NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for SMOKELESS TOBACCO

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Carcinogenicity

The oral use of Smokeless Tobacco is *known to be a human carcinogen* based on studies in humans which indicate a causal relationship between exposure to smokeless tobacco and human cancer (reviewed in IARC V. 38, 1985; Gross et al., 1995).

Smokeless tobacco has been determined to cause cancers of the oral cavity. Cancers of the oral cavity have been associated with the use of chewing tobacco as well as snuff which are the two main forms of smokeless tobacco used in the United States. Tumors often arise at the site of placement of the tobacco.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

In 1985 IARC determined there was inadequate evidence to indicate that smokeless tobacco is carcinogenic to experimental animals. Most reported studies had deficiencies in design. Subsequent studies have provided some evidence that snuff or extracts of snuff produce tumors of the oral cavity in rats. Smokeless tobacco products contain a variety of nitrosamines which have been shown to be carcinogenic to animals. The oral use of smokeless tobacco is estimated to be the greatest exogenous source of human exposure to these compounds. Nitrosamines are metabolically hydroxylated to form unstable compounds that bind to DNA. Extracts of smokeless tobacco have been shown to induce mutations in bacteria and mutations and chromosomal aberrations in mammalian cells. The oral cavity tissue cells of smokeless tobacco users have been shown to contain more chromosomal damage than those from nonusers.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 IDENTIFICATION

Chewing tobacco and snuff are the two main forms of smokeless tobacco used in the United States. Chewing tobacco consists of the tobacco leaf with the stem removed and various sweeteners and flavorings such as honey, licorice, and rum. Snuff consists of the entire tobacco leaf, dried and powdered or finely cut, menthol, peppermint oil, camphor, and/or aromatic additives such as attar of roses and oil of cloves (IARC, 1985).

Chewing tobacco and snuff contain known carcinogens such as volatile and nonvolatile nitrosamines, tobacco-specific *N*-nitrosamines (TSNAs), polynuclear aromatic hydrocarbons, and polonium-210 (^{210}Po). These carcinogenic TSNAs are present in twice or more the concentration found in other consumer products (Brunnemann et al., 1986).

TSNAs, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*-nitrosonornicotine (NNN), present in tobacco and tobacco smoke are formed from nicotine and tobacco alkaloids (Hecht and Hoffman, 1988). They are known carcinogens in laboratory animals. The concentrations of NNK and NNN, the most carcinogenic of the TSNAs, are high enough in tobacco and tobacco smoke that their total estimated doses to long-term snuff users and smokers are similar in magnitude to the total doses required to produce cancer in laboratory animals.

Snuff stored at ambient room temperature (37 °C) for 4 weeks has shown a significant increase in TSNA levels. The TSNA levels rose from 6.24 to 18.7 ppm, nitrosamino acid (NAA) rose from 3.13 to 16.3 ppm, and volatile *N*-nitrosamines (VNA) rose from 0.02 to 0.2 ppm.

2.0 HUMAN EXPOSURE

2.1 Use

The use of smokeless tobacco probably dates back 7000 years and is found throughout the world. Snuff also had early beginnings. It was used in many of the European and Asian countries and in many cases the way it was carried, e.g. snuff boxes, was a sign of wealth and rank (IARC, 1985). North America accepted chewing tobacco in favor of snuff around the 1850s because of their distaste for European habits, especially British.

IARC (1985, pp. 37-52; see Appendix A) detailed the use of smokeless tobacco in the United States and other countries. Figure 2-1 (Burns et al., 1997) shows trends in the consumption of smokeless tobacco products in the United States over the past years.

IARC (1985) gives peak year and peak per-capita U.S. annual consumption of chewing tobacco variously in the monograph on pp. 39-40, 44, and 57. To judge from Figure 2-1, a figure attributed by Burns et al. (1997) to an unspecified 1996 U.S. Department of Agriculture (USDA) publication (presumably one of the 1996 quarterly issues of the *Tobacco Situation and Outlook Report*), the peak year appears to be near 1890 (about 4 lb per person), with nearly comparable consumption about 1910. After reclassification of some chewing tobacco products as snuff by the USDA in 1982, male per-capita consumption of chewing tobacco was estimated to be 1.06 lb in 1983 (U.S. Department of Agriculture, 1984b; cited by IARC, 1985, p. 57).

Christen and Glover (1981; cited by IARC, 1985) reported an increase in chewing tobacco among young adult males during the 1960s and 1970s. Chewing tobacco did not carry the stigma of being linked to health issues, could be performed in areas where smoking was prohibited, and was advertised as being more economical than smoking. The tobacco industry has promoted

tobacco chewing as a recreational activity, with spitting contests, shirts, and clubs. This is ironic in view of the fact that smoking replaced chewing when spitting in public was banned as a health hazard after the introduction of the germ theory of infection in the late nineteenth century (IARC, 1985).

Snuff is the only smokeless tobacco product that has had increasing sales in the United States (Djordjevic et al., 1993). In the three leading brands of snuff that account for 92% of the U.S. market, concentrations of nicotine and TSNAs were significantly higher than in the fourth and fifth most popular brands (Hoffman et al., 1995).

Additional listings on smokeless tobacco consumption for selected countries are on pp. 59, 61-62 of the monograph (IARC, 1985).

2.2 Production

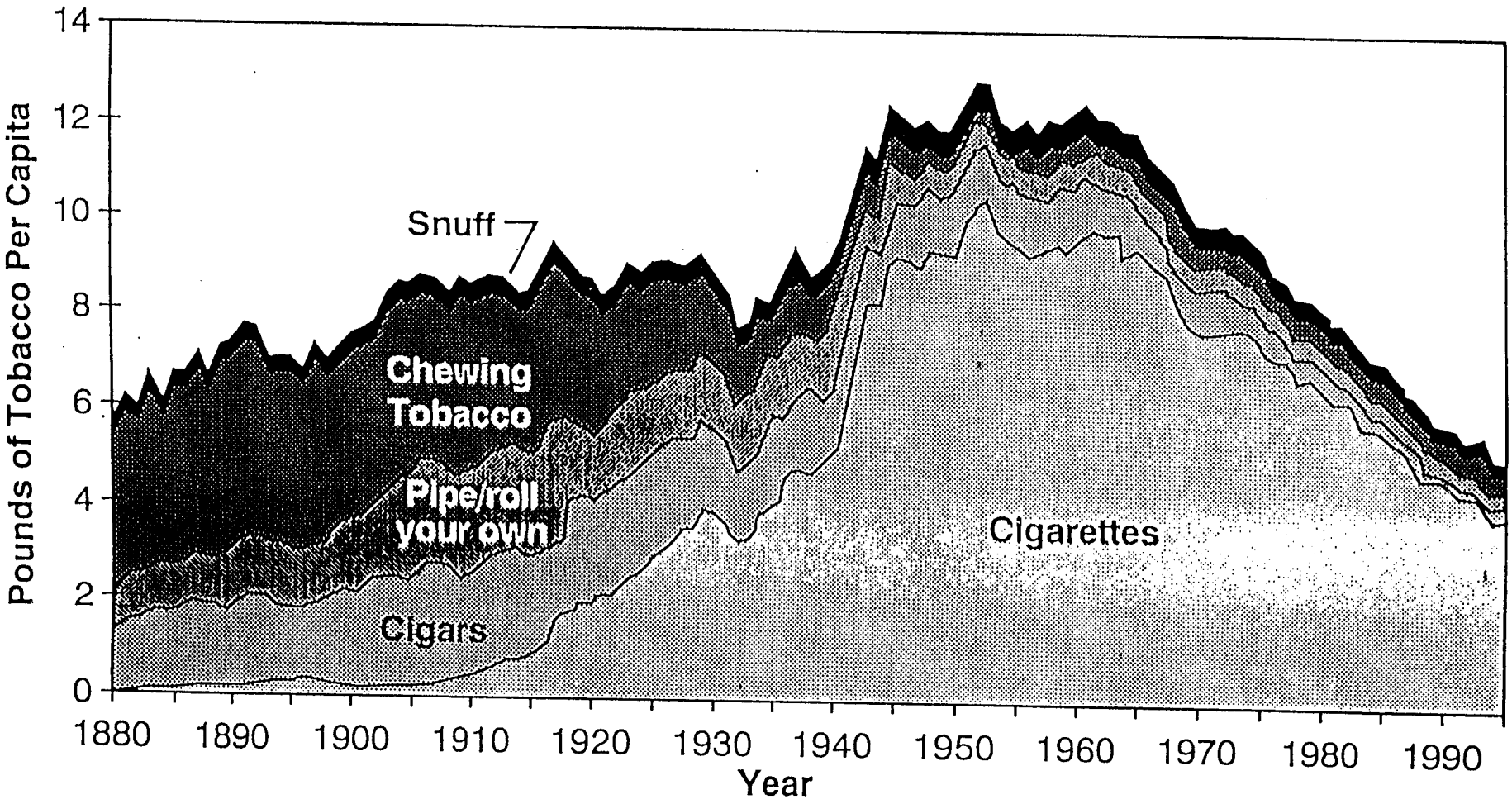
Smokeless tobacco production processes are explained in IARC (1985, pp. 52-55; see Appendix A).

Chewing tobacco production in 1983 was reported to be 39,300 Mg or metric tons (IARC, 1985). This included plug, moist plug, twist/roll, and loose leaf.

Snuff production increased between 1880 and 1930 from four million pounds (1800 Mg) to more than 40 million pounds (18,000 Mg) per year (Garner, 1951; cited by IARC, 1985).

