobacco product in the United States with increasing sales (an increase of 38.4% between 1981 and 1993) and with increased consumption, primarily by male adolescents aged 12-18 years old and young adults aged 19 years old or older. It is known from previous studies that levels of nicotine and the proportion of unprotonated (free) nicotine, as well as the pH, which affects nicotine delivery, vary considerably among the leading snuff brands. Whether concentrations of major carcinogens, such as the nicotine-derived tobacco-specific *N*-nitrosamines (TSNAs), like *N'*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), also vary among these brands has not been determined previously. _Purpose:_ Our purpose was threefold: 1) to determine the concentrations of major carcinogenic nicotine-derived *N*-nitrosamines in each of the five most popular moist snuff brands; 2) to analyze the quantitative differences in the various snuff components (e.g., NNN) between two major brand categories: a category comprising the brands known to have high levels of unprotonated nicotine (Copenhagen, Skoal fine cut, and Kodiak) versus a category comprising the brands known to have low levels (Hawken and Skoal Bandits); and 3) to compare the differences in the concentrations of nicotine (previously determined), NNN, NNK, and total TSNAs between these two major brand categories. _Methods:_ Three boxes of each of the five leading U.S. moist snuff brands were bought in July 1994 from retailers in six areas and transferred immediately to the analytical laboratory. After extraction, *N*-nitrosamino acids and TSNAs were determined on a gas chromatograph interfaced with a thermal energy analyzer (GC-TEA) and integrator. Each 5-g sample of ground, freeze-dried tobacco was extracted twice, and each extract was analyzed twice by GC-TEA. All *P* values reported are two sided. _Results:_ Copenhagen, Skoal fine cut, and Kodiak as a group had statistically significant higher levels of nicotine ($P$ = .0017), NNN ($P$<.0001), NNK ($P$ = .0119), and total TSNAs ($P$<.0001) than the Hawken and Skoal Bandits group. Concentrations (means ± SD) of nicotine, NNN, NNK, and total TSNAs comparing the two major brand categories are as follows: nicotine—11.6 ± 1.01 mg/g versus 6.96 ± 3.62 mg/g ($P$ = .0017), NNN—7.74 ± 1.70 µg/g versus 4.17 ± 1.35 µg/g ($P$<.0001), NNK—1.23 ± 0.68 µg/g versus 0.61 ± 0.41 µg/g ($P$ = .0119), and total TSNAs—14.3 ± 3.82 µg/g versus 6.3 ± 2.56 µg/g ($P$<.0001). _Conclusions:_ The three leading U.S. snuff brands (Copenhagen, Skoal fine cut, and Kodiak; making up 92% of the U.S. market) showed not only high levels of pH, nicotine, and unprotonated (free) nicotine, but also high concentrations of the strongly carcinogenic TSNAs in comparison with the fourth and fifth best selling moist snuff brands, Hawken and Skoal Bandits (3% of the U.S. market).** **[J Natl Cancer Inst 1995;87:1862-9]**

Snuff consumption has increased by 38.4% in the United States between 1981 and 1993, while the sales of all other tobacco products have declined as follows: chewing tobacco, by 28.4%; smoking tobacco, by 58.4%; cigars and cigarillos, by 43.7%; small cigars, by 12.6%; and cigarettes, by 24.2% (*1,2*). In Sweden, where per capita consumption of oral snuff is the highest, snuff sales have increased by 92%, although cigarette sales have decreased significantly between 1970 and 1993 (*3*). One major reason for the greater use of moist snuff is the growing prevalence of snuff dipping among male adolescents aged 12-18 years and young adults aged 19 years or older in both countries (*4,5*). These trends are alarming in view of the findings of the International Agency for Research on Cancer (IARC) (*6*), the U.S. Surgeon General (*7*), and the World Health Organization (WHO) (*8*), all of whom have concluded that the oral use of snuff induces gingival recession, as well as precancerous lesions in the oral cavity and pharynx, and that snuff is carcinogenic to humans. In view of the fact that there are about 10 million current snuff users and their numbers are growing in the United States and because more and more children and adolescents are taking up the snuff-dipping habit, it is of concern that the incidence of oral cancer will rise accordingly. It is especially disturbing that, among some Indian tribes in the United States and among Alaskan Eskimos, snuff dipping starts as early as in kindergarten and is practiced by more than 50% of the 9th and 10th graders (*9,10*). There are also indications for increased risks of cancer of the esophagus, the pancreas, the renal pelvis, and the urinary bladder among snuff users (*6,7,11,12*).

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*See* "Notes" section following "References."

The conclusions of the public health organizations were based primarily on case–control studies from the southern United States and from Sweden (*6-8*). These were strengthened by an additional report (*13*). The epidemiologic and clinical findings are supported by bioassays in rodents and by the identification of carcinogens in snuff. Daily instillation of moist snuff into a surgically created lip canal led to benign and malignant tumors of the oral cavity of rats (*14,15*). Identified carcinogens in snuff include some volatile aldehydes, certain inorganic compounds, radioactive elements, benzo[*a*]pyrene, volatile *N*-nitrosamines, *N*-nitrosamino acids (NAAs), and tobacco-specific *N*-nitrosamines (TSNAs), the most active group of carcinogens (Figs. 1 and 2) (*16-18*). Swabbing of the mouths of rats with an aqueous solution of the TSNAs *N′*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) led to tumors of the oral cavity (14). In snuff dippers, NNN and NNK

