variety of individual types of cancer (including laryngeal cancer) with the history of such use in persons with the remaining cancers thought not to be related to tobacco use *(25)*. Prior experience with smokeless tobacco was divided into two levels of exposure. The estimates of the relative risks were controlled for age, race, and smoking. Relative risks of laryngeal cancer in men of 2.0 and 1.7 were found among individuals with low and high levels, respectively, of exposure to chewing tobacco or snuff. These estimates were not significantly different from 1.0. They are based on 106 cases, 11 with relatively low exposure and 5 with higher exposure, and 2,102 controls of which 98 had low exposure and 71 had high exposure. Only 13 female laryngeal cases were available for analysis in this study, which was insufficient to provide any meaningful results.

A case-control study by Wynder and Stellman included 387 male cases of laryngeal cancer and 2,560 hospital controls *(13)*. The percentages that had previously used chewing tobacco and snuff were 11.9 and 3.9, respectively, for the cases, and 9.0 and 2.7, respectively, for the controls. Based on these findings, crude relative risks of 1.4 for chewing tobacco and 1.5 for snuff were obtained. Neither estimate differs significantly from 1.0. No control for smoking or alcohol was done, although the authors state that cigarette smoking in users and nonusers of chewing tobacco was similar.

Interviews with 560 laryngeal cancer patients and 2,000 controls from the general population of Bombay revealed significantly increased risks, compared to nonchewers, among chewers of betel without tobacco (relative risk 2.5) than with tobacco (relative risk 2.6) *(21)*. Laryngeal cancer was noted to comprise an unusually high proportion of all cancer diagnoses in a hospital series in eastern India where pan chewing is common, but no assessment of the role of tobacco was made *(26)*.

Stomach Cancer

Zacho et al. noted that, in Denmark, both gastric cancer and use of chewing tobacco and snuff are directly related to age, more common in men than women, more prevalent in rural than urban areas, and inversely related to socioeconomic status *(27)*. On the basis of these observations, they hypothesized that use of smokeless tobacco increases the risk of stomach cancer. Obviously, other differences among individuals within Denmark could also explain these findings.

Weinberg et al. conducted a case-control study of stomach cancer in a coal mining region of Pennsylvania *(28)*. Cases who had died of stomach cancer from 1978 through 1980 were compared with three control groups: persons who died of other cancers of the digestive system, persons who died of arterial sclerotic heart disease, and persons who lived in the same neighborhood as the case. All controls were matched to individual cases on age, sex, race, and location of residence. Data on the use

of various forms of tobacco were obtained by interviewing next-of-kin or (for neighborhood controls) the subjects themselves. About 16 percent of all men in the study had used chewing tobacco. This percentage did not differ significantly among the cases and the three control groups. No women in this study had chewed tobacco. This study provides some evidence to suggest that chewing tobacco does not increase the risk of gastric cancer, although a small increase in risk could have been missed due to lack of statistical power.

The case-control analysis of the interview data from the TNCS found a relative risk of stomach cancer of 1.7 in men in the highest level of use of chewing tobacco and snuff, no increase in men in the lower use category, and no increase in women *(25)*. These results are based on 120 male cases, 12 of which were users, and 82 female cases, 2 of which were users. The power of this analysis to detect a true increase in risk is obviously low. The relative risk of 1.7 was not significantly greater than 1.0. In an abstract describing a cohort mortality study of U.S. veterans, the standardized mortality ratio for stomach cancer among nonsmoking users of smokeless tobacco was 151, but no study details were provided *(16)*.

Urinary Tract Cancer

Constituents of smokeless tobacco can enter the blood stream, and some are excreted in the urine. The kidney and bladder are thus potentially exposed to these agents but presumably in lower concentrations than are tissues of the upper aerodigestive tract. In a hospital-based case-control study in Seattle, Washington, patients who chewed tobacco were reported to be at nearly a fivefold increased risk of renal cancer compared to nontobacco users *(29)*. Only 6 percent of the 88 male cases were chewers. No association between the use of smokeless tobacco products and either renal cell or renal pelvis cancer was reported in a case-control study of these tumors in England *(30)*. Among 106 renal cell cancer case-control pairs in this study, 10 cases versus 11 controls had at some time used smokeless tobacco. Among 33 renal pelvis cancer-control pairs, 2 cases and 3 controls reported ever using smokeless tobacco products. In a large population-based study in Minnesota involving 495 cases and 697 controls, a nonsignificantly increased relative risk of renal cell cancer of 1.7 (95-percent confidence interval 0.5-6.0) was found among snuff users after adjusting for smoking *(31)*. There was a deficit in risk, however, associated with ever using chewing tobacco (relative risk 0.4, 95-percent confidence interval 0.1-2.6).

A review of eight epidemiologic investigations revealed no consistent evidence that the risk of bladder cancer is altered in users of smokeless tobacco products (table 2) *(13,25,32-39)*. The National Bladder Cancer Study is the largest of the investigations of bladder cancer considered in this review *(37)*. Cases for this study were selected through 10 population-

TABLE 2.—Estimates of Relative Risks of Bladder Cancer in Persons Who Have Ever Used Chewing Tobacco and Snuff

First Author (ref.)	Years Case Diagnosed	Sex	Relative Risks		
			Chewing Tobacco	Both	Snuff
Wynder *(32)*	1957-63	Male	1.4*		0.7*
Dunham et al. *(33)*	1958-64	Male	5.3*†	0.9*†	—
		Female	1.1*†	—	0.3*†
Cole et al. *(34)*	1966-68	Both	1.1*		1.0*
Williams and Horm *(25)*	1969-71	Male-level 1		1.61	
		level 2		1.15	
		Female-level 1		0	
		level 2		1.78	
Wynder and Stellman *(13)*	1974-75	Males	0.9		0.7
Howe et al. *(36)*	1974-76	Males	0.9		
Hartge et al. *(37)*	1977-78	Males	1.02		0.77†

* Estimated from published report.
† Based on analysis of nonsmokers only.

based cancer registries in the United States. Controls were a random sample of the same population from which the cases came. Information was obtained from interviews of 2,982 cases and 5,782 controls. Analyses of smokeless tobacco use were restricted to the 340 cases and 1,227 controls who claimed never to have smoked cigarettes. Of these, 11 percent of the cases and 10 percent of the controls had ever used chewing tobacco, and 3 percent of the cases and 4 percent of the controls had ever used snuff. The relative risks of bladder cancer in users of chewing tobacco and snuff were estimated to be 1.0 (0.7-1.5) and 0.8 (0.4-1.6), respectively.