FTC (1997), in its sixth biennial report to Congress mandated by the Comprehensive Smokeless Tobacco Health Education Act of 1986, compiled U.S. sales figures for smokeless tobacco collected from the five largest manufacturers (99% of the market). Annual U.S. sales between 1985 and 1995 fluctuated between 114.4 million lb (51,900 Mg [metric tons]) in 1988 and 121.4 million lb (55,100 Mg) in 1985. The total 116.4 million lb (52,800 Mg) sold in 1995 comprised 54.6 million lb (24,800 Mg) loose leaf/chewing tobacco, 4.2 million lb (1900 Mg) plug/twist chewing tobacco, 4.5 million lb (2000 Mg) Scotch snuff/dry snuff, and 53.1 million lb (24,100 Mg) moist snuff. Moist snuff has shown the strongest increase in sales—nearly 50%—since 1986; it has been advertised the most heavily among the smokeless tobacco products.

Figure 2-1. Per capita consumption of different forms of tobacco in the United States, 1880-1995



Attributed to an unspecified 1996 U.S. Department of Agriculture report by Burns et al. (1997, p. 13).

2.3 Regulations

Applicable regulations are given in detail in the Regulations table. Federal regulations related to tobacco products that concern taxation, customs duties, and the potential for hand-to-mouth transfer of toxic substances when using tobacco in the workplace are not addressed in this section.

The U.S. Food and Drug Administration (FDA) regulates nicotine-containing cigarettes and smokeless tobacco products as nicotine-delivery medical devices under 21 CFR Part 897 "to reduce the number of children and adolescents who use these products and to reduce the life-threatening consequences associated with tobacco use." Measures to reduce the appeal of and access to cigarettes and smokeless tobacco products include numerous restrictions on advertising, including promotional items and event sponsorship. Tobacco-product-dispensing vending machines and self-service displays are prohibited except in adult establishments that do not allow children on the premises at any time. Retailers must request that persons up to the age of 27 present photographic identification bearing their birth date. Free distribution of tobacco products is prohibited. Each package and advertisement must bear the label "Nicotine-Delivery Device for Persons 18 or Older." Cigarettes may not be sold in packages of fewer than 20.

Analyses of FDA jurisdiction over tobacco products (cigarettes and smokeless tobacco products) have been published in the *Federal Register*, including 60 FR 41453-41787, August 11, 1995, with a correction at 60 FR 65349-65350; 61 FR 44615 ff., August 28, 1996; and 61 FR 45219-45222, August 28, 1996. FDA published Children and Tobacco Executive Summaries (U.S. FDA, 1996a,b), which are available free on the Internet and by mail.

The Federal Trade Commission (FTC) of the Department of Commerce administers and enforces the Comprehensive Smokeless Tobacco Health Education Act of 1986, Public Law 99-252 (FTC, 1998). Regulations published in 16 CFR Part 307 include the requirement that one of three warning messages in regular rotation and distribution throughout the United States on packages of smokeless tobacco products and in their advertisements. One of the messages is "WARNING: THIS PRODUCT MAY CAUSE MOUTH CANCER." The requirements are given in detail in the Regulations table.

The Federal Communications Commission (FCC) shares responsibility with FTC for the ban of advertisements of cigarettes and smokeless tobacco on radio and television (FTC, 1998). A CFR citation was not located for 15 U.S.C. Sec. 4402(f), which banned, effective August 1986, advertising for smokeless tobacco products on any electronic communication medium subject to FCC jurisdiction.

The Centers for Disease Control and Prevention's (CDC) Office on Smoking and Health (OSH) is the delegated authority to implement major components of the DHHS's tobacco and health program, which comprises programs of information, education, and research. CDC's authority includes collection of tobacco ingredients information to facilitate HHS's overall goal of reducing death and disability from use of tobacco products (CDC, 1997). Manufacturers, packagers, and importers of smokeless tobacco products are required by the Comprehensive Smokeless Tobacco Health Education Act of 1986 (Public Law 99-252) to report to the Secretary of HHS the ingredients, including nicotine, in smokeless tobacco products. HHS is authorized to undertake research on the health effects of ingredients. CDC has published requests for comments in the *Federal Register* on its proposed data collection in 61 FR 49145-49147,

September 18, 1996, and 62 CFR 24115-24116, May 2, 1997. CDC has also requested comments on an analytical protocol proposed for measuring the quantity of nicotine in smokeless tobacco products (62 FR 24116-24119, May 2, 1997, and 62 FR 29729, June 2, 1997). (These regulations were not final as of January 31, 1999.)

HHS, under 45 CFR Part 96—Subpart L—Substance Abuse Prevention and Treatment Block Grant, requires that to be eligible for Block Grants to support substance abuse prevention and treatment services, each State must have in effect and strictly enforce a law that prohibits sale or distribution of tobacco products to persons under age 18 by manufacturers, distributors, or retailers.

Federal agencies have issued regulations to implement Public Law 104-52, the Prohibition of Cigarette Sales to Minors in Federal Buildings and Lands. Some agencies have not restricted their corresponding regulations to cigarettes. For example, the General Services Administration (41 CFR) and the Treasury Department (31 CFR) prohibit the vending and free distribution of tobacco products on property under their jurisdictions.