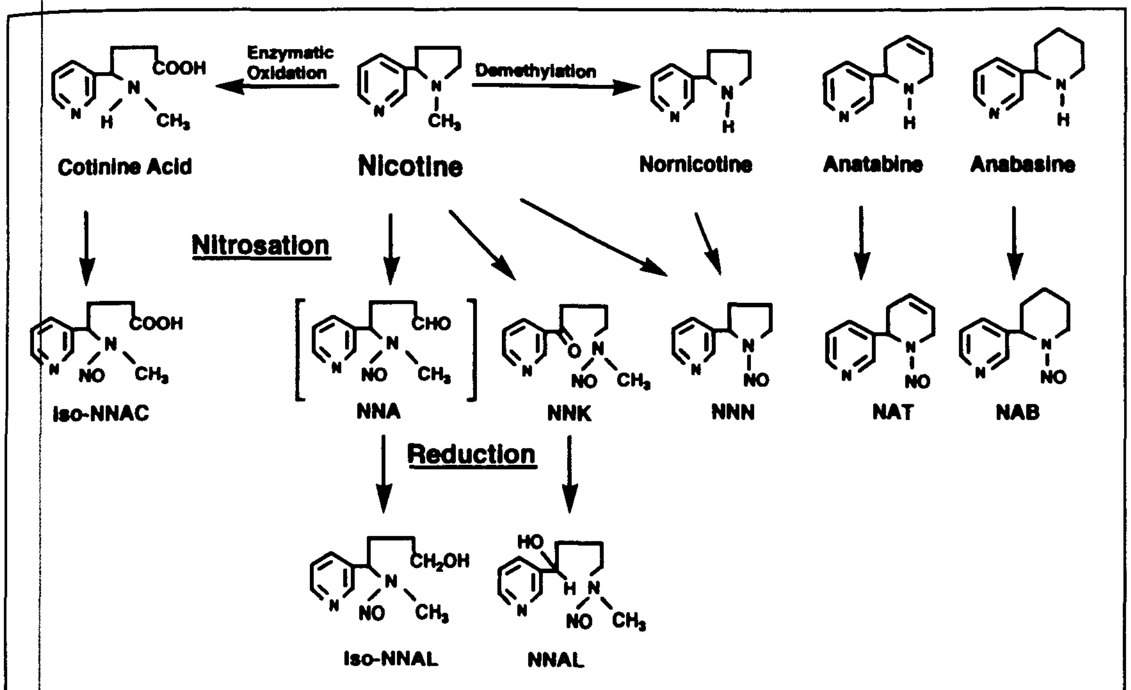


**Fig. 1.** Formation of tobacco-specific *N*-nitrosamines. Iso-NNAC = 4-(methylnitrosamino)-4-(3-pyridyl)butyric acid; NNA = 4-(methylnitrosamino)-4-(3-pyridyl)butanal; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN = *N′*-nitrosonornicotine; NAT = *N′*-nitrosoanatabine; NAB = *N′*-nitrosoanabasine; Iso-NNAL = 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.

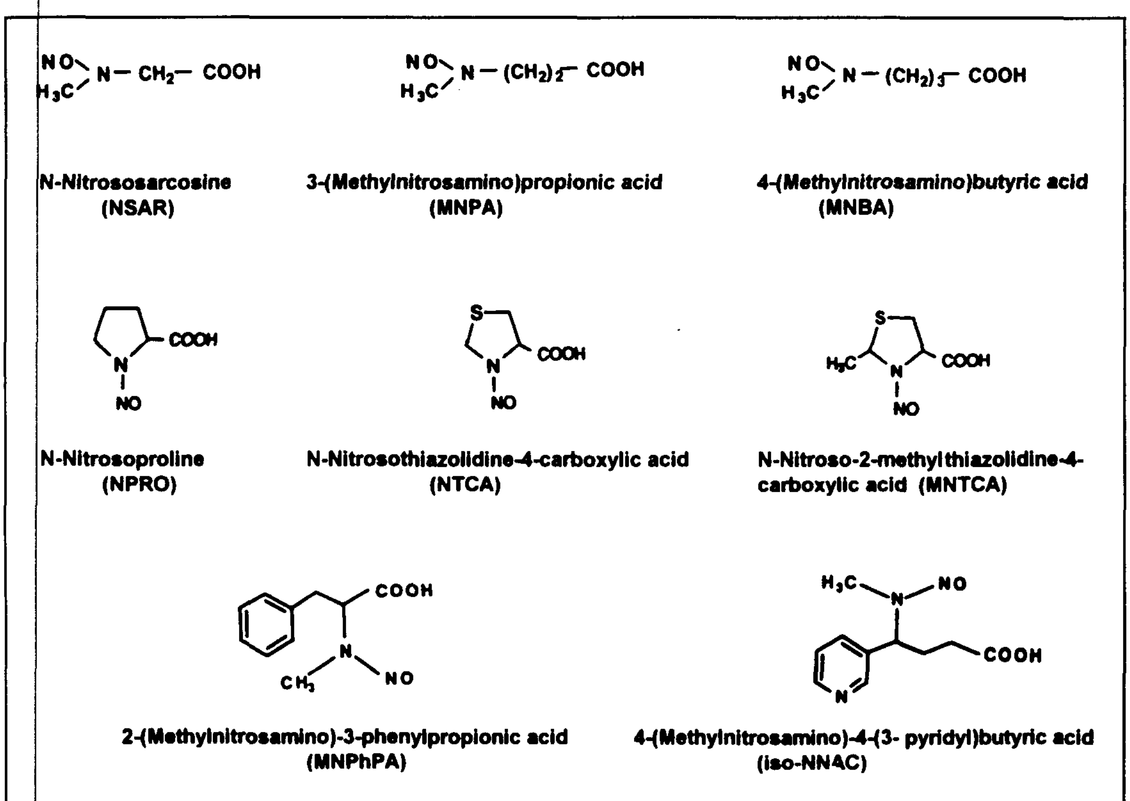


**Fig. 2.** *N*-Nitrosamino acids in moist snuff tobacco.

are metabolically activated and form protein adducts, including those with hemoglobin that permits their quantitative assessment (*19*). It is strongly indicated that NNN and NNK are major contributors to the carcinogenic activity of snuff (*20,21*).

In 1987, we (*16*) reported on the concentrations of volatile aldehydes, volatile nitrosamines, benzo[*a*]pyrene, several inorganic compounds, polonium 210, and TSNAs in five moist snuff brands and in three dry snuff brands purchased in the United States in 1985 and 1986. A systematic analysis of all moist snuff brands on the U.S. market, however, had not been published until recently, when we (*22*) reported on the range of nicotine, moisture, pH, and nonionized nicotine in 17 moist snuff brands bought in Westchester County, NY, in 1994 and in the leading five moist snuff brands purchased in six areas across the United States. These five brands accounted for 95% of the U.S. moist snuff market in 1993. The averaged values for moisture, pH, nicotine, and unprotonated nicotine (Table 1) demonstrate the availability of snuff brands with low, medium, and high nicotine deliveries (*22*). These data and the sales figures support the concept that the product design may be aimed at creating and maintaining nicotine dependence (*23*). A recent report (*4*) is consistent with the hypothesis that snuff dippers initially use brands with low nicotine dosage and then switch to brands with high nicotine dosage, particularly Copenhagen. The U.S. Surgeon General (*24*) concluded in 1988 that "... nicotine is the drug in tobacco that causes addiction."