Wynder et al. conducted a hospital-based study of 300 male bladder cancer cases *(32)*. Eleven percent of the 300 cases and 8 percent of the 300 hospital controls had ever used chewing tobacco; 2 percent of the cases and 3 percent of the controls had used snuff. The percentage of users was not significantly different in cases and controls, and no attempt was made to analyze the data further.

Dunham et al. interviewed 493 bladder cancer patients and 527 hospitalized controls in New Orleans *(33)*. Among nonsmokers, there was an increased relative risk associated with chewing tobacco use among males but a deficit in risk associated with snuff use among females, but the numbers of cases involved were small (four males and three females).

Cole et al. interviewed 470 cases from the Boston area and 500 population-based controls *(34)*. Forty-six of the cases had used chewing

tobacco and three had used snuff. Based on the prior experience with smokeless tobacco in the controls (controlling for age and sex), 42.3 and 7.9 cases would have been expected to have used chewing tobacco and snuff, respectively. Some increase in the risk of bladder cancer was found in the TNCS survey, but none of the risks from this study are significantly different from 1.0 (table 1) *(25)*. In addition, no evidence of a dose response is seen.

In a second hospital-based case-control study *(13)* of similar design to the first *(32)*, Wynder and Stellman found that 8 percent and 1.9 percent of 586 cases had used chewing tobacco and snuff, respectively, compared to 9 percent and 2.7 percent of 2,560 controls who had used these two products. When analyses were restricted to nonsmokers in a continuation of this study, a significant excess risk of bladder cancer was associated with snuff use among women, but only 3 of 76 cases were users *(35)*.

A population-based case-control study was conducted in three Canadian provinces by Howe et al. *(36)*. Controls were matched to individual cases on neighborhood, age, and sex. The ratio of male pairs discordant for the use of chewing tobacco was 29/34, giving a relative risk of 0.9 (95-percent confidence interval, 0.5-1.6). This estimate was not altered by controlling for smoking. No female cases or controls gave a prior history of use of smokeless tobacco.

In Denmark, 165 male and 47 female patients with cancer of the urinary bladder from a hospital serving a specific geographic area were interviewed, as were geographically-matched controls *(38,39)*. The estimated relative risk associated with tobacco chewing was 2.0 (1.2-3.4) based on 39 exposed cases. In a logistic model containing variables for tobacco chewing, smoking, and other major correlates of bladder cancer, the relative risk associated with chewing was 1.7 and statistically significantly higher than 1.0. The authors estimated that tobacco chewing might account for 9 percent of the bladder cancer diagnoses in the area.

Although two studies did report elevated relative risks associated with smokeless tobacco use, on balance these studies provide little evidence to suggest that smokeless tobacco alters the risk of bladder cancer. It is possible that a small increase in risk has not been detected by the studies not reporting increases due to lack of statistical power.

Other Cancers

All other organs of the body are likely exposed to even lower concentrations of products of smokeless tobacco via the blood.

In a large prospective study in Norway, 16,713 individuals were interviewed to obtain information on the use of tobacco and alcohol and were followed up for development of pancreatic cancer *(40)*. Sixty-three persons in the cohort developed this neoplasm during a 10-year followup.

After controlling for cigarette smoking and alcohol consumption, a relative risk of 2.9 was observed in regular users of chewing tobacco or snuff (compared to nonusers). The 95-percent confidence limits of this value include 1.0. Risk was greater in regular users than former or occasional current users, and a trend of increasing risk with amount used was of borderline statistical significance (P=.06). The case-control analysis of the interview data from the TNCS *(24)* with respect to pancreas cancer is based on only 91 male cases (3 exposed to smokeless tobacco) and 85 female cases (none exposed); and although no increase in relative risk of pancreatic cancer in relation to smokeless tobacco was observed, the power of this study to detect such an increase is low.

Other cancer sites were found to be related to the use of smokeless tobacco in the case-control analysis of the interview data from the TNCS *(24)*. Relative risks for colon cancer at low and high levels of exposure were found to be 0.9 and 1.5 for men and 0.4 and 2.0 for women, respectively. Relative risks of cervical cancer in users of these two levels of exposure were 3.1 and 2.3. No studies have been conducted to confirm or refute these findings. In view of the large numbers of possible associations investigated, these results should be considered of value only in generating hypotheses for further investigation.

Summary

The epidemiologic studies showing an association between the use of snuff and oral cancers indicate that topical exposure of tissues to smokeless tobacco can cause cancers at the site of the exposure. Case reports of neoplasms developing in the ear and nose of individuals who used snuff at these sites raise the possibility that direct exposure may increase the risk in locations besides the oral cavity. Other tissues that come in contact with constituents of smokeless tobacco in more dilute concentrations include the linings of the esophagus, larynx (supraglotic portion), and stomach. Results of studies of cancers of these three sites in relation to smokeless tobacco are inconclusive; many are of limited power to detect small increases in risk and did not control for relevant, potentially confounding variables. However, some studies of these three cancers do show an increase in risk in relation to the use of smokeless tobacco. Constituents of smokeless tobacco can enter the bloodstream, and some are excreted in the urine. The kidney and bladder are thus potentially exposed to these products and their metabolites but presumably in lower concentrations than are tissues of the upper aerodigestive tract. Evidence suggests that the risk of bladder cancer is not altered to any large extent in users of smokeless tobacco products, but results from studies of kidney cancer are inconsistent. Information regarding the risks of other cancers in relation to smokeless tobacco use is sparse.