Under 32 CFR 85.6, health promotion efforts in each military service should include smoking prevention and cessation programs. Health care providers are encouraged to take the opportunity at routine medical and dental examinations to apprise service personnel of tobacco use risks (including smokeless tobacco) and how to get help to quit.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 801—PART 801—LABELING. SUBPART D—Exemptions From Adequate Directions for Use.	Subchapter H covers 21 CFR parts 800-898 and governs medical devices. Cigarettes and smokeless tobacco as defined in 21 CFR 897 are exempt from section 502(f)(1) of the FFD&C Act.
	21 CFR 801.126—Sec. 801.126 Exemptions for cigarettes and smokeless tobacco. Promulgated: 61 FR 44615, Aug. 28, 1996.	
	21 CFR 803.19—Sec. 803.19 Exemptions, variances, and alternative reporting requirements. Promulgated: 60 FR 63597, Dec. 11, 1995, as amended at 61 FR 44615, Aug. 28, 1996.	Manufacturers of cigarettes and smokeless tobacco products are required to submit medical device reports for serious adverse effects that are not well known or well documented by the scientific community.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 897—PART 897—CIGARETTES AND SMOKELESS TOBACCO. Promulgated: 61 FR 44615, Aug. 28, 1996, unless otherwise noted. U.S. Code: 21 U.S.C. 352, 360, 360h, 360i, 360j, 371, 374, 393.	Part 897 regulates nicotine-containing cigarettes and smokeless tobacco as medical devices.
	21 CFR 897.1—Sec. 897.1 Scope.	Failure to comply with any applicable provision in this part in the sale, distribution, and use of cigarettes and smokeless tobacco renders the product misbranded under the FFD&C Act.
	21 CFR 897.2—Sec. 897.2 Purpose.	Restrictions on the sale, distribution, and use of cigarettes and smokeless tobacco are established "to reduce the number of children and adolescents who use these products and to reduce the life-threatening consequences associated with tobacco use.
	21 CFR 897.3—Sec. 897.3 Definitions.	This section defines cigarettes, smokeless tobacco, manufacturers, distributors (common carriers excluded), and packages. Retailers are any persons who sell cigarettes or smokeless tobacco to individuals for personal consumption or who operate a facility where vending machines or self-service displays are permitted (see 21 CFR 897.16). Smokeless tobacco means any product that consists of cut, ground, powdered, or leaf tobacco that contains nicotine and that is intended to be placed in the oral cavity.
	21 CFR 897—Subpart B—Prohibition of Sale and Distribution to Persons Younger Than 18 Years of Age.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 897.10—Sec. 897.10 General responsibilities of manufacturers, distributors, and retailers.	Each manufacturer, distributor, and retailer must ensure that the cigarettes and smokeless tobacco products it manufactures, labels, advertises, packages, distributes, sells or otherwise holds for sale comply with all applicable requirements under this part.
	21 CFR 897.12—Sec. 897.12 Additional responsibilities of manufacturers.	Manufacturers shall remove self-service displays, advertising, labeling, and other items that do not comply.
	21 CFR 897.14—Sec. 897.14 Additional responsibilities of retailers.	Except as allowed under Sec. 897.16(c)(2)(ii), a retailer may sell cigarettes and smokeless tobacco only in direct, face-to-face exchange. A retailer may not sell cigarettes or smokeless tobacco to any person younger than 18 years of age and must verify age for persons under the age of 26 by photographic identification containing the bearer's date of birth. Retailers may not offer for sale these products in units smaller than the smallest package distributed by the manufacturer for individual customer use. Self-service displays, etc., that do not comply with requirements must be removed or brought into compliance.
	21 CFR 897.16—Sec. 897.16 Conditions of manufacture, sale, and distribution.	Brand or trade names of new cigarette or smokeless tobacco products introduced after January 1, 1995, may no longer use the name of a nontobacco product. The minimum number of cigarettes allowed per package is 20. Vending machines and self-service displays are permitted only when located in establishments that do not allow entry at any time of persons under 18 years of age. Mail-order sales are permitted except for redemption of coupons. Free sample distribution is not permitted.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 897—Subpart C—Labels.	Appropriate names for smokeless tobacco products as provided in Section 502 of the act are loose leaf, plug, or chewing tobacco and moist or dry snuff.
	21 CFR 897.24—Sec. 897.24 Established names for cigarettes and smokeless tobacco.	
	21 CFR 897.25—Sec. 897.25 Statement of intended use and age restriction.	Each package shall bear the statement "Nicotine-Delivery Device for Persons 18 or Older."
	21 CFR 897—Subpart D—Labeling and Advertising.	
	21 CFR 897.30—Sec. 897.30 Scope of permissible forms of labeling and advertising.	Manufacturers, distributors, and retailers who advertise and label media other than those specified must provide 30-days' notice to FDA, giving the medium and discussing the extent to which persons younger than 18 years of age may see the advertisement or label. Outdoor advertising, including billboards, must not be placed within 1000 feet of any elementary or secondary school, public playground, or playground area (including baseball diamonds and basketball courts) in a public park.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 897.32—Sec. 897.32 Format and content requirements for labeling and advertising.	This section excludes print advertising inside retail establishments where vending machines and self-service displays are permitted and in adult publications such as newspapers, magazines, and periodicals of limited distribution to persons younger than 18 years of age (fewer than 2 million or less than 15% of the total readership). Audio and video formats exclude music and sound effects. Video formats must be static black text on a white background. The advertisement must append the statement "Nicotine-Delivery Device for Persons 18 or Older" after the appropriate product name as specified in 21 CFR 897.24.
	21 CFR 897.34—Sec. 897.34 Sale and distribution of nontobacco items and services, gifts, and sponsorship of events. Effective Date Note: At 61 FR 44617, Aug. 28, 1996, in Sec. 897.34, paragraph (c) [regarding event sponsorship] was added, effective Feb. 28, 1998. At 61 FR 47550, Sept. 9, 1996, the effective date was corrected to Aug. 28, 1998.	"No manufacturer and no distributor of imported cigarettes and smokeless tobacco may market, license, distribute, or sell items or services" (or cause these actions by others) that bear the brand name, logo, symbol, motto, selling message, recognizable color or pattern of colors, or other indicia of product identification associated with any brand of cigarettes or smokeless tobacco. These product-associated restrictions also apply to sponsorship of any athletic, musical, artistic, or other social or cultural event or any entry or team in any event by any manufacturer, distributor, or retailer. (The sponsor may use the name of the company if the corporate name and corporation were registered before January 1, 1995, and does not include the brand name, etc.) Manufacturers, distributors, and retailers may not offer or cause to be offered gift or redemption items other than cigarettes or smokeless tobacco.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F T C	16 CFR CHAPTER I—FEDERAL TRADE COMMISSION.	
	16 CFR 307—PART 307—REGULATIONS UNDER THE COMPREHENSIVE SMOKELESS TOBACCO HEALTH EDUCATION ACT OF 1986. Promulgated: 51 FR 40015, Nov. 4, 1986. Redesignated and amended at 56 FR 11662, 11663, Mar. 20, 1991; through 61 FR 45886, Aug. 30, 1996. U.S. Code: 15 U.S.C. 4401 et seq. (Title 15—Commerce and Trade. Chapter 70—Comprehensive Smokeless Tobacco Health Education Act of 1986. Public Law 99-252, Sec. 2, February 27, 1986, 100 Stat. 30).	FTC both administers and enforces this act, including the ban on broadcast advertising of smokeless tobacco products on radio and television advertising (FTC, 1998). The regulations stipulate rotation of three warning statements on smokeless tobacco products and advertising: WARNING: THIS PRODUCT MAY CAUSE MOUTH CANCER WARNING: THIS PRODUCT MAY CAUSE GUM DISEASE AND TOOTH LOSS WARNING: THIS PRODUCT IS NOT A SAFE ALTERNATIVE TO CIGARETTES The act governs label and advertising disclosures and requires submission of rotation plans. In addition, FTC must submit biennial reports to Congress on smokeless tobacco advertising and promotion [15 U.S.C. 4407(b)].
	16 CFR 307.2—Sec. 307.2 Required warnings.	No other statements shall be required by Federal, state, or local statute or regulation.
	16 CFR 307.3—Sec. 307.3 Terms defined.	A smokeless tobacco product means any finely cut, ground, powdered, or leaf tobacco that is intended to be placed in the oral cavity, including snuff, chewing tobacco, and plug tobacco.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F T C	16 CFR 307.4—Sec. 307.4 Prohibited acts.	Manufacturers, packagers, and importers shall not distribute or cause to be distributed smokeless tobacco in packages that do not bear one of the warning statements.
	16 CFR 307.5—Sec. 307.5 Language requirements.	The package warning statement must appear in English. Warning statements in printed advertisements must be in the predominant language of the publication in which the advertisement appears.
	16 CFR 307.6—Sec. 307.6 Requirements for disclosure on the label.	This section stipulates warning statement placement and point size, depending on package type so that the warning will be prominent and conspicuous.
	16 CFR 307.7—Sec. 307.7 Requirements for disclosure in print advertising.	Print advertisements such as periodicals, point-of-sale and non-point-of-sale promotional materials, posters, and placards (but not billboards) must carry a prominent and conspicuous warning statement in capital letters in a "circle and arrow format."
	16 CFR 307.8—Sec. 307.8 Requirements for disclosure in audiovisual and audio advertising.	Audiovisual advertisements must display the warning statement conspicuously in a "circle and arrow format" at the end and simultaneously announce the warning. If the advertisement is only audio, the statement must be clearly audible and given at the end of the advertisement.
	16 CFR 307.9—Sec. 307.9 Requirements for disclosure on utilitarian objects.	On objects such as t-shirts, the warning statement must be conspicuously displayed and its permanence must be equivalent to that of the smokeless tobacco product brand name, logo, or selling message.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F T C	16 CFR 307.10—Sec. 307.10 Cooperative advertising.	Advertisements paid for in whole or in part, directly or indirectly, by manufacturers, packagers, or importers must carry the warning. Retailers are allowed to make in-store announcements (if in print: 4 $in.^2$ or less) so long as they merely state product name or other identifier and the price.
	16 CFR 307.11—Sec. 307.11 Rotation, display, and distribution of warning statements on smokeless tobacco packages.	Rotation of each of the three warning statements must be evenly and randomly distributed to all parts of the United States. Plans must be submitted to the Commission for approval.
	16 CFR 307.12—Sec. 307.12 Rotation, display, and distribution of warning statements on smokeless tobacco advertising.	The regulation is similar to that for rotation of the statements on packages. Allowance will be made for the practical constraints on the production and distribution of advertising.
H H S	45 CFR—TITLE 45—PUBLIC WELFARE. SUBTITLE A—DEPARTMENT OF HEALTH AND HUMAN RESOURCES.	
	45 CFR 96—PART 96—BLOCK GRANTS—Subpart L—Substance abuse prevention and treatment. Promulgated: 58 FR 17070, March 31, 1993 with tobacco-related amendments 61 FR 1491-1509, January 19, 1996. U.S. Code: 42 U.S.C. 300x-21 to 300x-35 and 300x-51 to 300x-64.	The amendments promulgated January 19, 1996, implement section 1926 of the Public Health Service (PHS) Act regarding the sale and distribution of nicotine-containing tobacco products to minors by requiring, as a condition of eligibility for Block Grants, that individual States have in effect and enforce a law that prohibits such sales and distribution to minors.
	45 CFR 96.122—Sec. 96.122 Application content and procedures.	This section requires States applying for Block Grants to provide a copy of the state law described in Sec. 96.130 and a description of enforcement strategies.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
H H S	45 CFR 96.123—Sec. 96.123 Assurances.	Applications for Block Grants must include Assurances that the State has a law in effect that makes it unlawful to sell or distribute tobacco products to minors and enforces the law in a manner reasonably expected to reduce the extent to which tobacco products are available to persons younger than age 18.
	45 CFR 96.130—Sec. 96.130 State law regarding the sale of tobacco products to individuals under age of 18.	Since fiscal year 1994 (in some cases fiscal year 1995), for States to be eligible for Block Grants to assist State programs providing substance-abuse prevention and treatment services, they must have in effect a law making it unlawful for manufacturers, distributors, or retailers to sell or distribute tobacco products to minors. Prohibitions include over-the-counter and vending-machine sales to minors. States must conduct annual, random, unannounced inspections to ensure compliance. The report to the HHS Secretary must include descriptions of enforcement activities, including inspection methodology and overall success. Annual reports should include a plan for improving enforcement and should document progress in reducing availability to minors.
O T H E R	41 CFR—TITLE 41—PUBLIC CONTRACTS AND PROPERTY MANAGEMENT. SUBTITLE C—FEDERAL PROPERTY MANAGEMENT REGULATIONS.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
OTHER	41 CFR 101-20—MANAGEMENT OF BUILDINGS AND GROUNDS. Promulgated: 61 FR 2121-2122, Jan. 25, 1996. U.S. Code: 40 U.S.C. 486nt. Public Law 104-52, Sec. 636 (Prohibition of Cigarette Sales to Minors in Federal Buildings and Lands Act).	The General Services Administration (GSA) prohibited the sale of tobacco products in vending machines and the distribution of free samples in federal government-owned and -leased space. When promulgated, GSA intended to remove vending machines selling tobacco products from government property.
	31 CFR—TITLE 31—MONEY AND FINANCE: TREASURY.	
	31 CFR 12—PART 12—RESTRICTION OF SALE AND DISTRIBUTION OF TOBACCO PRODUCTS. Promulgated: 61 FR 25396, May 21, 1996. Public Law 104-52.	To implement Public Law 104-52, tobacco products sales from vending machines and free distribution are prohibited in federal buildings under the jurisdiction of the Secretary of the Treasury.
	32 CFR—TITLE 32—NATIONAL DEFENSE. CHAPTER I—OFFICE OF THE SECRETARY OF DEFENSE. PART 85—HEALTH PROMOTION.	
	32 CFR85.6—Sec. 85.6 Procedures.	Health promotion efforts in each military service should include smoking prevention and cessation programs. Military personnel at initial entry and permanent transfer should be informed about smoking's health consequences as well as those of alcohol and drug abuse. During routine physical and dental examinations, health care providers are encouraged to advise of risks of tobacco use, including smokeless tobacco; advise of the health benefits of abstinence; and advise how to get help to quit.