We have extended our earlier studies by assessing the range of concentrations of the major carcinogens in snuff, the NAAs and the TSNAs in the five leading moist snuff brands. We also determined nitrate and nitrite concentrations in the snuff brands because nitrate is reduced primarily by bacteria to nitrite during tobacco processing and during aging of snuff (*25,26*). Nitrite is the major nitrosating agent in tobacco; its reaction with secondary and tertiary amines leads to *N*-nitrosamines, many of which are carcinogenic (*6,27*).

Our purpose in conducting this study was threefold: 1) to determine the concentrations of major carcinogenic TSNAs in each of the five most popular moist snuff brands [95% of the U.S. market in 1993 (*28*)]; 2) to analyze the quantitative differences in the various snuff components (e.g., NNN) between two major brand categories (Copenhagen, Skoal fine cut, and Kodiak versus Hawken and Skoal Bandits) that show distinct differences in the content of unprotonated nicotine; and 3) to compare the differences in the concentrations of nicotine (previously determined), NNN, NNK, and total TSNAs between these two major brand categories.

## Materials and Methods

### Sampling

Three boxes of each of the five leading U.S. moist snuff brands, manufactured by the U.S. Tobacco Company (Copenhagen, Skoal, and Skoal Bandits) and by the Conwood Company (Kodiak and Hawken), were bought in July 1994 from retailers in Westchester County, NY; Boston, MA; Lexington, KY; Denver, CO; Alameda, CA; and Lansing, MI. These six areas were chosen because they represent six different regions of the U.S. mainland with distinct climates. The snuff from the three boxes from each location was combined and analyzed in duplicate. After purchase, the samples were express mailed to our analytical laboratory in Valhalla, NY. Without exception, the boxes with the moist snuff-containing sachets (Skoal Bandits) and those with moist snuff of different cuts had been shelved in air-conditioned stores. When the products were received in the laboratory, they were immediately stored in a cold room (4 °C), where they were kept until the time of analysis. Under these conditions, no changes were observed in pH and the levels of nitrate, nitrite, TSNAs, and NAAs (*29*).

### Reagents and Standards

Chemicals and solvents used in the processes discussed below and identified in the studies cited were analytical reagents of the highest purity from M. T. Baker Chemical Co., Phillipsburg, NJ, and Fisher Scientific Co., Fair Lawn, NJ. 4-(Methylnitrosamino)-4-(3-pyridyl)butyric acid (iso-NNAC) was synthesized from cotinine according to an earlier published method (*17*). 3-(methylnitrosamino)-propionic acid (MNPA) was prepared by reaction of methylamine with β-propiolactone (*30*) followed by *N*-nitrosation (*17,31*), and 4-(methylnitrosamino)butyric acid (MNBA) was prepared by nitrosation of 4-(methylamino)butyric acid hydrochloride with sodium nitrite (*17*). *N*-Nitrosoguvacoline (NG), which served as an internal standard for the analysis of TSNAs and NAAs, was prepared according to a previously published method (*32*). NNN and NNK were also synthesized as reported earlier (*33,34*). Their purity (>99.5%) and that of the purchased *N*-nitrosamines (the latter include *N*′-nitrosoanatabine [NAT], *N*′-nitrosoanabasine [NAB], *N*-nitrososarcosine [NSAR], and *N*-nitrosoproline [NPRO]—all from the NCI Chemical Carcinogen Repositories, Midwest Research Institute, Kansas City, MO) were verified by capillary gas chromatography (GC) using flame ionization detection and mass spectrometry analysis.

### Analyses

The determination of the TSNAs and NAAs in moist snuff was completed according to an earlier published method (*17*). It is based on the extraction of 5 g of ground, freeze-dried tobacco with 150 mL water, to which 5 mL of 20% am-

**Table 1.** Nicotine, moisture, and pH levels of the five leading* U.S. moist snuff brands: average values of same brands bought in six areas in different regions of the United States†

| | | Mean ± standard deviation | | | | |
|---|---|---|---|---|---|---|
| Snuff brand | U.S. market share, 1993, % | Moisture, % | pH of suspension | Nicotine, mg/g | Unprotonated nicotine,% |
| Copenhagen | 42 | 58.8 ± 1.78 | 8.00 ± 0.31 | 12.0 ± 0.7 | 49.0 ± 16.7 |
| Skoal (Original, Fine Cut, Wintergreen) | 39‡ | 57.9 ± 0.90 | 7.46 ± 0.14 | 11.9 ± 1.3 | 22.0 ± 5.73 |
| Kodiak (Wintergreen) | 11 | 56.5 ± 2.00 | 8.19 ± 0.11 | 10.9 ± 0.8 | 59.7 ± 6.01 |
| Hawken (Wintergreen) | 1 | 28.8 ± 2.60 | 5.71 ± 0.10 | 3.2 ± 0.2 | 0.5 ± 0.11 |
| Skoal Bandits (Straight) | 2 | 54.8 ± 6.30 | 5.37 ± 0.12 | 10.1 ± 0.8 | 0.23 ± 0.05 |

*By percent market share (*28*).
†Used with permission from Djordjevic et al., 1995 (*22*).
‡39% includes Skoal original and various Skoal long cuts.