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CHEMICAL CONSTITUENTS, INCLUDING CARCINOGENS, OF SMOKELESS TOBACCO

Chemical Composition of Smokeless Tobacco

To date, at least 2,500 known compounds have been identified in processed tobacco *(1).* Besides polysaccharides and protein, tobacco contains *Nicotiana* alkaloids (0.5-5.0 percent), alkanes (0.1-0.4 percent), terpenes (0.1-3.0 percent), polyphenols (0.5-4.5 percent), phytosterols (0.1-2.5 percent), carboxylic acids (0.1-0.7 percent), aromatic hydrocarbons, aldehydes, ketones, amines, amides, nitriles, N- and O-heterocyclic compounds, chlorinated organic compounds, alkali nitrates (0.2-5.0 percent), and at least 30 metal compounds *(2,3).*

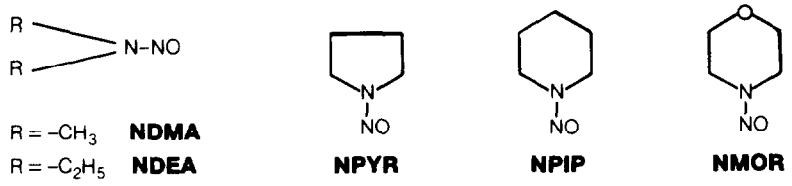
The most important habituating agent in tobacco is nicotine, the major representative of the alkaloids that constitute 0.5-5 percent of the leaf depending on the strain, variety, and agricultural practices that are employed during the tobacco cultivation. In total, the alkaloids are composed of 85 to 95 percent nicotine *(4)* and of other major alkaloids such as the secondary amines nornicotine, anatabine, and anabasine with lesser amounts of cotinine, myosmine, nicotyrine, 2,3'-dipyridyl, and N'-oxynicotine *(5).*

Carcinogens in Smokeless Tobacco

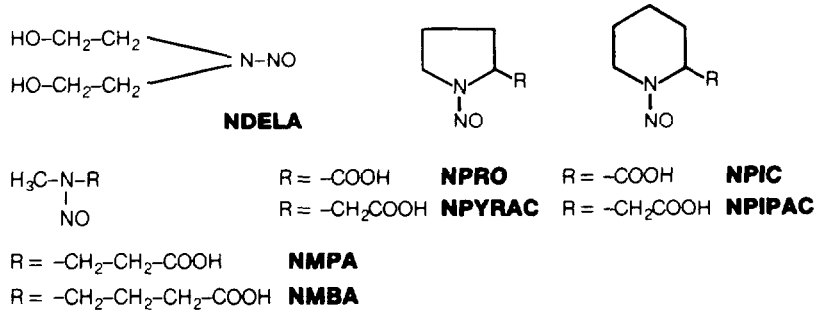
At present, three classes of carcinogens are known to occur in smokeless tobacco products: N-nitrosamines, polynuclear aromatic hydrocarbons (PAH), and polonium-210 (^{210}Po). Although chemical-analytical

FIGURE 1.—N-Nitrosamines in Smokeless Tobacco

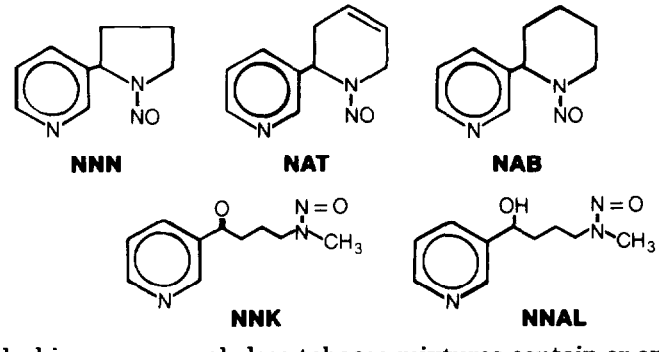
1. **Volatile Nitrosamines**



2. **Nonvolatile Nitrosamines**



3. **Tobacco-Specific Nitrosamines**

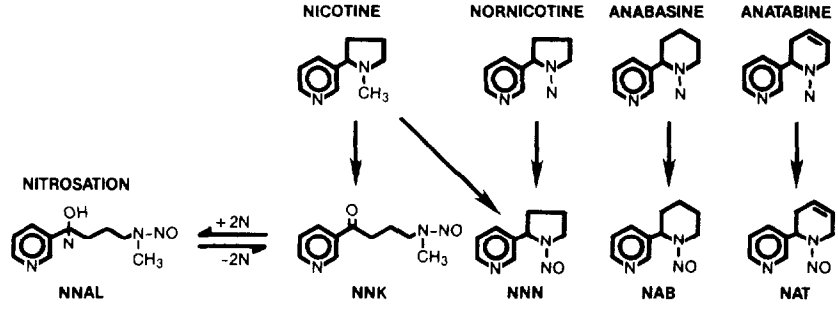


data are lacking, some smokeless tobacco mixtures contain or are suspected to contain traces of cadmium and nickel compounds *(6)*, formaldehyde, and coumarin, all of which are known animal carcinogens *(7,8)*.