[a]Regulations considered for inclusion in the table and discussion were examined in the 1998 editions of titles 16, 19-21, 29, 31, 32, 40, and 41 and in the 1997 editions of titles 42, 45, and 47 of the *Code of Federal Regulations* and in 1997, 1998, and 1999 issues of the *Federal Register* through January 31, 1999.

3.0 HUMAN STUDIES

3.1 Studies Reviewed in IARC (1985)

IARC (1985) concluded that there is sufficient evidence that oral use of smokeless tobacco, including snuffs and chewing tobacco, is carcinogenic to humans. The human studies evaluated are described in the IARC monograph (see Appendix A, pp. 92-116).

3.2 Studies Published Post-IARC (1985)

U.S. epidemiological studies of the association between the risk of oral cancer and the use of smokeless tobacco report relative risks from 2.05 to 11.2 (Gross et al., 1995; Gupta et al., 1996).

Gross et al. (1995) reviewed the analytic epidemiological studies, published from 1952 to 1993, on the relationship between oral cancer and smokeless tobacco. They then used meta-analysis methods to summarize the major findings of these studies and concluded that the results were variable but "indicate an apparent association between the risk of oral cancer and the use of [smokeless tobacco] in the United States." The meta-analysis of the U.S. data published through 1993 indicates that the overall relative risk (RR) is 1.74 [95% confidence interval (CI) = 1.32-2.31] for the association of smokeless tobacco use and oral cancer. The meta-analysis also identified possible study and publication biases.

More recent studies are summarized below and in **Table 3-1**. A cohort study (Heineman et al., 1995) evaluated the relationship between mortality from rectal or colon cancer and the use of chewing tobacco or snuff, after a 26-year follow-up. This study compared colon or rectal cancer deaths among American veterans who reported use of chewing tobacco or snuff (n = 39), excluding current or ex-cigarette smokers, to the mortality from these cancers among veterans who had never used tobacco (n = 782). The relative risk (as maximum likelihood estimate of hazard ratio) for rectal cancer, 1.9 (95% CI = 1.2-3.1), indicated a risk almost double that for unexposed veterans, but the risk was higher for those who described their tobacco use as "never heavy use" than for those who reported "ever heavy use," suggesting a lack of dose response. The risk of colon cancer was not greater among users of chewing tobacco and snuff (relative risk = 1.2; 95% CI = 0.9-1.7). The estimated relative risks were adjusted for age, calendar time, year of questionnaire response, and physical activity. The influence of other factors that may affect colon and rectal cancer rates was not examined.

Additional studies associate chewing tobacco or snuff with cancer at sites other than the head and neck. Muscat et al. (1995) reported an association between the use of chewing tobacco and renal cell carcinoma. This multicenter, hospital-based case-control study found that 2.6% of males (n = 543) with renal cell carcinoma and 1.0% of controls (patients with conditions unrelated to tobacco use; n = 529) had chewed tobacco regularly for at least one year. The odds ratio (OR) (adjusted for age and education) for "ever use" of chewing tobacco was 3.2 (95% CI = 1.1-8.7). The risk of renal carcinoma among men increased with frequency of use, with an OR of 2.5 (95% CI = 1.0-6.1) for use $\leq$ 10 times per week and an OR of 6.0 (95% CI = 1.9-18.7) for use > 10 times per week. No adjustment was made for cigarette smoking.

In contrast, McLaughlin et al. (1995) showed a weak relationship between use of smokeless tobacco and renal cell cancer. This international, multicenter, population-based, case-control study (1732 cases and 2309 controls) reported that 11 renal cell carcinoma cases and 13

controls had used smokeless tobacco. An estimated RR of 1.3 (95% CI = 0.6-3.1) was found for renal cell cancer and use of smokeless tobacco after adjustment for age, sex, study center, and body-mass index.

Hayes et al. (1994) examined the association between tobacco use and prostate cancer in a multicenter, population-based, case-control study (981 cases and 1315 controls). An increased risk of prostate cancer was associated with snuff use [OR = 5.5 (95% CI = 1.2-26.2)] but not with other tobacco uses, including cigarette smoking, pipe smoking, cigar smoking, or use of chewing tobacco. The authors suggested that the association with snuff use was a chance finding.

Male tobacco chewers were reported by Muscat et al. (1997) to have an increased risk of pancreatic cancer, but this was based on a small number of cases and controls. This hospital-based, case-control study identified six male cases and five male controls who chewed tobacco regularly for at least one year and did not currently smoke cigarettes (crude OR of 3.6; 95% CI = 1.0-12.8; compared to never users and long-term quitters). There was no association found for snuff use.

The association of prostate cancer with use of smokeless tobacco was examined in the Lutheran Brotherhood cohort study with a 20-year follow-up (Hsing et al., 1990). The cases (n = 149) were white males with fatal prostate cancer. Data on the use of smokeless tobacco was obtained from mailed questionnaires in 1966. The calculated RR for fatal prostate cancer was significantly higher for men who had ever used smokeless tobacco (snuff or chewing tobacco; RR = 2.1; 95% CI = 1.1-4.1) and especially for regular users of smokeless tobacco (RR = 2.4; 95%CI = 1.3-4.9). These RRs were adjusted for age and cigarette smoking because some of the users of smokeless tobacco also reported they smoked cigarettes. A dose-response relationship could not be evaluated, nor could possible differences between snuff and chewing tobacco.

Table 3-1. Recent Human Studies of Effects of Exposure to Smokeless Tobacco

Design	Population Groups	Exposure	Effects	Potential Confounders	Reference
Cohort	**Cases:** 39 deceased U.S. veterans who reported use of chewing tobacco or snuff **Controls:** 782 deceased U.S. veterans who reported no tobacco use	**Estimation:** data from mailed questionnaire; followed prospectively for 26 years	**Evaluation:** Calculated relative risk (RR) of fatal colon and rectal cancer, after adjustment for potential cofounders **RR (95% CI) for colon cancer:** 1.2 (0.9-1.7) for users of chewing tobacco or snuff **RR (95% CI) for rectal cancer:** 1.9 (1.2-3.1) for users of chewing tobacco or snuff	age, calendar time, year of questionnaire response, physical activity	Heineman et al. (1995)
Case-control; multicenter hospital-based	**Cases:** 788 hospital patients with renal cell carcinoma **Controls:** 779 patients without kidney cancer and who were not hospitalized for conditions related to tobacco use	**Estimation:** direct interview with structured questionnaire **Categories:** never, ≤10 times/wk, > 10 times/wk	**Evaluation:** Calculated Odds Ratios (OR) from multiple logistic regression estimates, after adjustment for potential confounders **OR (95% CI) for renal cell carcinoma:** 3.2 (1.1-8.7) for male tobacco chewers compared to men who had never chewed tobacco 2.5 (1.9-18.7) for use 10 or fewer times/wk 6.0 (1.9-18.7) for use more than 10 times/wk	age, education	Muscat et al. (1995)
Case-control; international multicenter population-based	**Cases**: 1732 total (1050 men, 682 women); aged 20-79 years **Controls:** 2309 total (1429 men, 880 women); aged 20-79 years; matched to cases by sex and 5-year age groups	**Estimation:** direct interview with questionnaire and protocol; same protocol used at study centers in five countries (Australia, Denmark, Germany, Sweden, United States)	**Evaluation:** Calculated RR for renal-cell cancer, after adjustment for potential confounders **RR (95% CI; no. cases/controls) for renal cell carcinoma:** 1.3 (0.6-3.1; 11/13) for use of smokeless tobacco	age, sex, center, and body-mass index.	McLaughlin et al. (1995)

Table 3-1. Recent Human Studies of Effects of Exposure to Smokeless Tobacco (Continued)

Design	Population Groups	Exposure	Effects	Potential Confounders/Effects	Reference
Case-control; population-based	**Cases:** 981 pathologically confirmed prostate cancer (479 Blacks, 502 Whites) from three population-based cancer registries in U.S.; aged 40-79 **Controls:** 1315 from same geographic areas and proportional to expected age, gender, and race distribution of cases	**Estimation:** Direct interview of cases and controls **Categories:** former user or current user	**Evaluation:** Calculated OR by logistic regression analysis and adjusted for potential confounders **OR (95% CI) for prostate cancer risk:** 1.0 (0.6-1.5) for former users (total Black and White) of chewing tobacco 0.5 (0.2-1.0) for current users (total Black and White) of chewing tobacco 0.6 (0.3-1.4) for former users (total Black and White) of snuff 5.5 (1.2-26.2) for current users (total Black and White) of snuff	age, race, study site	Hayes et al. (1994)
Case-control hospital-based	**Cases:** 484 male and female patients with pancreatic cancer **Controls:** 954 male and female patients who did not have pancreatic cancer and were hospitalized for conditions unrelated to tobacco use	**Estimation:** Direct interview with structured questionnaire	**Evaluation:** Calculated OR from multiple logistic regression analysis; not adjusted for potential confounders **OR (95% CI; no. cases/controls) for pancreatic cancer** 3.6 (1.0-12.8; 6/5) for male tobacco chewers compared to never users and long-term quitters	age, education, cigarette smoking	Muscat et al. (1997)
Cohort	**Cases:** 149 white males who died of prostate cancer; aged 35 and above **Controls:** 19 white males who died of prostate cancer; aged 35 and above both cases and controls from cohort of 17,633	**Estimation:** mailed questionnaire; 20-year follow-up **Categories:** ex-users, occasional, regular	**Evaluation:** Calculated RR for fatal prostate cancer after adjustment **RR (95% CI):** 2.1 (1.1-4.1) for ever- users of smokeless tobacco 1.8 (0.8-3.9) for ex-users of smokeless tobacco 1.4 (0.5-3.9) for occasional users of smokeless tobacco 2.4 (1.3-4.9) for regular users of smokeless tobacco	cigarette smoking (some smokeless tobacco users also smoked cigarettes); age	Hsing et al. (1990)

Abbreviations: OR = odds ratio; RR = relative risk; CI = confidence interval

4.0 EXPERIMENTAL CARCINOGENICITY

4.1 Animal Studies Reviewed by IARC (1985)

IARC (1985, pp.78-85, see Appendix A) reviewed and evaluated animal studies with chewing tobacco, snuff, and nass and concluded that there is inadequate evidence to evaluate the carcinogenicity of these substances in experimental animals. In the United States and Europe, chewing tobacco and snuff are the primary smokeless tobacco products used (Gupta et al., 1996), so selected animal studies with these substances are summarized below. The IARC Working Group noted that most of these published studies had various deficiencies, e.g., lack of quantitative and qualitative information on the nature of the tobacco extracts.