monium sulfamate solution in 3.6 N $H_2SO_4$ was added to prevent artifactual formation of *N*-nitrosamines. The tobacco was extracted overnight by shaking on the wrist-action shaker. The pH of the acidic aqueous tobacco extract was adjusted to 2.0 with 5 N NaOH. This solution was repeatedly extracted with ethyl acetate to remove the NAAs. After the removal of NAAs, the acidic layer was adjusted to pH 9 with 5 N NaOH, and the TSNAs were extracted four times with ethyl acetate. The alkaline, aqueous tobacco extract was then brought to pH 4 with 1 N HCl; iso-NNAC was extracted repeatedly with ethyl acetate. The ethyl acetate extracts (NAAs, TSNAs, and iso-NNAC) were dried ($Na_2SO_4$) and evaporated to dryness in vacuo. The extracts obtained at pH 2 and pH 4 were methylated with diazomethane and were then analyzed by GC with a thermal energy analyzer (GC-TEA) as methyl esters of NAAs, while the TSNAs were determined directly by GC-TEA according to earlier published methods (*17*). Before GC-TEA analyses, a solution of 3.2 ppm of NG in acetonitrile was added to each sample as an internal standard. Since some TSNAs, especially the most polar NNK, were already partially extracted at pH 2, the final concentrations reported in this study were obtained by adding up the amounts of individual compounds obtained at pH 9 and pH 2. The same was done with the NAA data. (In this case, the values obtained by extraction at pH 2 and pH 4 were combined.) For the TSNAs and the NAAs, the standard deviation was ±5%; for iso-NNAC, the standard deviation was ±8% (*17*). The detection limit for each individual NAA and TSNA was 1 ng/g snuff.

Nitrite–nitrogen and nitrate–nitrogen were determined with autoanalyzer methods according to the techniques of Crutchfield and Burton (*35*) and Armstrong et al. (*36*), respectively.

For quality control, each sample was extracted at least twice. In addition, each extract was analyzed by GC-TEA several times. Therefore, each value is the average of multiple analyses.

### Statistical Methods

The association between various snuff components and nitrites was evaluated by use of analysis of covariance (*37*). In this model, the dependent variable was the level of the snuff component, the covariate was nitrite, and the factors included the six areas from which the samples were obtained as well as two major brand categories (Copenhagen, Skoal fine cut, and Kodiak versus Hawken and Skoal Bandits). There were two replicates for the measure of each snuff component. In this analysis, the F test of interest was the statistical significance of the association between nitrites versus NNN, NNK, NAT, NAB, and total TSNAs, with the effects of brand and areas simultaneously adjusted.

As was discussed earlier, there are primarily two types of moist snuff brands on the U.S. market: those with relatively high amounts of unprotonated nicotine (>2 mg/g tobacco) and those with low amounts of unprotonated nicotine (<1 mg/g tobacco). Unprotonated nicotine in the leading three brands of moist snuff (Copenhagen, Skoal fine cut, and Kodiak) ranges between 2.6 and 6.5 mg/g tobacco, while the fourth and fifth ranking brands (Hawken and Skoal Bandits) contain only between 0.016 and 0.023 mg unprotonated nicotine per gram of tobacco. For these reasons, we evaluated the significance of differences in various snuff components in these two categories of snuff by means of analysis of variance (*37*). Statistical comparisons of the measured levels of nicotine, NNN, NNK, and total TSNAs between the two major brand categories were done using a two-tailed Student's *t* test.

Data were analyzed by use of SAS/STAT (*38*) on a Sun Sparc workstation. All *P* values reported represent two-sided tests of statistical significance.

## Results

Table 2 presents quantitative data for the TSNAs (i.e., NNN, NNK, NAT, and NAB) as well as for nitrate–nitrogen and nitrite–nitrogen in samples taken from six U.S. areas for each of the five leading moist snuff brands. Each value is an average of two analyses. We observed highly significant variations in the levels of individual TSNAs as well as nitrate–nitrogen and nitrite–nitrogen concentrations in identical snuff brands that were bought in six areas. We found the coefficient of variation to range between 9.8% and 20.3% for NNN and between 10.4% and 32.9% for NNK. These wide ranges are likely due to origins of samples from different production batches and/or the fact that these samples may have been kept under different storage conditions for varying lengths of time. We (*29*) reported earlier that storage for 4 weeks or longer and at ambient or elevated temperatures significantly raised the concentrations of the NNN (≥65%), NNK (≥390%), and nitrite (≥850%). There were remarkably different concentrations of the strong carcinogens NNN, NNK, and total TSNAs in the five snuff brands. These concentrations were on the low level in Hawken with 3.07 ± 0.30 µg/g, 0.23 ± 0.04 µg/g, and 4.08 ± 0.44 µg/g, respectively, and on the high level in Copenhagen with 8.73 ± 1.44 µg/g, 1.89 ± 0.62 µg/g, and 17.2 ± 2.97 µg/g, respectively. The major nitrosating agent in snuff, nitrite, was 1.4 ± 0.8 µg/g in Hawken and 672.0 ± 296.8 µg/g in Copenhagen; however, nitrate concentrations did not differ greatly from one another, being 461.8 ± 16.2 µg/g and 512.5 ± 120.6 µg/g, respectively. The lower moisture content of Hawken (28.8% versus an average of 57% for the other four brands) can also, at least partially, explain the lower TSNA levels in this brand. Andersen et al. (*39*) reported that the TSNA levels in snuff containing 55.5% moisture and stored at ambient temperature (24 °C) increased during 48 weeks about 17-fold over TSNA levels in the same product containing 21.9% moisture. These findings support the view that the production of snuff (especially during fermentation) and storage conditions influence the reduction of nitrate to nitrite and thereby affect the yields of carcinogenic *N*-nitrosamines.

Table 3 lists the association between nitrite and NNN, NNK, NAT, NAB, and total TSNAs. These data reveal that the concentrations of nitrite and not those of nitrate are important for the formation of TSNAs. Nitrate is reduced to nitrite by the microflora, primarily during fermentation of the tobacco leaves (*26*). The statistical evaluation showed that the TSNA concentrations in the two major snuff brand categories (Copenhagen, Skoal fine cut, and Kodiak versus Hawken and Skoal Bandits) are significantly higher in the first group of snuff brands than in the second group of snuff brands ($P$ = .05-.0001, Table 4).