N-Nitrosamines

Tobacco leaves contain an abundance of amines in the form of proteins and alkaloids. Tobacco also contains up to 5 percent nitrates and traces of nitrite. Thus there is the potential for the formation of N-nitrosamines from the nitrate, nitrite, and amines during the processing of smokeless tobacco products. In tobacco, we distinguish between volatile nitrosamines, nonvolatile nitrosamines, and tobacco-specific nitrosamines (figure 1). With the exception of some N-nitrosamino

FIGURE 2.—Formation of Tobacco-Specific Nitrosamines



acids, the nitrosamines in tobacco are animal carcinogens that are formed after harvesting of the tobacco during curing, fermentation, and/or aging. The N-nitrosamino acid, N-nitrosoproline, occurs in processed food and can also be formed in humans by endogenous nitrosation of proline. This nitrosamino acid is not carcinogenic on the basis of presently available data *(9-12)*. Table 1 summarizes the available data for the volatile nitrosamines in smokeless tobacco. Only one of the volatile nitrosamines, NDMA, has been found in U.S. looseleaf tobacco, but four nitrosamines have been found in American snuff. N-Nitrosomorpholine is formed during tobacco processing or aging from morpholine, a cyclic amine that is not known to occur in uncontaminated tobacco *(13,14)* but originates from packing materials and/or flavor additives. Table 2 lists the presently known nonvolatile nitrosamines in smokeless tobacco. N-Nitrosodiethanolamine (NDELA) in U.S. tobacco originates primarily from residues on tobacco leaves of the sucker-growth inhibitor maleic hydrazidediethanolamine (MH-30). Use of this formulation of the agricultural spray was banned in the United States in 1981, and the concentration of NDELA in smokeless tobaccos has markedly decreased since then *(14,15)*.

Figure 2 presents the formation of the tobacco-specific N-nitrosamines (TSNA) from the alkaloids. There is progressive nitrosation of the alkaloids during curing and processing and even during the shelf life of the commercial products *(16)*. Table 3 summarizes the presently available quantitative data for four out of five TSNA's that are present in smokeless tobacco. The nitrosamines are detectable in snuff and tobacco products from various parts of the world. Analyses of Swedish snuff brands manufactured between 1980 and 1985 have revealed a significant decrease of the levels of TSNA; such a trend has not been observed for U.S. snuff brands *(14,16,17)*. It has been suggested that the lowering of TSNA levels in Swedish snuff brands is due to better control of the bacterial content of the tobacco products. Reduced bacterial activity will probably reduce nitrite levels and, consequently, inhibit nitrosamine

TABLE 1.—Volatile Nitrosamines in Smokeless Tobacco (ppb)*

Product	NDMA	NPYR	NPIP	NMOR	Reference
U.S.					
Looseleaf†	ND - 380 (4)	ND - 1.2 (4)	ND (4)	ND - 2.5 (4)	*13,14,17,34*
Snuff	ND - 215 (26)	ND - 291 (16)	ND - 107 (16)	ND - 690 (26)	*13,14,17,20, 29,34-37*
Sweden					
Chewing Tobacco	ND - 0.6 (4)	0.9 - 3.7 (4)	ND (2)	ND - 0.8 (2)	*17,36*
Snuff	ND - 60 (53)	ND - 210 (27)	ND - 0.5 (37)	ND - 1.2 (53)	*14,17,36*
Canada					
Snuff	23 - 72.8 (2)	321 - 337 (2)			*14*
Denmark					
Chewing Tobacco	ND - 8.6 (6)	7.0 - 25.5 (6)	ND (4)	ND - 32.8 (6)	*17,36*
Norway					
Chewing Tobacco	37 - 220 (2)	84.0 - 280 (2)	2.8 - 15 (2)	28 - 37 (2)	*17*
India					
Chewing Tobacco	ND - 0.56 (4)	1.55 - 4.48 (4)		ND (4)	*14*
U.S.S.R.					
Nass‡	ND (4)	1.74 - 8.82 (4)		ND (4)	*14*

* Number in parentheses, number of samples analyzed.

† One sample also contained 8.6 ppb NDEA.

‡ Also contained ND - 69.6 NDEA *(14)*.

TABLE 2.—Nonvolatile Nitrosamines in Smokeless Tobacco (ppb)*

Tobacco Product	NDELA	NMPA	NMBA	NPRO	NPYRAC	NPIC	NPIPAC	Reference
U.S.								
Looseleaf	224 - 680 (3)			450 - 463 (2)				*13,14,34*
Snuff	160 - 6,800 (13)	1,250 - 7,420 (5)	120 - 2,240 (5)	500 - 50,900 (13)	ND - 2,000 (5)	ND - 6,100 (5)	ND - 1,500	*13-15, 34, 38,39*
Sweden								
Snuff	230 - 390 (8)	510 - 4,400 (12)	ND - 260 (12)	890 - 29,500 (12)	100 - 300 (5)	ND - 5,560 (12)	100 - 200 (5)	*14,15,38,40*
Canada								
Plug Tobacco	110 (1)			100 (1)				*14*
Snuff	1,180 - 2,720 (3)			8,800 - 16,600 (2)				*14*
Germany								
Plug Tobacco	50 (2)			500 - 700 (2)				*14*
Belgium								
Chewing Tobacco		1,600 (1)	100 (1)	3,300 (1)	200 (1)	100 (1)	200 (1)	*40*
U.S.S.R.								
Nass	40 (4)			ND - 180 (4)				*14*
India								
Chewing Tobacco	30 - 110 (4)			190 - 410 (4)				*14*

* Number in parentheses, number of samples analyzed.