4.1.1 Tobacco

Several studies with mice examined tumor incidence after oral administration, skin application, or application to the oral mucosa or cheek pouch. Lung adenocarcinomas or hepatocellular carcinomas were observed in male Swiss mice following oral administration (intubation) of a commercially available Indian chewing tobacco extract diluted with distilled water, and in male Swiss mice given a diet with a tobacco extract for up to 25 months. Controls received only distilled water by intubation. The tumor incidence at 15 to 20 months was 0/4, 8/15, 4/10 in the controls, 1:50 dilution, and 1:25 dilution groups, respectively. The tumor incidence at 21 to 25 months was 1/20 and 8/10 for controls and animals fed a diet with a tobacco extract, respectively. Specific rates of lung and liver tumor incidences were not reported (Bhide et al., 1984; cited by IARC ,1985).

One study evaluated cancer incidence after skin application of tobacco extracts (Ranadive et al., 1963; cited by IARC, 1985). Groups of 11 to 36 hybrid mice received skin applications of two separate extracts (partially alkaloid-free and totally alkaloid-free) for up to 95 weeks followed by weekly applications of croton oil. Controls received acetone followed by croton oil. A statistically significant increase in the incidence of papillomas and carcinomas was seen at the site of application in mice that were treated with the tobacco extracts between 61 and 95 weeks after the start of treatment. The papilloma incidence was 3/19, 10/21, 22/35 for controls, partially alkaloid-free extract, and totally alkaloid-free extract, respectively. The carcinoma incidence was 0/19, 6/21, 10/35 for the same respective groups.

Other studies observed no increased cancer incidence in animals after application of tobacco to the oral mucosa or cheek pouch. In a study with groups of 9 to 16 mice, starting at age 2 to 3 months old, six separate extracts of an Indian chewing tobacco were applied daily to the oral mucosa for up to 18 months of age (Mody and Ranadive, 1959; cited by IARC, 1985, p. 81). No difference in cancer incidence was observed among the different groups. A study with a group of 22 Wistar rats examined effects of painting the oral mucosa with an alkaloid-free extract of tobacco (Gothoskar et al., 1975; cited by IARC, 1985). The extracts were applied in acetone twice a week for life; 10 to 14 controls were untreated. No tumors were observed at the site of application in either group. Several studies with Syrian hamsters reported no neoplastic changes after chronic application of various tobacco extracts to the cheek pouch (IARC, 1985, p. 81).

4.1.2 Snuff

Early studies of snuff with rats or hamsters yielded insufficient data for evaluation (IARC, 1985, p. 111). One study with mice (Hamazaki and Murao, 1969; cited by IARC, 1985) suggested that inhalation of fine tobacco powder may influence the development of lung cancer. A group of 80 mice (Strain A) as exposed to tobacco leaf powder (dose unspecified) by inhalation on alternate days for 30 months. The control group (n = 80) was exposed by inhalation to tobacco leaf powder that was washed in water until cessation of the nicotine reaction. Lung cancer, identified as alveolar cell carcinoma, squamous-cell carcinoma, or malignant adenomas, was observed in 12 of 75 of the experimental mice and 1 of 80 of the control mice. The incidence of leukemia was 11/75 and 2/80 in treated and control groups, respectively. Hepatocellular carcinoma was found in 3/75 treated animals and 0/80 control animals.

4.2 Animal Studies Published Post-IARC (1985)

Experimental animal carcinogenicity studies published post-IARC (1985) are summarized below and in **Table 4-1.**

Hecht et al. (1986) investigated tumor induction by snuff and TSNAs in the oral cavity of rats. The study examined multi-site tumor incidence in male F344 rats after swab application to the oral cavity or lips of test solutions, or after insertion of test preparations into test canals in the lower lip.

Three test solutions included an aqueous snuff extract, an aqueous snuff extract enriched with two nitrosamines [NNN = *N*-nitrosonornicotine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone], or an aqueous solution of NNN and NNK. The control group was swabbed with water only. Groups of 30 test rats and 21 control rats, aged 10 weeks, were treated daily with 0.5 mL of the test solutions until study termination at week 131. After a complete necropsy on all rats, tumors were found in the oral cavity of rats treated with NNN and NNK (8/30) and snuff extract enriched with NNN and NNK (3/30). No oral cavity tumors were detected in rats treated with snuff extract or water only. Tumors were observed at other sites in control and all treated groups. A statistical analysis of differences between tumor incidence at other sites in treatment and control groups was not presented.

Snuff preparations were inserted into surgically prepared test canals in the lower lip of male F344 rats aged 13 weeks. These preparations, inserted 5 times weekly for 116 weeks, were snuff (n = 32), extracted snuff (n = 21), or snuff enriched with a snuff extract (n = 32). The extracted snuff was prepared by aqueous extraction. The enriched snuff was prepared by treatment with the aqueous extract. Control rats (n = 10) received no insertion after surgery. Oral cavity tumors were observed in all treated groups—snuff (3/32), extracted snuff (2/21), and enriched snuff (1/32)—but not in the control group (0/10). Tumors were observed at other sites in control and all treated groups. A statistical analysis of differences between tumor incidence at other sites in treatment and control groups was not presented.

Another study reported an increased incidence of oral cavity or lip carcinomas in rats treated with snuff (Johansson et al., 1989). Groups of 30 male Sprague-Dawley rats were treated (by application to a surgically created lower lip canal) with snuff, 4-nitroquinoline *N*-oxide in propylene glycol, or snuff after initiation with 4-nitroquinoline *N*-oxide. Control groups received a cotton pellet dipped in saline or propylene glycol. All groups received the treatment for 104

weeks, except the 4-nitroquinoline *N*-oxide or propylene glycol groups, which were treated for only 4 weeks.

In the group treated with snuff, squamous-cell carcinoma was observed (6/29) in several sites, including the lip, hard palate, nasal cavity and forestomach, while squamous-cell papilloma was seen (3/29) in the lip, hard palate, and nasal cavity. Initiation with 4-nitroquinoline *N*-oxide prior to snuff application resulted in 8/28 carcinomas of the hard palate, tongue, nasal cavity, and forestomach, but only 1/28 squamous-cell papilloma of the tongue. In the group treated with 4-nitroquinoline, squamous-cell carcinomas (7/29) and papillomas (2/29) were detected. No tumors were observed at these sites in rats of either control group. The difference in the incidence of squamous-cell tumors between the three treated groups and the two control groups was statistically significant.

Table 4-1. Recent Experimental Carcinogenicity Studies of Smokeless Tobacco

Species, Strain, Age	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose, Route	Exposure Duration	Results/Comments	Reference
F344 male rats; age 10 wk	Groups of 30	Group of 21 given only water on swab	1) aqueous snuff extract 2) snuff extract enriched with NNN (*N*-nitrosonornicotine) and NNK [4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone] 3) solution of NNN and NNK; purity not reported	0.5 mL applied by swab to oral cavity	Daily for 131 wk	Oral cavity tumors in group treated with NNN and NNK (8/30) and in group treated with snuff extract enriched with NNN and NNK (3/30) No oral cavity tumors in rats treated with snuff extract or water only Tumors were observed at other sites in the control group and all treated groups, but differences were not analyzed	Hecht et al. (1986)
F344 male rats; age 13 wk	Groups of 32 rats for snuff and enriched snuff; group of 21 rats for extracted snuff	Group of 10 rats that received no application to test canals	1) snuff 2) snuff after aqueous extraction 3) snuff enriched with an aqueous snuff extract	Preparation inserted into surgically prepared test canals in lower lip 5 times weekly	5 times/wk for 116 wk	Oral cavity tumors in snuff group (3/32), extracted snuff group (2/21), and enriched snuff group (1/32), but not in control group (0/10) Tumors were observed at other sites in the control group and all treated groups but differences were not analyzed	Hecht et al. (1986)

Table 4-1. Recent Experimental Carcinogenicity Studies of Smokeless Tobacco (Continued)

Species, Strain, Age	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose Route	Exposure Duration	Results/Comments	Reference
Male Sprague-Dawley rats; age 3 mo	Groups of 30	Two groups of 30; 1) received cotton pellet dipped in saline 2) propylene glycol applied to palate with sable hair brush	Snuff (commercially available U.S. brand) 4-Nitroquinoline *N*-oxide (4-NQO); purity unspecified	Three treatments applied to surgically-created canal in lower lip 1) snuff ($\geq$ 100 mg each application) 2) 4-Nitroquinoline *N*-oxide (4-NQO) (0.5%) in propylene glycol 3) 4-NQO for first 4 weeks; then snuff	 Snuff applied daily 5 d/wk for 104 wk 4-NQO applied 3 times/wk for 4 wk 4-NQO applied 3 times/wk for 4 wk followed by snuff application for 104 wk	 Snuff: squamous-cell carcinoma (6/29) in lip, hard palate, nasal cavity, and forestomach; squamous-cell papilloma (3/29) in lip, hard palate, and nasal cavity 4-NQO: squamous-cell carcinoma (7/29); squamous-cell papilloma (2/29) Snuff initiated with 4-NQO: squamous-cell carcinoma (8/28) in hard palate, tongue, nasal cavity, and forestomach; squamous-cell papilloma (1/28) in tongue No tumors in rats of either control group	Johansson et al. (1989)

5.0 GENOTOXICITY

Studies of the genotoxic effects of smokeless tobacco and snuff as reviewed by the IARC monograph (1985, pp. 87-89; see Appendix A) are summarized below. More recent studies adding new information to the results summarized in IARC are also summarized

5.1 Studies Reviewed in IARC (1985)

Ethanol extracts of tobacco, containing polar (hydrophilic) constituents, were positive for the induction of gene mutations in *Salmonella typhimurium* strain TA98 in the presence, but not in the absence, of metabolic activation.