One earlier study (*40*) had reported a statistically significant association of the concentration of nicotine with NNN and NNK in tobacco; however, this could not be confirmed in other investigations (*41-43*). We did not find statistically significant associations between nicotine, NNN, NNK, and total TSNAs by comparing individual brands with each other; however, in comparisons of the major snuff brand categories (Copenhagen, Skoal fine cut, and Kodiak versus Hawken and Skoal Bandits), statistically significant higher concentrations of nicotine ($P$ = .0017), NNN ($P$<.0001), NNK ($P$ = .0119), and total TSNAs ($P$<.0001) were observed (Table 5). Concentrations (means ± SD) of nicotine, NNN, NNK, and total TSNAs comparing the two major brand categories are as follows: nicotine—11.6 ± 1.01 mg/g versus 6.96 ± 3.62 mg/g ($P$ = .0017), NNN—7.74 ± 1.70 µg/g versus 4.17 ± 1.35 µg/g ($P$<.0001), NNK—1.23 ± 0.68 µg/g versus 0.61 ± 0.41 µg/g ($P$ = .0119), and total TSNAs—14.3 ± 3.82 µg/g versus 6.3 ± 2.56 µg/g ($P$<.0001).

The data for the three carcinogenic NAAs (*44,45*) (i.e., NSAR, MNPA, and MNBA) and for the noncarcinogenic NPRO and iso-NNAC are presented in Table 6 together with quantitative data for total NAAs. In addition to NSAR, MNPA, MNBA, NPRO, and iso-NNAC, the total NAAs include *N*-

**Table 2.** Analyses of tobacco-specific *N*-nitrosamines (TSNAs) and nitrate and nitrite (expressed as µg/g dry weight) in the five leading* U.S. snuff brands—1994†

| Snuff brand | Manufacturer (% market share) | TSNAs‡ | Area§ Westchester County, NY | Boston, MA | Lexington, KY | Denver, CO | Alameda, CA | Lansing, MI | Mean | Standard deviation | Coefficient of variation, % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Copenhagen | U.S. | NNN | 7.93 | 7.86 | 10.78 | 9.60 | 9.72 | 6.47 | 8.73 | 1.44 | 16.5 |
| | Tobacco | NNK | 1.45 | 1.55 | 3.20 | 1.46 | 2.05 | 1.61 | 1.89 | 0.62 | 32.9 |
| | Co. | NAT | 5.88 | 5.03 | 7.50 | 6.87 | 6.81 | 4.68 | 6.13 | 1.02 | 16.7 |
| | (42) | NAB | 0.34 | 0.34 | 0.65 | 0.54 | 0.57 | 0.57 | 0.50 | 0.12 | 23.7 |
| | | Total TSNAs | 15.60 | 14.78 | 22.13 | 18.47 | 19.15 | 13.33 | 17.24 | 2.97 | 17.3 |
| | | Nitrate–nitrogen | 470.0 | 532.0 | 512.5 | 730.0 | 512.5 | 318.0 | 512.5 | 120.6 | 23.5 |
| | | Nitrite–nitrogen | 428.0 | 466.0 | 782.5 | 321.0 | 1180 | 855.0 | 672.0 | 296.8 | 44.2 |
| Skoal, | U.S. | NNN | 6.05 | 7.07 | 10.21 | 8.34 | 8.94 | 8.48 | 8.18 | 1.33 | 16.2 |
| Original, | Tobacco | NNK | 1.21 | 1.09 | 1.47 | 1.11 | 1.29 | 1.31 | 1.25 | 0.13 | 10.4 |
| Fine | Co. | NAT | 3.82 | 4.25 | 7.01 | 5.04 | 5.47 | 5.00 | 5.10 | 1.01 | 19.9 |
| Cut, | (39)‖ | NAB | 0.26 | 0.33 | 0.54 | 0.36 | 0.42 | 0.33 | 0.37 | 0.09 | 23.6 |
| Wintergreen | | Total TSNAs | 11.34 | 12.74 | 19.23 | 14.85 | 16.12 | 15.12 | 14.90 | 2.50 | 16.8 |
| | | Nitrate–nitrogen | 1099 | 1199 | 1058 | 1090 | 991 | 1024 | 1077 | 66.0 | 6.1 |
| | | Nitrite–nitrogen | 20.7 | 29.4 | 33.1 | 62.9 | 127.8 | 113.3 | 64.5 | 41.9 | 64.9 |
| Skoal, Long | U.S. | NNN | 3.12 | 2.95 | N/A | N/A | N/A | N/A | 3.04 | | |
| Cut, Classic | Tobacco | NNK | 0.55 | 0.38 | N/A | N/A | N/A | N/A | 0.47 | | |
| | Co. | NAT | 1.73 | 1.31 | N/A | N/A | N/A | N/A | 1.52 | | |
| | | NAB | 0.12 | 0.22 | N/A | N/A | N/A | N/A | 0.17 | | |
| | | Total TSNAs | 5.52 | 4.86 | N/A | N/A | N/A | N/A | 5.19 | | |
| Kodiak, | Conwood | NNN | 6.27 | 5.84 | 4.63 | 5.99 | 7.03 | 8.05 | 6.30 | 1.06 | 16.8 |
| Wintergreen | Co. | NNK | 0.84 | 0.37 | 0.53 | 0.49 | 0.42 | 0.62 | 0.55 | 0.15 | 28.2 |
| | (11) | NAT | 4.38 | 2.79 | 1.91 | 3.46 | 4.42 | 5.79 | 3.79 | 1.25 | 33.0 |
| | | NAB | 0.39 | 0.23 | 0.15 | 0.29 | 0.40 | 0.45 | 0.32 | 0.10 | 33.0 |
| | | Total TSNAs | 11.88 | 9.23 | 7.22 | 10.23 | 12.27 | 14.91 | 10.96 | 2.44 | 22.3 |
| | | Nitrate–nitrogen | 1367 | 1391 | 1038 | 1449 | 1384 | 1417 | 1341 | 138 | 10.3 |
| | | Nitrite–nitrogen | 2.20 | 2.00 | 1.90 | 2.20 | 3.20 | 5.10 | 2.77 | 1.13 | 40.7 |
| Hawken, | Conwood | NNN | 3.21 | 2.88 | 3.44 | 3.29 | N/A | 2.53 | 3.07 | 0.30 | 9.8 |
| Wintergreen | Co. | NNK | 0.27 | 0.31 | 0.19 | 0.22 | N/A | 0.18 | 0.23 | 0.04 | 19.2 |
| | (1) | NAT | 0.90 | 0.47 | 0.58 | 0.73 | N/A | 0.48 | 0.63 | 0.15 | 23.8 |
| | | NAB | 0.10 | 0.13 | 0.16 | 0.16 | N/A | 0.10 | 0.13 | 0.02 | 18.8 |
| | | Total TSNAs | 4.48 | 3.79 | 4.37 | 4.40 | N/A | 3.29 | 4.08 | 0.44 | 10.8 |
| | | Nitrate–nitrogen | 483.0 | 432.0 | 469.0 | 472.0 | N/A | 453.0 | 461.8 | 16.2 | 3.5 |
| | | Nitrite–nitrogen | 0.8 | 0.8 | 1.1 | 1.1 | N/A | 3.1 | 1.4 | 0.8 | 57.1 |
| Skoal Bandits, | U.S. | NNN | 6.33 | 5.29 | 5.15 | 5.77 | 5.02 | 3.00 | 5.09 | 1.03 | 20.3 |
| Straight | Tobacco | NNK | 0.83 | 0.81 | 0.93 | 1.43 | 0.95 | 0.58 | 0.92 | 0.26 | 27.9 |
| | Co. | NAT | 3.00 | 1.36 | 1.78 | 2.47 | 2.31 | 1.36 | 2.05 | 0.60 | 29.4 |
| | (2) | NAB | 0.15 | 0.12 | 0.14 | 0.16 | 0.15 | 0.06 | 0.13 | 0.03 | 25.9 |
| | | Total TSNAs | 10.31 | 7.58 | 8.00 | 9.83 | 8.43 | 5.00 | 8.19 | 1.72 | 21.0 |
| | | Nitrate–nitrogen | 1915 | 1840 | 2211 | 2190 | 2011 | 1971 | 2023 | 136 | 6.7 |
| | | Nitrite–nitrogen | 0.9 | 1.3 | 1.1 | 1.1 | 1.4 | 2.0 | 1.3 | 0.4 | 27.0 |