TABLE 3.—Tobacco-Specific N-Nitrosamines in Smokeless Tobacco (ppb)*

Product	NNN	NNK	NAT	NAB	Reference
U.S.					
Looseleaf	620-8,200 (9)	ND-380 (4)	130-2,300 (5)	ND-140 (5)	*14,17,41,42*
Plug Tobacco	3,400-4,300 (3)				*43*
Snuff	1,600-135,000 (21)	100-13,600 (21)	1,560-338,000 (21)	10-6,700 (12)	*6,14,16,17,38,42,43*
Sweden					
Snuff	3,050-154,000 (34)	510-2,950 (34)	1,600-21,400 (34)	110-150 (19)	*14,16,17,38*
Plug Tobacco	350-2,090 (3)	ND-240 (3)	690-1,580 (3)	ND-100 (3)	*14,17*
Canada					
Snuff	50,420-79,100 (2)	3,200-5,800 (2)	152,000-170,000 (2)	4,000-4,800 (2)	*14*
Norway					
Snuff	13,000-29,000 (2)	2,700-3,900 (2)	9,100-16,000 (2)	1,000-2,400 (2)	*17*
Denmark					
Snuff	4,460-8,000 (3)	1,350-7,030 (3)	2,680-6,170 (3)		*16*
Chewing Tobacco	210-1,400 (4)	ND-210 (4)	300-2,800 (4)	ND-60 (4)	*17*
Germany					
Plug Tobacco	1,420-2,130 (2)	30-40 (2)	330-500 (2)	30-50 (2)	*14*
Snuff	6,080-6,700 (2)	1,500-1,540 (2)	3,920-4,370 (2)		*16*
U.S.S.R.					
Nass	120-520 (4)	20-130 (4)	32-300 (4)	8-30 (4)	*14*
India					
Chewing Tobacco	470-2,400 (5)	130-230 (4)	300-450 (4)	30-70 (4)	*14,41*
Belgium					
Chewing Tobacco	7,380 (1)	970 (1)	130 (1)		*38*

* Number in parentheses, number of samples analyzed.

TABLE 4.—Estimated Exposure of U.S. Residents to Nitrosamines*

Source 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 Whiskey	NDMA	Ingestion	0.03	
Cigarette Smoking	VNA†	Inhalation	0.3	
	NDELA	Inhalation	0.5	
	NNN	Inhalation	6.1	
	NNK	Inhalation	2.9	16.2
	NAT+NAB	Inhalation	7.2	
Snuff Dipping‡	VNA	Ingestion	3.1	
	NDELA	Ingestion	6.6	
	NNN	Ingestion	75.0	
	NNK	Ingestion	16.1	164.5
	NAT+NAB	Ingestion	73.4	

* From the National Research Council *(18)*, amended by data for snuff dipping *(13)*. In addition, it has been established that upon inhalation of the air in cars with new leather upholstery daily exposure amounts to 0.50 µg of NDMA and 0.20 µg of NDEA *(18)*.

† VNA, NDMA + NEMA + NDEA + NPYR *(37)*.

‡ Brunnemann et al. *(13)*; average values from the leading five U.S. fine-cut tobaccos used for snuff dipping in 1981; assumed daily consumption 10 g/day of snuff; VNA = NDMA + NPYR + NMOR.

formation *(17)*. NNK and NNN are powerful carcinogens in mice, rats, and hamsters, NAB is moderately carcinogenic, and NAT is inactive in rats in doses up to 9 mmol/kg (table 3, page 82) *(3)*.

The daily exposure of an "average" snuff dipper to carcinogenic N-nitrosamines exceeds by at least two orders of magnitude the estimated exposure of U.S. residents to nitrosamines in products other than tobacco products (table 4) *(18,19)*. Furthermore, the concentrations of carcinogenic nitrosamines in snuff exceed very significantly the permissible limits for individual nitrosamines in consumer products (table 5).

During snuff dipping or chewing of tobacco, the TSNA's are extracted by the saliva. Consequently, the saliva of snuff dippers is reported to contain 5.0-420 ppb of NNN, up to 96 ppb of NNK, and 6.6-555 ppb of NAT *(16)*. The saliva analyses of Indian tobacco chewers showed the presence of 1.2-220 ppb of NNN, 3.2-51.7 ppb of NAT, and up to 2.3 ppb of NNK *(20,21)*. Recently, three additional TSNA's have been isolated from U.S. commercial snuff: 4-(methylnitrosamino)-1-(3-pyridyl)butanol-1 (NNAL), 4-(methylnitrosamino)-1-(3-pyridyl) butene-1 (NNO), and 4-(methylnitrosamino)-4-(3-pyridyl)butanol-1 (Red NNA) (figure 3) *(22)*. Additional amounts of TSNA's are most likely also formed by nitrosation processes that occur in the oral cavity during chewing *(19-21,23)*.

TABLE 5.—Permissible Limits for Individual N-Nitrosamines in Consumer Products

Product	Permissible Limit ppb (µg/kg)	Agency
Bacon (Meat)	5	USDA*
Beer	5	FDA†
Rubber Nipples of Baby Bottles	10	FDA‡
Range of Individual Nitrosamines Present in Snuff Tobaccos ppb (µg/kg)		
NNN	5,800 - 64,000	Range in the leading 5 U.S. brands (1984-85)
NNK	100 - 3,100	
NAT	3,300 - 215,000	
NAB	200 - 6,700	
NDELA	160 - 6,800	Range in 13 U.S. brands (1980-1985)

* No "confirmable levels of nitrosamines" *(44)*.

† Regulation set for N-nitrosodimethylamine *(45)*.

‡ Regulation set for any individual volatile N-nitrosamine *(46)*.

Polynuclear Aromatic Hydrocarbons

A number of naphthalenes have been identified in processed tobacco and especially in Latakia, which is flavor enriched by treatment with wood smoke *(24,25)*. While smoking tobaccos were found to contain 300-5,000 ppb of phenanthrene, 110-4,200 ppb of anthracene, 76-1,800 ppb of pyrene, 15-14,000 ppb of fluoranthene, and 8.5 ppb of benzo(a)pyrene (BaP) *(26,27)*, analyses of British snuff in 1957 showed levels of 260 ppb of pyrene, 335 ppb of fluoranthene, and 72 ppb of BaP *(28)*. In the five most popular snuff brands in the United States that were analyzed in 1985, BaP ranged from < 0.1 to 63 ppb *(29)*.