In mammalian systems *in vitro*, ethanol extracts of tobacco induced gene mutations in Chinese hamster ovary (CHO) cells both with and without S9 activation. Ethyl acetate extracts of tobacco, containing nonpolar (lipophilic) constituents, were positive for the induction of sister chromatid exchanges (SCE) in human lymphocytes and lymphoblastoid cells, but did not induce gene mutations in Chinese hamster lung V79 fibroblast cells. Both ethanol and ethyl acetate extracts induced morphological transformation in Syrian hamster embryo (SHE) cells. Aqueous extracts of tobacco were positive for chromosomal aberrations in CHO cells in the absence, but not in the presence, of metabolic activation. Likewise, tobacco alkaloids induced SCE in CHO cells in the absence, but not in the presence, of S9.

Powdered tobacco fed to *Drosophila melanogaster* did not induce sex-linked recessive lethal mutations, sex-chromosome loss, or autosomal translocations. In mammalian systems *in vivo*, ethanol extracts induced micronuclei in the bone marrow erythrocytes of Swiss mice.

In humans, a significant increase in micronuclei was induced in the exfoliated lip mucosa, buccal, and sublingual cells of smokeless tobacco users when compared to nonuser controls.

5.2 Studies Reviewed Post-IARC (1985)

The frequency of micronuclei in squamous epithelial cells was significantly increased in cells from the oral mucosa of smokeless tobacco users, compared to micronuclei in oral mucosa from nonusers (Livingston et al., 1990). In contrast, there was no significant difference in the frequency of peripheral lymphocyte SCE between users and nonusers. Oral mucosa samples were obtained from persons (n = 24) who reported regular use of smokeless tobacco, and from an equal number of nonusers who were age- and sex-matched to the users. Exposure to smokeless tobacco was indicated by determination of a nicotine metabolite (cotinine) in saliva samples from both groups.

Another investigator (Shirnamé-Moré, 1991) examined the mutagenic activity of smokeless tobacco by application of tobacco water extracts to human cell lines. Two human lymphoblast cell lines (TK-6; AHH-1) were treated with aqueous extracts of smokeless tobacco (two commercial brands) and incubated for 28 hours prior to determination of the mutant fraction. Both lines showed a significantly higher mutant fraction than historical controls.

6.0 OTHER RELEVANT DATA

6.1 Absorption, Distribution, Metabolism and Excretion

These processes are summarized by IARC (1985, p. 88, see Appendix A), Hoffman et al. (1994), Hecht et al. (1994), and Nair et al. (1996).

6.1.1 Absorption

The saliva of users of snuff and chewing tobacco extracts nicotine, cotinine, nitrite, and endogenously formed TSNAs at the parts-per-billion to parts-per-million level and all may be found in the urine and blood after use of these products. Nicotine may be readily absorbed from the mouth, but some oral snuff users have been reported to have almost no absorption. Nicotine was also detected in the blood of subjects after inhalation of a single pinch of snuff. This concentration was comparable to the concentration of nicotine found in heavy smokers (IARC, 1985, p. 88). High concentrations of nitrosamines were found in the saliva within a few minutes of insertion of the product into the mouth. The nitrosamine concentration rapidly decreased after removal of the product (Hoffman et al., 1994).

6.1.2 Distribution

Hemoglobin adducts of nitrosamines were also detected in the blood of tobacco chewers at higher levels than were measured in nonsmokers (Hoffman et al., 1994; Hecht et al., 1994).

6.1.3 Metabolism

TSNAs are believed to be a major class of direct carcinogenic chemicals. Two of these nitrosamines probably cause cancers of the lung, oral cavity, esophagus, and pancreas in humans as a result of the use of tobacco products (Hecht et al., 1994). Analyses of body fluids from tobacco chewers show that chewers can metabolically activate nitrosamines to intermediates that bind to cellular macromolecules. Smokeless tobacco was shown to increase endogenous nitrosation in the oral cavity, a site where chewing habits are causally associated with cancer. Several nitrosamines were detected in chewing tobacco and saliva incubated under simulated gastric conditions, and in the saliva of subjects given betel quid and smokeless tobacco (Nair et al., 1996).

Hoffman et al. (1994) reviewed the chemistry and biochemistry of TSNAs, the procarcinogenic agents derived from leaf tobacco. The nitrosamine yield *in vivo* depends upon the nitrate/nitrite content and processing after placement in the mouth. The nitrosamines are metabolically activated by α-hydroxylation. The hydoxylation products lead to the formation of an unstable compound that is reactive with cellular macromolecules, including DNA and hemoglobin. The formation of endogenous nitrosamines occurs at a higher rate in users of smokeless tobacco than in persons who do not use tobacco products (Hoffman et al., 1994). Biomarkers of exposure to nitrosamines in smokeless tobacco users include TSNAs in saliva, NNK metabolites in urine, and NNK/NNN hemoglobin adducts in blood (Hoffman et al., 1994; Hecht et al., 1994). (DNA adducts have been found in the lungs of smokers.)

6.1.4 Excretion

In an analysis of NNK urinary metabolites, Carmella et al. (1997) concluded that glucuronidation and, to a lesser extent, pyridine *N*-oxidation are the primary pathways of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) detoxification in humans. Metabolites of TSNAs were detected in the urine of smokeless tobacco users at levels similar to those in found in the urine of smokers (Kresty et al., 1996).

6.2 Cell Transformation

An *in vitro* study with human cells showed that morphologic changes occurred after treatment with smokeless tobacco extracts and TSNAs (Murrah et al., 1993). Epithelial cells from human labial and gingival mucosa were treated for one hour with aqueous extracts of smokeless tobacco or with certain TSNAs. Both the TSNA and tobacco extracts prolonged the life of the labial mucosal cells in culture, indicative of the early stages of cell transformation. A similar but less pronounced effect was observed with gingival epithelial cells.

7.0 MECHANISMS OF CARCINOGENESIS

Several types of chemicals that are known animal carcinogens are contained in tobacco (Hoffman and Hoffman, 1995). Some of these chemicals are direct-acting carcinogens because they cause DNA damage if they are not metabolized, others must be metabolized prior to initiation of cancer, and other chemicals act as initiators or promoters of the cancer process.

Hoffman et al. (1994) and Hecht et al. (1996) reviewed the biochemistry of metabolically activated TSNAs. In animals administered a nitrosamine, hydroxylation of a methylene or methyl group at an alpha carbon leads to formation of unstable compounds that react with DNA. Hydroxylation of an alpha methyl group produces methylated bases that have been quantified in the lung, nasal, and liver DNA of rats and in the liver DNA of mice. A recent study shows that a DNA adduct inhibits the repair of the methylguanine lesion. Methylguanine lesions in human lung, formed by a TSNA and possibly other methylating agents in tobacco smoke, are higher in cigarette smokers than in nonsmokers. These methylguanine lesions in human lung DNA can cause miscoding, which can lead to adenoma or adenocarcinoma in mouse and hamster lung DNA.

Genetic mutations induced by tobacco extracts without chemical transformation (without metabolic activation) indicate that direct-acting genotoxic chemicals are present in tobacco (see Section 5).

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APPENDIX A

Excerpts from the IARC Monograph on the Evaluation of the Carcinogenic Risks of Chemicals to Humans Volume 37 (Tobacco Habits Other Than Smoking) 1985, pp. 37-136

CONTENTS

TOBACCO HABITS OTHER THAN SMOKING

1. Description of the Habits

1.1 Introduction

Habits associated with the use of smokeless tobacco are found worldwide, with countless variations in the nature of the product used, as well as in the customs associated with its use. The tobacco is often processed and treated with additives and flavouring agents. It may be taken alone or in combination with one or a variety of other ingredients. The predominant use of smokeless tobacco is oral, although it may be placed in or inhaled into the nasal cavity. The saliva produced during oral use may be swallowed or expectorated, according to custom or personal preference.

Tobacco grown for the manufacture of smokeless products is of two species within the genus *Nicotiana* (Solanaceae), *N. tabacum* and *N. rustica*. It is believed that the former originated in Brazil and the latter in Mexico. The vast majority of smokeless-tobacco products are made from *N. tabacum*; all of those manufactured in North America and western Europe are made from this species. *N. rustica* is used in the USSR and, to a limited extent, in India. The alkaloid, nicotine, is the factor that creates dependence in the continued use of tobacco.