*By percent market share (*28*).

†Each sample was extracted two times, and each extract was analyzed twice by a gas chromatograph interfaced with a thermal energy analyzer. Values reported here are the means of 2 × 2 determination.

‡NNN = *N*′-nitrosonornicotine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT = *N*′-nitrosoanatabine; NAB = *N*′-nitrosoanabasine.

§TSNA values in columns are expressed as micrograms per gram dry weight. N/A = not analyzed.

‖39% included Skoal original and various Skoal long cuts.

nitrosothiazolidine-4-carboxylic acid, *N*-nitroso-2-methylthiazolidine-4-carboxylic acid, and 2-(methylnitrosamino)-3-phenylpropionic acid (Fig. 2). Whereas we found (*see* Table 2) the highest concentrations of the carcinogenic NNN and of total TSNAs in the best selling brands Copenhagen (NNN = 8.73 µg/g and total TSNAs = 17.24 µg/g), Skoal (NNN = 8.18 µg/g and total TSNAs = 14.90 µg/g), and Kodiak (NNN = 6.30 µg/g and total TSNAs = 10.96 µg/g) and we found the lowest TSNA levels in the snuff brands with the lower rate of sales (i.e., Skoal Bandits: NNN = 5.09 µg/g, total TSNAs = 8.19 µg/g; Hawken: NNN = 3.07 µg/g, total TSNAs = 4.08 µg/g), the values for some NAAs, especially MNPA, were reversed. The best selling brands (Table 6), i.e., Copenhagen (MNPA = 2.62 µg/g and total NAAs = 10.47 µg/g), Skoal (MNPA = 2.39 µg/g and total NAAs = 8.15 µg/g), and Kodiak (MNPA = 2.23 µg/g and total NAAs = 5.70 µg/g), had the lowest concentrations of NAAs. The lowest selling brands (Skoal Bandits and Hawken) contained significantly higher amounts of MNPA ($P$ = .0076) and total NAAs ($P$ = .0221) (MNPA = 10.96 µg/g and 5.62 µg/g, respectively; total NAAs = 13.45 µg/g and 11.56 µg/g, respectively). This observation cannot be readily explained. Perhaps, the acyclic NAAs with a secondary amino group [e.g., 3-(methylamino)propionic acid] are more readily nitrosatable than the tertiary amine nicotine or the heterocyclic secondary amines

**Table 3.** Association between nitrite and tobacco-specific *N*-nitrosamines (TSNAs) in five leading* U.S. snuff brands†

| TSNAs‡ | Association with nitrite, *P*§ |
|---|---|
| NNN | .007 |
| NNK | .001 |
| NAT | .0005 |
| NAB | .0001 |
| Total TSNAs | .0002 |

*By percent market share (*28*).
†Using analysis of covariance.
‡NNN = *N*′-nitrosonornicotine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT = *N*′-nitrosoanatabine; NAB = *N*′-nitrosoanabasine.
§Adjusted for six areas, two categories of brand (Copenhagen, Skoal fine cut, and Kodiak versus Hawken and Skoal Bandits), and two test samples.

**Table 4.** Tobacco-specific *N*-nitrosamines (TSNAs) in five leading* U.S. snuff brands compared† by two major brand categories‡ (Copenhagen, Skoal fine cut, and Kodiak versus Hawken and Skoal Bandits)

| TSNAs§ | Difference between two groups of brands, P‖ |
|---|---|
| NNN | .0001 |
| NNK | .05 |
| NAT | .0001 |
| NAB | .0001 |
| Total TSNAs | .0001 |

*By percent market share (*28*).
†Using factorial analysis of variance.
‡Determined by differences in levels of unprotonated nicotine.
§NNN = *N*′-nitrosonornicotine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT = *N*′-nitrosoanatabine; NAB = *N*′-nitrosoanabasine.
‖Adjusted for six areas of origin and two test samples.

nornicotine, anatabine, and anabasine (*see* Figs. 1 and 2). It is also possible that the five snuff brands differ in the available precursors for the NAAs, the amino acids with a secondary amino group, as a result of influences such as curing, fermenting, and aging and/or because additives have been used. This is being studied.

The analyses of Skoal were completed with the fine cut product; however, the sales figures for Skoal (1993: 39%) also include the long cut product. Tables 2 and 6 show the TSNA and NAA contents for fine cut and long cut Skoal bought in Westchester County, NY, and in Boston, MA, respectively.