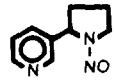
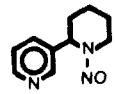
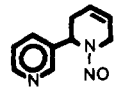
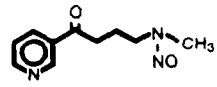
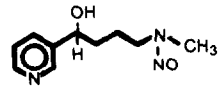
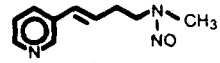
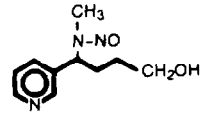
Polonium-210

This alpha-emitting element has long been incriminated as a human carcinogen *(30)*. The levels of ^{210}Po in dozens of U.S. and foreign cigarette tobaccos were between 0.1 and 1.0 pCi/g *(31)*. In recent samples of the five leading U.S. snuff brands, ^{210}Po ranged from 0.16 to 1.22 pCi/g *(29)*. It appears that ^{210}Po in tobacco leaves stems partially from certain types of fertilizers and airborne particles that are taken up by the trichomes (glandular hair) of the tobacco leaf *(31-33)*.

Summary

In processed tobacco, more than 2,550 chemical compounds have been identified. Among these are traces of known carcinogens such as

FIGURE 3.—Tobacco Specific N-Nitrosamines in Snuff U.S. Brands, 1985

Nitrosamines	Relative Carcinogenicity in Rats*	Concentration in Snuff (µg/g) (Dry Weight)	
		A	B
NNN 	+++	3.3	64
NAB 	+	1.1	6.7
NAT 	±	44	215
NNK 	+++	1.8	3.1
NNAL† 	+	0.3	0.14
NNO† 	?	trace‡	trace‡
Red NNA 	?	1.3	1.8

* +++ Tumors with 1 mmol/kg; + tumors with 9 mmol/kg; (for type of tumors induced see table 4, page 38); ± insignificant number of tumors with 9 mmol/kg; ? not tested.

† Isolated amounts only.

‡ < 0.01 µg/g.

PAH, ^{210}Po, and N-nitrosamines. The most prevalent organic carcinogens are the tobacco-specific N-nitrosamines that are formed from the *Nicotiana* alkaloids during the processing of tobacco leaves. Their concentrations in snuff exceed the levels of nitrosamines in other consumer products by over one hundredfold. During snuff dipping or chewing of tobacco, the nitrosation process continues within the mouth stimulated by oral bacteria.

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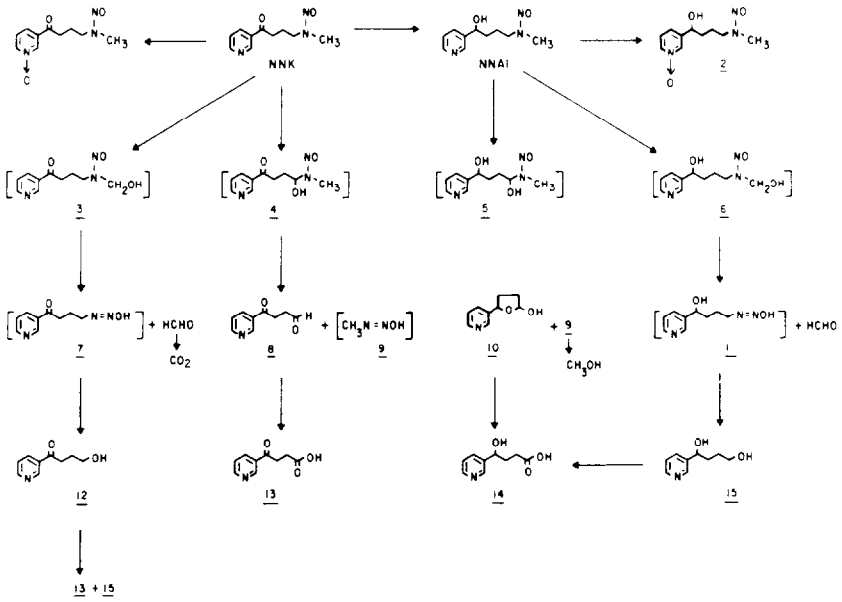
Abbreviations

BaP	Benzo(a)pyrene
NAB	N'-Nitrosoanabasine
NAT	N'-Nitrosoanatabine
ND	Not detected
NDEA	Nitrosodiethylamine
NDELA	Nitrosodiethanolamine
NDMA	Nitrosodimethylamine
NMBA	Nitrosomethylbutyric acid
NMOR	Nitrosomorpholine
NMPA	Nitrosomethylpropionic acid
NNAL	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	N'-Nitrosonornicotine
NNO	4-(Methylnitrosamino)-1-(3-pyridyl)butene-1
NPIC	Nitrosopipecolic acid
NPIP	Nitrosopiperidine
NPIPAC	Nitrosopiperidine-acetic acid
NPRO	Nitrosoproline
NPYR	Nitrosopyrrolidine
NPYRAC	Nitrosopyrrolidine-acetic acid
PAH	Polynuclear aromatic hydrocarbons
^{210}Po	Polonium-210
Red NNA	4-(Methylnitrosamino)-4-(3-pyridyl)-1-butanol
TSNA	Tobacco-specific nitrosamines

METABOLISM OF CONSTITUENTS OF SMOKELESS TOBACCO

The tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN) are quantitatively the major known carcinogens that are present in snuff and other types of smokeless tobacco. Molecular changes that are induced in the genetic material of tobacco chewers are most likely to arise from the metabolism of these two nitrosamines. Although present in similar quantities, N'-nitrosoanabasine (NAB) and N'-nitrosoanatabine (NAT) are less carcinogenic than NNK and NNN and are less likely to play an important role in the induction of oral cancer in man. Some snuff products contain considerable amounts of N-nitrosomorpholine (NMOR) and N-nitro-