In Europe and the USA, the smokeless-tobacco products used are predominantly chewing tobacco and snuff. Within these groups are several types, differentiated by formulation and treatment of the tobacco. During the past few years in the USA, there has been a reclassification of products within the two major categories, and some types of fine-cut smokeless tobacco that were classified as 'chewing tobacco' prior to 1981 are now categorized as 'moist/fine-cut snuff'. In general, *chewing tobaccos*, as the name implies, require the consumer to take a portion of the tobacco product and chew it and/or place it between the buccal mucosa and gum for varying periods of time.

Snuff is available in Europe and the USA as products with different particle sizes and moisture contents. The majority of snuff used today has a relatively high moisture content and is finely cut rather than pulverized; as with chewing tobacco, it is used orally and is placed between the buccal mucosa and gum. The other type of snuff, which is dry and pulverized, is for oral or nasal use. The major use involves placing a suitable amount between the lower lips and gum or between the gum and buccal mucosa; although dry snuff may also be sniffed through the nasal cavities, this technique is a minor use pattern. In some countries, snuff has become available in small packets wrapped in porous paper (like 'tea-bags') which are placed between the buccal mucosa and gum; such preparations appear to appeal to young adult users in Scandinavia and the USA.

In many Asian countries, tobacco is commonly added to betel quid, as described elsewhere in this volume. Tobacco may also be used alone, with lime and in various other combinations.

Nasal use of snuff is widely practised among the Bantu population in South Africa.

Although many of these habits are practised by millions of people, most of the available information is from North America, Europe and South-East Asia. Published estimates of the total number of persons practising the habits do not exist or are of variable reliability.

1.2 Historical overview

(a) *Tobacco chewing*

The tobacco plant is thought to have originated on the mainland between North and South America and had already spread throughout the two continents at the time of its discovery. However, cultivation of the plant probably dates back at least 7000 years; tobacco seeds were discovered during archaeological excavations in both Mexico and Peru, and findings in remains of permanent settlements built around 3500 BC show that tobacco was an article of established value to the inhabitants. It would appear that people who frequently lacked sufficient food alleviated their hunger pangs by chewing tobacco (Voges, 1984).

American Indians were probably the first people to smoke, chew and snuff tobacco, as early as the 1400s (Christen *et al.*, 1982). In 1499, Amerigo Vespucci found Indians on Margarita Island, a small island off the coast of Venezuela, chewing a green herb that was carried in a gourd around their necks. Since there was a scarcity of water on the island, Vespucci assumed that the green herb, known as tobacco, was chewed to quench thirst, since it produced an increase in salivary flow; he also reported that the Indians chewed these leaves to whiten their teeth (Heimann, 1960; Stewart, 1967).

According to a report written in the late 1500s by Garcilasso de la Vega, the practice of tobacco chewing was widespread in parts of South America (Voges, 1984); Samuel de Champlain, the founder of Québec, reported the use of plug tobacco in Santo Domingo during the sixteenth century, and Columbus, in 1571, observed men in Veragua, later known as Costa Rica, putting a dry herb in their mouths and chewing it (Heimann, 1960). Tobacco chewing seems to have been common, especially when long distances had to be covered, and it has been reported that an Indian could trek for two or three days with no other support against hunger, thirst and fatigue than tobacco. Several American tribes mixed either lime or finely-powdered and burned, fresh- or salt-water molluscs with their chewing tobacco (Curtis, 1935).

Among native Americans, chewing tobacco was thought to have several medicinal uses, some of which included alleviating toothache and disinfecting cuts by spitting the tobacco juice and saliva mixture onto the wound; it was also thought to relieve the effects of snake, spider and insect bites (Axton, 1975).

By 1531, the Spaniards were growing tobacco commercially in the West Indies and maintained a monopoly over the European markets until 1575, at which time the Portuguese began to grow large quantities of the commodity. Tobacco was soon grown in Europe as both a decorative and medicinal plant. In 1559, Jean Nicot, in whose honour the genus *Nicotiana* was named, was ambassador to Sebastian, King of Portugal. He grew tobacco and promoted the product in Europe for its magic 'cure-all' properties. By 1613, tobacco had become one of the major exports of the American colonies. In the 1600s in southern Africa, people sold land and slaves for tobacco (Christen *et al.*, 1982).

Tobacco arrived in Turkey in 1605, Russia in 1634 and Arabia in 1663; Spaniards transported tobacco seeds to the Philippines, from whence it was shipped to China, on to Siberia and across the Bering Sea back to Alaskan Eskimos. Reports indicate that the leaf reached the Australian aborigines and even the Andaman Islanders. On the western coast of Africa, tobacco became a commercial enterprise (Axton, 1975).

Along the eastern coast of the USA, no evidence of tobacco chewing was found until 1704; it became popular only during the first half of the nineteenth century (Gottsegen, 1940), when chewing tobacco was known in the Connecticut Valley as 'fudgeon' (Brooks, 1952).

Tobacco chewing was not confined to the Americas. When smoking was forbidden on British naval vessels because of the fire hazard, sailors turned to chewing tobacco and snuff. In Europe, tobacco was regarded as an excellent prophylactic during the plague and, for those who did not like smoking, chewing was an alternative. General George Monck, Duke of Albemarle, who was reponsible for aiding the return of the Stuarts to the British monarchy, was a tobacco chewer, as were many of his troops. Charles II chewed tobacco, as did Queen Caroline of England. Tobacco chewing was recommended for cleaning the teeth of women and children (Brooks, 1952).

In 1797, Adam Clarke, a famous Methodist minister, begged all tobacco consumers and especially religious followers, for the sake of their health and their souls, to avoid tobacco. He was particularly disturbed by the fact that it had become unsafe to kneel when praying because chewers had made the floors unsanitary for the devout (Brooks, 1952).

By the mid 1850s, tobacco chewing had been accepted by North Americans, following two centuries of pipe smoking and snuff use, for two reasons: firstly, Americans rejected European habits in general, and British habits in particular, that entailed snuff boxes and formality; and secondly, tobacco chewing was more convenient for Americans trekking westward in their wagons to build new homesites and develop the land (Heimann, 1960).

During the 1860s, tobacco was chewed in the form of either a plug or a twist. Of the 348 tobacco factories listed in the 1860 Census for Virginia and North Carolina, only seven manufactured smoking products (Heimann, 1960). American pioneers resorted to the use of a home-made sweet plug, so-named because the leaf was wadded into a hole in a log and laced with a sweetening agent, usually brandy or cane sugar, which, after removal of the fermented leaf, resulted in a tasty chew (Axton, 1975).

During the latter part of the nineteenth century, the 'germ theory of infection' changed the course of chewing in America, and it was felt that expectorating on the floor and into a brass cuspidor could be a source of contamination and spreading of disease. By the 1890s, public outcry made tobacco chewing socially unacceptable behaviour and unlawful in most public places (Christen *et al.*, 1982). Anti-spitting laws were passed in New York and Philadelphia in 1896 and in Toronto, Canada, in 1904 (Kozlowski, 1981).

In 1945, cuspidors were removed from all federal buildings by order of the US District Court in Washington DC (Brooks, 1952). The apparent decline in tobacco chewing is exemplified by a memorandum of 14 September 1955 to the American Tobacco Company, stating, 'It has become impossible to hire persons in the New York area to clean and maintain cuspidors ... it will be necessary to remove them promptly from the premises' (Heimann, 1960).

The market for chewing tobacco passed its peak in 1890, when some three pounds (about 1.5 kg) of plug, twist or fine-cut chewing tobacco were chewed annually per capita in the

USA (Heimann, 1960). Chewing remained the dominant form of tobacco usage in America until the expansion of the cigarette industry in 1918 (Maxwell, 1980).

During the second half of the 1960s and continuing through the 1970s, however, a resurgence in tobacco chewing occurred in the USA. Chewing tobacco has now become popular among young adult males. Some persons may have selected chewing as an alternative to smoking since it does not, as yet, have the stigma of being linked to health issues. Chewing can be performed in areas where smoking is prohibited, and it is purported to be more economical: advertisements claim that chewers can keep a wad of tobacco 'alive' for several hours, and it is claimed that a three-ounce (85-g) pouch of loose-leaf chewing tobacco can last a week or more for the average chewer (Christen & Glover, 1981).

The tobacco industry is promoting tobacco chewing as a recreational activity, with spitting contests, shirts and clubs. The trend toward the 'western cowboy' image of masculinity is being promoted in advertising by connecting tobacco chewing with western clothes. On one college campus, a chewing club claims the membership of athletes, the president of the student body, and a number of intellectual students; free samples of chewing tobacco are being handed out at other colleges. Commercial advertisements use public figures for extolling the virtues of chewing tobacco (Christen & Glover, 1981).

(*b*) *Snuff taking*

This topic has been reviewed recently (Christen *et al.*, 1982).

The Indians of Brazil were the first people known to use snuff. Using a cup made from a block of rosewood and a pestle of the same wood, the tobacco leaves were ground into a powder and acquired the delicate aroma of the wood. The resulting snuff was placed in ornately decorated bone tubes, one end of which was plugged to preserve the fragrance (Curtis, 1935).

Friar Ramón Pané, a Franciscan monk who travelled with Christopher Columbus on his second voyage to the New World in 1493, reported that the Carib Indians of the lesser Antilles used snuff (Christen *et al.*, 1982). In Haiti, snuff powder was used by medicine men for clearing nasal passages and as an analgesic (Stewart, 1967). Friar Pané's return to Spain with snuff signalled the arrival in Europe of a habit that was to last for several centuries.