## Discussion

In this study, we have analyzed samples from the five leading U.S. moist snuff brands that were purchased in July 1994 in six U.S. areas with distinctly different climates. These five brands accounted for 95% of the U.S. market in 1993 (*28*) and were Copenhagen (42%), Skoal (39%), Kodiak (11%), Hawken (1%), and Skoal Bandits (2%). Copenhagen, the leading snuff brand, has dominated the U.S. market for more than 10 years with at least 40% of the sales each year. A survey in 1993 has shown that, among users aged 10-22 years, the percentage of Copenhagen as preferred brand increases with the years of snuff dipping (*4*).

Copenhagen, Skoal fine cut, and Kodiak, the three leading snuff brands, are not only highest in nicotine, pH values, and unprotonated nicotine (*22*), but also higher in concentrations of the potent carcinogenic TSNAs than the fourth and fifth ranking U.S. snuff brands, Hawken and Skoal Bandits (Table 5).

The precursors for TSNAs are nicotine and the minor tobacco alkaloids nornicotine, anatabine, and anabasine (*46*). The major nitrosating agent in snuff tobacco is nitrite. The latter is formed from nitrate during the processing of tobacco to snuff and during aging of snuff (*25,26,29,39*). The nitrite concentrations, but not the nitrate content of the tobacco, are significantly associated with the concentrations of each of the TSNAs. For example, Hawken, with the lowest nitrite concentration (1.4 ± 0.8 µg/g) of the five snuff brands, had the lowest concentration of the highly carcinogenic NNK (0.23 ± 0.04 µg/g) and also of the other three individual TSNAs and of the total TSNAs (4.08 ± 0.44 µg/g), whereas Copenhagen, with the highest nitrite concentration (672 ± 297 µg/g), showed the highest levels of NNK (1.89 ± 0.62 µg/g), of other individual TSNAs, and of total TSNAs (17.24 ± 2.97 µg/g).

The higher concentrations of MNPA in Hawken and Skoal Bandits moist snuff are of concern. In a lung adenoma bioassay in A/J mice, in which MNPA was administered by intraperitoneal injection, MNPA had about one tenth the carcinogenic potency of the highly potent NNK (*45*). The results of DNA alkylation studies were in line with the bioassay data (*47*). When MNPA was applied at a 10-fold higher dose than NNK,

**Table 5.** Differences in concentrations of nicotine, NNN,* NNK,* and total TSNAs* between the two major brand categories† (Copenhagen, Skoal fine cut, and Kodiak versus Hawken and Skoal Bandits)‡

| | Concentration, mean ± standard deviation | | | |
|---|---|---|---|---|
| Snuff brand | Nicotine,§ mg/g | NNN, µg/g | NNK, µg/g | Total TSNAs, µg/g |
| Copenhagen, Skoal (Original, Fine Cut, Wintergreen), and Kodiak (Wintergreen) | 11.6 ± 1.01 | 7.74 ± 1.70 | 1.23 ± 0.68 | 14.3 ± 3.82 |
| Hawken (Wintergreen) and Skoal Bandits (Straight) | 6.96 ± 3.62 | 4.17 ± 1.35 | 0.61 ± 0.41 | 6.3 ± 2.56 |
| *P* | .0017 | <.0001 | .0119 | <.0001 |

*NNN = *N*′-nitrosonornicotine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; TSNAs = tobacco-specific *N*-nitrosamines.
†Determined by differences in levels of unprotonated nicotine.
‡Based on two-tailed Student's *t* test.
§Data used with permission from Djordjevic et al., 1995 (*22*).

**Table 6.** Analyses of *N*-nitrosamines (NAAs) (expressed as μg/g dry weight) in the five leading* U.S. snuff brands—1994†