FIGURE 1.—Metabolic Pathways of NNK

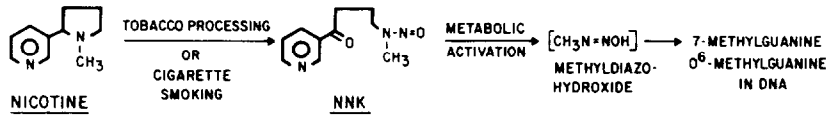


sodiethanolamine (NDELA); the former is a potent carcinogen. The levels of benzo[a]pyrene (BaP) and ^{210}Po in snuff tobacco are low compared to those of the nitrosamines (see previous section). This section will focus on the routes of metabolic activation of the compounds that are most likely to be involved in the induction of tumors that are related to snuff use—NNK, NNN, and NMOR.

Metabolism of NNK

The overall metabolic scheme for NNK, as determined by *in vivo* and *in vitro* studies in F-344 rats, Syrian golden hamsters, and A/J mice, is illustrated in figure 1 *(1-4)*. A key feature of this metabolic scheme is the conversion of NNK to the alpha-hydroxy intermediate *4*, which is unstable and undergoes spontaneous conversion to the keto aldehyde *8* and, most likely, methyl diazohydroxide *9*. The latter is a methylating agent that is well known for its ability to methylate DNA forming 7-methylguanine, 06-methylguanine, 4-methylthymidine, and a spectrum of other products *(5)*. Among these, 06-methylguanine, which is generated from precursors such as N-methylnitrosourea (NMU) or N-nitrosodimethylamine, has been unequivocally shown to be able to induce miscoding during DNA replication, and the resulting point mutation is sufficient to activate proto-oncogenes *(6,7)*. Many studies have demonstrated a correlation between 06-methylguanine persistence in replicating tissues and the initiation of the carcinogenic process, although it is clear in other cases that additional factors are also involved *(8,9)*.

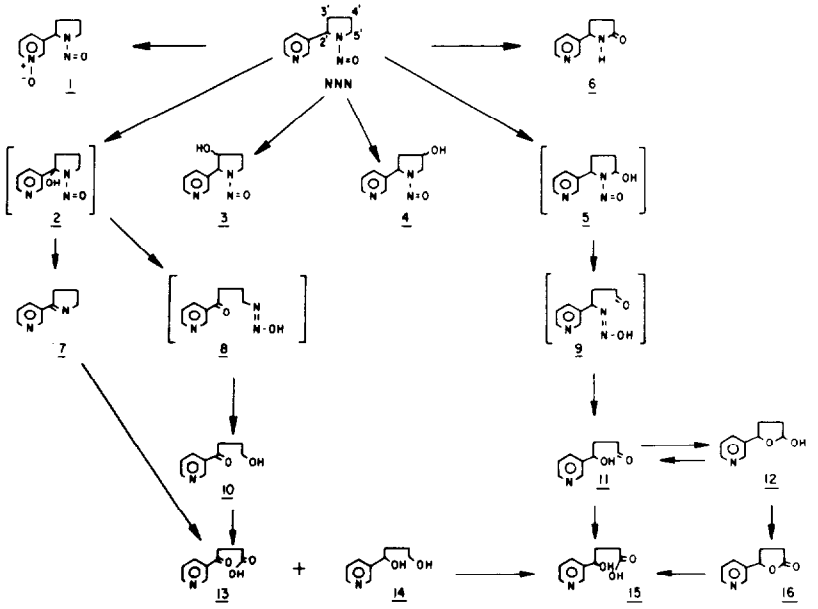
FIGURE 2.—Scheme Linking Nicotine to Formation of the Promutagenic DNA Adduct, O^6-Methylguanine



Recent studies have demonstrated that NNK can methylate target tissue DNA of rats; 7-methylguanine and 05-methylguanine have been detected in the DNA of rat lung, nasal mucosa, and liver but not in the nontarget tissues, kidney, and esophagus *(10-14)*. These studies have also shown that, in the case of NNK, 06-methylguanine formation alone is not sufficient for tumor induction since persistent levels of 06-methylguanine in the lung were less than those observed upon treatment with equivalent quantities of N-nitrosodimethylamine, but the latter did not induce lung tumors *(13)*. It is clear from these, and related studies with NNN, that DNA adducts are also formed via pyridyloxobutylation or related processes. Regardless of the mechanism, it is significant that NNK causes DNA methylation; this creates a mechanistic link between nicotine, the habituating factor in tobacco, and 06-methylguanine formation in DNA, as illustrated in figure 2. Immunoassay methods are currently being developed to detect 06-methylguanine in the exfoliated oral cells of snuff dippers. Its presence can be inferred from the animal studies that are discussed above and by the demonstration that human tissues, including buccal mucosa, can metabolize NNK by alpha-hydroxylation *(15)*. In this respect, it is significant that injection of Syrian golden hamsters with the methylating agent MNU, combined with irritation of the buccal mucosa, resulted in the induction of oral cavity tumors *(16)*.

The pathway of NNK metabolism leading to the alpha-hydroxy intermediate 3 is also considered to be important in NNK carcinogenesis. This pathway gives rise to the electrophilic diazohydroxide 7. The properties of this intermediate have been investigated by using a model compound, 4-(carbethoxynitrosamino)-1-(3-pyridyl)-1-butanone (CNPB). Generation of *7* from CNPB is strictly analogous to the well-known ability of NMU to generate methyl diazohydroxide. Mutagenicity assays in *S. typhimurium* of CNPB have shown that it is more mutagenic than NMU *(17)*. Chemical model studies have demonstrated that it modifies the N2-position of deoxyguanosine *(18)*. This adduct and other adducts that may be formed from the diazohydroxide 7 and related intermediates are likely to play an important role in tumor induction by NNK. Autoradiographic studies have demonstrated that radioactivity from [carbonyl-14C]NNK is firmly bound to target tissues of rats and hamsters *(4,19)* and to tissues of the marmoset monkey *(20)*.

FIGURE 3.—Metabolic Pathways of NNN

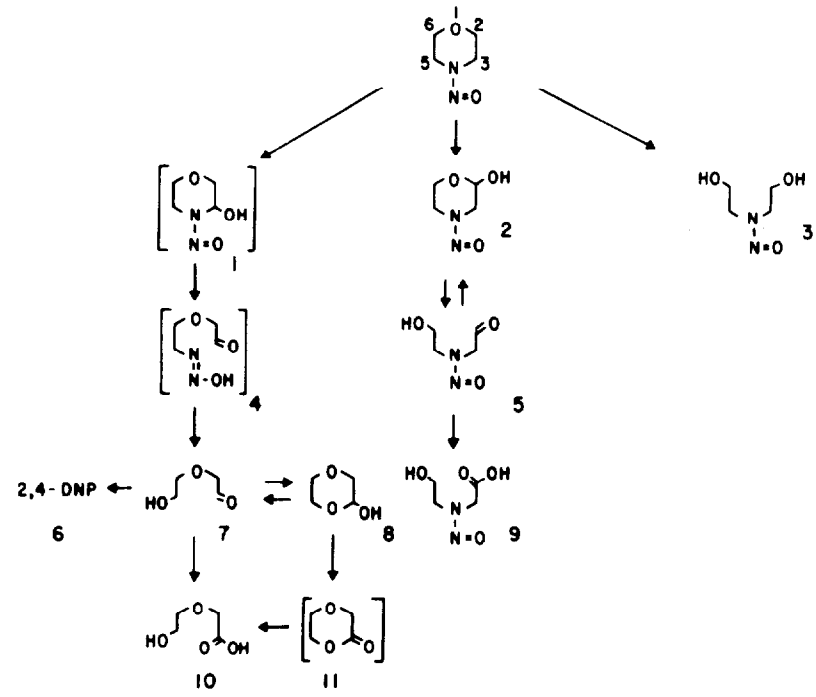


A third key feature of NNK metabolism is its rapid conversion in vivo and in cultured tissues from experimental animals and humans to its reduced form, NNA1, which has similar tumorigenic activity to that of NNK *(1,3,4,15,21)*. NNA1 is slowly metabolized as indicated in figure 1 and also by reconversion to NNK. Like NNK, it methylates DNA *in vitro* and *in vivo*. While the full details of the NNK-NNA1 equilibrium have not yet been elucidated, it is clear that NNA1 can act as a circulating source of NNK metabolites. It may play an important role in tissue-specific carcinogenesis by NNK.

Metabolism of NNN

Metabolic pathways of NNN are illustrated in figure 3. These pathways have been elucidated by *in vivo* and *in vitro* studies in rats, hamsters, and mice *(2,3,22-29)*. The stable metabolite NNN-1-N-oxide *(1)* has tumorigenic activity somewhat less than that of NNN but is still an effective carcinogen in F-344 rats *(30)*. Metabolism of NNN to the 2'- and 5'-hydroxy intermediates *2* and *5* constitutes a major pathway *in vivo* and *in vitro* in experimental animals, human liver microsomes *(31)*, and cultured human tissues, including buccal mucosa *(15)*. Of particular interest is the ability of two NNN target tissues, lingual mucosa and esophageal mucosa, to carry out preferential 2'-hydroxylation of NNN *(27,32)*. The intermediate that is formed by 2'-hydroxylation of NNN is diazohydroxide *8*, which is identical to that formed by methyl hydroxylation of NNN (*7*, figure 1). As described above, this intermediate is

FIGURE 4.—Metabolic Pathways of NMOR



highly mutagenic, and this or related intermediates appear to play an important role in carcinogenesis by both NNN and NNK. The intermediate *9* is significantly less mutagenic than *8* in *S. typhimurium (33)*, and various lines of evidence indicate that it is less important in NNN tumorigenesis than is *8 (33,34)*. Autoradiographic studies have demonstrated that radioactivity from [2'-14C]NNN is bound to tissues of mice, rats, and marmoset monkeys *(20,35-37)*. Immunoassays are currently being developed for the putative DNA adducts that are produced by 2'-hydroxylation of NNN and methyl hydroxylation of NNK; it will be important to assess the levels of these adducts in the exfoliated oral cells of snuff dippers. Their levels may relate to the susceptibility of individuals to the effects of smokeless tobacco. The metabolic pathways that lead to these intermediates can be affected by alcohol consumption and dietary components *(32,38-43)*.

Metabolism of NMOR

The metabolic pathways of NMOR are illustrated in figure 4. These have been elucidated by *in vitro* and *in vivo* studies in rats *(44-47)*. Structure activity studies had shown that 3-hydroxylation of NMOR, leading

to intermediate *4*, was likely to be important in NMOR carcinogenesis *(48)*. This pathway could result in the formation of glyoxal-deoxyguanosine adducts *(49)*; 2-hydroxylation of NMOR also occurs, giving the mutagenic product *2*. The latter also forms glyoxal-deoxyguanosine adducts *(50)*. These adducts, which are likely to have miscoding properties, also should be present in the DNA of snuff dippers since human tissues are capable of metabolizing NMOR *(51)*.

Summary

Persuasive evidence exists that the carcinogenic nitrosamines that are present in high quantities in snuff and other forms of smokeless tobacco are metabolized by target tissues of experimental animals and by human tissues to intermediates that can modify the genetic material of the cell.

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