| | | | Area§ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Snuff brand | Manufacturer (% market share) | NAAs‡ | Westchester County, NY | Boston, MA | Lexington, KY | Denver, CO | Alameda, CA | Lansing, MI | Mean | Standard deviation | Coefficient of variation, % |
| Copenhagen | U.S. Tobacco Co. (42) | NSAR | 0.07 | 0.04 | 0.06 | 0.05 | 0.07 | 0.04 | 0.06 | 0.01 | 22.9 |
| | | MNPA | 3.34 | 2.49 | 3.48 | 2.25 | 2.48 | 1.67 | 2.62 | 0.62 | 23.8 |
| | | MNBA | 0.36 | 0.30 | 0.51 | 0.27 | 0.37 | 0.20 | 0.34 | 0.10 | 28.9 |
| | | NPRO | 5.75 | 4.24 | 7.33 | 6.08 | 6.84 | 3.77 | 5.67 | 1.29 | 22.7 |
| | | iso-NNAC | 0.22 | 0.18 | 0.48 | 0.33 | 0.45 | 0.21 | 0.31 | 0.12 | 37.9 |
| | | Total NAAs‖ | 9.52 | 7.07 | 11.38 | 8.65 | 15.68 | 10.49 | 10.47 | 2.70 | 25.8 |
| Skoal, Original, Fine Cut, Wintergreen | U.S. Tobacco Co. (39)¶ | NSAR | 0.04 | 0.04 | 0.04 | 0.03 | 0.04 | 0.03 | 0.04 | 0.00 | 12.9 |
| | | MNPA | 2.16 | 2.58 | 2.90 | 2.63 | 2.12 | 1.93 | 2.39 | 0.34 | 14.2 |
| | | MNBA | 0.18 | 0.26 | 0.29 | 0.29 | 0.21 | 0.14 | 0.23 | 0.06 | 24.7 |
| | | NPRO | 3.77 | 3.87 | 6.12 | 5.04 | 4.22 | 4.60 | 4.60 | 0.80 | 17.4 |
| | | iso-NNAC | 0.10 | 0.12 | 0.20 | 0.21 | 0.13 | 0.00 | 0.13 | 0.07 | 55.0 |
| | | Total NAAs‖ | 6.70 | 7.62 | 10.56 | 9.13 | 7.48 | 7.43 | 8.15 | 1.30 | 15.9 |
| Skoal, Long Cut, Classic | U.S. Tobacco Co. | NSAR | 0.03 | 0.20 | N/A | N/A | N/A | N/A | 0.12 | | |
| | | MNPA | 1.46 | 1.45 | N/A | N/A | N/A | N/A | 1.46 | | |
| | | MNBA | 0.11 | 0.09 | N/A | N/A | N/A | N/A | 0.10 | | |
| | | NPRO | 2.95 | 1.61 | N/A | N/A | N/A | N/A | 2.28 | | |
| | | iso-NNAC | 0.13 | 0.10 | N/A | N/A | N/A | N/A | 0.12 | | |
| | | Total NAAs‖ | 5.11 | 3.60 | N/A | N/A | N/A | N/A | 4.36 | | |
| Kodiak, Wintergreen | Conwood Co. (11) | NSAR | 0.03 | 0.04 | 0.03 | 0.03 | 0.04 | 0.05 | 0.04 | 0.01 | 20.3 |
| | | MNPA | 1.60 | 2.48 | 2.28 | 2.37 | 2.08 | 2.56 | 2.23 | 0.32 | 14.3 |
| | | MNBA | 0.17 | 0.24 | 0.17 | 0.22 | 0.22 | 0.14 | 0.19 | 0.04 | 18.3 |
| | | NPRO | 1.21 | 2.12 | 2.80 | 2.74 | 2.31 | 3.16 | 2.39 | 0.63 | 26.2 |
| | | iso-NNAC | 0.13 | 0.16 | 0.14 | 0.10 | 0.16 | 0.14 | 0.14 | 0.02 | 14.7 |
| | | Total NAAs‖ | 3.51 | 5.56 | 5.96 | 6.42 | 5.81 | 6.91 | 5.70 | 1.07 | 18.8 |
| Hawken, Wintergreen | Conwood Co. (1) | NSAR | 0.09 | 0.08 | 0.07 | 0.06 | N/A | 0.07 | 0.07 | 0.01 | 14.9 |
| | | MNPA | 6.75 | 5.84 | 4.53 | 5.02 | N/A | 5.96 | 5.62 | 0.71 | 12.6 |
| | | MNBA | 0.46 | 0.33 | 0.26 | 0.29 | N/A | 0.30 | 0.33 | 0.06 | 19.4 |
| | | NPRO | 5.60 | 4.34 | 4.63 | 4.34 | N/A | 5.55 | 4.89 | 0.52 | 10.6 |
| | | iso-NNAC | 0.16 | 0.17 | 0.14 | 0.09 | N/A | 0.16 | 0.14 | 0.03 | 18.2 |
| | | Total NAAs‖ | 13.76 | 11.12 | 10.09 | 10.26 | N/A | 12.57 | 11.56 | 1.28 | 11.1 |
| Skoal Bandits, Straight | U.S. Tobacco Co. (2) | NSAR | 0.04 | 0.02 | ND | 0.01 | ND | 0.02 | 0.02 | 0.01 | 39.9 |
| | | MNPA | 14.03 | 9.50 | 8.34 | 11.98 | 10.97 | 10.94 | 10.96 | 1.80 | 16.4 |
| | | MNBA | 0.20 | 0.21 | 0.11 | 0.03 | 0.01 | 0.05 | 0.10 | 0.08 | 78.0 |
| | | NPRO | 2.11 | 2.48 | 1.64 | 2.24 | 1.24 | 1.71 | 1.90 | 0.42 | 21.8 |
| | | iso-NNAC | 0.09 | 0.09 | 0.06 | 0.04 | 0.06 | 0.08 | 0.07 | 0.02 | 26.1 |
| | | Total NAAs‖ | 17.15 | 12.64 | 10.51 | 14.79 | 12.50 | 13.09 | 13.45 | 2.07 | 15.4 |

*By percent market share (*28*).

†Each sample was extracted two times, and each extract was analyzed twice by gas chromatography with a thermal energy analyzer. Values reported are the means of 2 × 2 determinations.

‡NSAR = *N*-nitrososarcosine; MNPA = 3-(methylnitrosamino)propionic acid; MNBA = 4-(methylnitrosamino)butyric acid; NPRO = *N*-nitrosoproline; iso-NNAC = 4-(methylnitrosamino)-4-(3-pyridyl)butyric acid.

§NAA values in columns are expressed as micrograms per gram dry weight. N/A = not analyzed; ND = not detected.

‖Includes also *N*-nitrosothiazolidine-4-carboxylic acid, *N*-nitroso-2-methylthiazolidine-4-carboxylic acid, and 2-(methylnitrosamino)-3-phenylpropionic acid.

¶39% includes Skoal original and various Skoal long cuts.

about the same levels of $N^7$- and $O^6$-methylguanine adducts were obtained in both mouse lung and liver. Considering that the levels of MNPA in Hawken and Skoal Bandits (Straight) moist snuff brands exceed those of NNK by about 24- and 12-fold, respectively, it is likely that this NAA contributes to the overall carcinogenic potential of these two brands.

This study has also shown that the fine cut snuff (Skoal) is significantly richer in TSNAs and NAAs than the long cut (*see* Table 2). This is not surprising, since one can assume that chemical reactions, including *N*-nitrosation of amines, occur more extensively during fermentation and aging of the fine cut because of the greater surface area. In support of this concept, compared with long cut, fine cut had higher levels not only of TSNAs or NAAs but also of both types of *N*-nitrosamines.

According to the National Research Council (*48*), the estimated daily exposure of U.S. residents to carcinogenic nitrosamines is less than 1 μg from food sources, alcoholic beverages, and cosmetic products; a smoker of 20 cigarettes per day inhales in addition 16.2 μg of carcinogenic nitrosamines. Our findings indicate that a snuff dipper who consumes an average 10 g of snuff per day is exposed to 24-46 μg of the strong carcinogenic TSNAs and to 11-50 μg of the weakly carcinogenic NAAs.

It has been reported that, during the past 15 years, the concentrations of the highly carcinogenic NNN and NNK in the two leading U.S. snuff brands have been reduced by more than 70% (*49*); nevertheless, further reductions are feasible. The consumer has choices among moist snuff brands that have different concentrations of nicotine and different concentrations of the carcinogenic TSNAs. It needs to be emphasized, however, that abstinence from snuff is the only way to prevent cases of cancer attributable to use of this form of smokeless tobacco and that snuff use is not a safe alternative to tobacco smoking.

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