In 1519, Ocaranza found that Mexican Indians used tobacco powder to heal burns and wounds, and in 1525 Herrera observed Mexican Indians holding tobacco powder in their mouth to send them to sleep and reduce pain (Stewart, 1967). The Indians inhaled powdered tobacco through a hollow Y-shaped piece of cane or pipe by placing the forked ends into each nostril and the other end near the powdered tobacco. This instrument was called a '*tobago*' or '*tobaca*'. The word was later changed by the Spaniards to 'tobacco' (Christen *et al.*, 1982).

Jean Nicot is credited with introducing snuff to Catherine de Medici, Queen of France, to cure her headaches (Christen *et al.*, 1982).

The Dutch, who named the powdered tobacco 'snuff', were also using the product by 1560 (Christen *et al.*, 1982).

By the early 1600s, snuff had become an expensive commodity and its use had spread throughout South America, China, Japan and Africa. The origin of the process terms 'carotte'

and 'rappee' goes back to the 1600s when tobacco for snuff was prepared in the form of a carrot to be rasped in the quantity desired for use (Curtis, 1935). In 1620, the Royal Snuff Factory was established in Seville, and this became the centre of manufacturing and development of this product (Voges, 1984). Snuff use expanded through Japan to China (Ching Dynasty) in the 1650s: palace artisans produced exquisitely-carved, inlaid enamelled or painted snuff bottles with a tiny spoon attached to the bottle stopper; a small portion of snuff was placed on the left thumbnail and inhaled through the nose. The Chinese believed that snuff cured pains in the eyes and teeth, alleviated throat ailments, constipation and cold symptoms, and promoted sweating (Christen *et al.*, 1982).

By 1650, snuff use had also spread from France to England, Scotland and Ireland. The Irish called snuff 'powder' or 'smutchin'; the Scots called it 'sneeshin' (Harrison, 1964).

Snuff use reached a peak in England during the reign of Queen Anne (1702-1714), and was called the 'final reason for the human nose'. It was at this time that ready-made snuff became available in England. It continued to be popular during the reign of George III, and his wife, Charlotte (1760-1820), referred to as 'Snuffy Charlotte', had an entire room in Windsor Castle devoted to her snuff stock. Lord Nelson, the Duke of Wellington, Marie Antoinette, Disraeli, Pope and Samuel Johnson all used snuff (Harrison, 1964). In diplomatic intrigue, poisons were sometimes placed in snuff. The aristocratic popularity of snuff led to a minor art form, in that snuff boxes became symbols reflecting the wealth and rank of their owner. The dandy, Lord Petersham, was said to own an annual set of 365 snuff boxes (Christen *et al.*, 1982).

The leading snuff supplier of the time, whose shop still exists in The Haymarket, London, provided King George IV with his own special blends, King's Morning Mix, King's Plain, and King's Carotte (Ryan, 1980). Home-made snuff was common. The tightly-rolled tobacco leaves (carotte) were often soaked in cinnamon, lavender, or almond oils; tobacco was dried and ground by means of an iron hand-grater that resembled a modern cheese-grater. The proper manner of inhaling snuff was to place a small quantity on the back of the hand and sniff it up the nostrils to induce a sneeze (Christen *et al.*, 1982).

Although hundreds of varieties of snuff existed in Europe by the 1800s, these consisted of three basic types: Scotch snuff, which was a dry, strong, unflavoured and finely-ground powder; Maccaboy, a moist and highly-scented snuff; and Rapee, also known as Swedish snuff, a coarsely-grated snuff (Heimann, 1960).

Snuff was introduced into Sweden in the middle of the seventeenth century, but its popularity among aristocrats reached a height during the eighteenth century, when use of nasal snuff became the highest fashion at the court of King Gustav III, among both men and women. The habit subsequently spread to the general Swedish population.

In many Swedish cities, snuff has been manufactured since the beginning of the eighteenth century. In Gothenburg, which is considered to be the centre of snuff production, manufacture started in about 1650 (Loewe, 1981). In 1795, Samuel Fiedler established a snuff mill in Gothenburg and began a small business, which later developed into three separate companies. At the end of the nineteenth century, the leading producer was Jacob Ljunglöf in Stockholm; his leading brand 'Ettan' became well known throughout Europe (Loewe, 1981). In 1914, the production of snuff in Sweden was taken over by the Swedish tobacco monopoly, which restored Gothenburg as the centre of snuff production in Sweden. A large factory was built around 1920, and expanded in 1979, for the production of snuff and chewing tobacco.

Since the beginning of the twentieth century, snuff has mainly been used orally in Sweden.

Snuff made its way to North America in 1611 by way of John Rolfe, husband of Pocahontas. Rolfe introduced the better Spanish variety of tobacco to ensure the survival of the Jamestown Colony in Virginia. Although most of the colonists in America never fully accepted the English style of snuff use, American aristocrats used snuff, and Dolly Madison was known to distribute samples of snuff to White House guests. During the 1800s until the mid 1930s, a communal snuff box was installed for members of the US Congress. The colonists also found it more to their taste to place snuff in their mouths rather than to sniff it (Christen *et al.*, 1982).

The first snuff mills in America were constructed in Virginia in about 1730 (Heimann, 1960). The snuff was made from New England tobacco and its quality was said to equal that of the native Scottish varieties (Robert, 1949). Pierre Lorillard, a Huguenot, established a snuff mill in New York in 1760 and carefully guarded the secret of his ingredients and blends (Christen *et al.*, 1982).

Between 1880 and 1930, the production of snuff in the USA increased from four million pounds (1.8 million kg) to more than 40 million pounds (18 million kg) per year (Garner, 1951). By 1945, the American Snuff Company in Memphis, Tennessee, claimed to be the largest snuff manufacturer in the world (Christen *et al.*, 1982). Snuff was made predominantly from dark, air- and fire-cured leaves. Stems and leaves were aged in hogsheads and conditioned before being cut into strips of one to two inches (2.5-5 cm) in width. The chopped leaves underwent further fermentation for about two months, during which time the tobacco lost its creosote-like odour and became more aromatic. It was next dried by passing through steam-heated containers and then ground to a fine powder in a revolving steel drum. The powder was passed over silk cloth containing as many as 96 threads per inch (38/cm). The coarse residue was returned to the mill for additional grinding before being packed into 100-pound (45-kg) bags for storage prior to repacking in smaller containers for retail sale. The dry and moist snuffs were used for dipping and placing in the mouth. Rappee or French snuff was used for inhaling, and Maccaboy snuff was both sucked and inhaled (Garner, 1951).

(c) *Tobacco and health*

In the past, tobacco use was considered by some to be beneficial. During the nineteenth and early twentieth centuries in America, dental snuff was advertised to relieve toothache, to cure neuralgia, bleeding gums and scurvy, and to preserve and whiten teeth and prevent decay (Christen *et al.*, 1982).

The use of tobacco, including smokeless tobacco, has been controversial since its introduction. Therefore, a history of smokeless-tobacco use is not complete without a discussion of the attacks on tobacco by various groups. In 1590 in Japan, tobacco was prohibited, and users lost their property or were jailed. King James VI of Scotland, who took over the British throne in 1604, was a strong anti-smoking advocate and increased taxes on tobacco by 4000% in an attempt to reduce the quantity imported into England. In 1633, the Sultan Murad IV of Turkey made any use of tobacco a capital offence, punishable by death from hanging, beheading or starvation, maintaining that tobacco caused infertility and reduced the fighting capabilities of his soldiers. The Russian Czar Michael Fedorovich, the first Romanov (1613-1645), prohibited the sale of tobacco, stating that users would be subjected to physical punishment; persistent users would be killed. A Chinese law in 1638 threatened that anyone possessing tobacco would be beheaded (Christen *et al.*, 1982).

During the mid 1600s, Pope Urban VIII banned the use of snuff in churches, and Pope Innocent X attacked its use by priests in the Catholic Church. Other religious groups banned snuff use: John Wesley (1703-1791), the founder of Methodism, attacked its use in Ireland; similarly, the Mormons, Seventh-Day Adventists, Parsees and Sikhs of India, Buddhist monks of Korea, members of the Tsai Li sect of China, and some Ethiopian Christian sects forbade the use of tobacco (Christen *et al.*, 1982).

In Germany (Bavaria) in 1652, tobacco was available only on a doctor's prescription; Frederick the Great, King of Prussia, prevented his mother, the Dowager Queen of Prussia, from using snuff at his coronation in 1790. Louis XV, ruler of France from 1723-1774, banned snuff use from the Court of France (Christen *et al.*, 1982).

In 1761, John Hill, a London physician and botanist, concluded that nasal cancer could develop as a consequence of tobacco snuff use. He reported five cases of 'polypusses, a swelling in the nostril that was hard, black and adherent with the symptoms of an open cancer' (Redmond, 1970).

1.3 Current practices

(*a*) *Tobacco chewing*

In the USA and Europe, the three main types of chewing tobacco are firm plug/moist plug, loose-leaf and twist/roll (Rizio, 1984). Each of these types is produced by using different tobaccos and additives, and by using different manufacturing processes.

Plug or pressed-leaf tobacco is made from enriched tobacco leaves or leaf fragments (spinner) wrapped in fine tobacco and pressed into flat bars or rolls before being packed in clear cellophane (Rizio, 1984). Plug can be either firm or moist: firm plug is more common and has a moisture content of less than 15%; moist plug is kept in a pouch or 'soft' and has a moisture content of 15% or more (US Department of Agriculture, 1982).

Loose-leaf tobacco, formerly called 'scrap', is made of fermented cigar leaf tobacco. Some brands are lightly sweetened, while others carry large amounts of sugars, syrups, liquorice and other flavouring materials. The treated tobacco is not compressed, but is packaged as a batch of loose pieces or cut strips. It is sold in a 3-oz (about 85-g) foil-lined pouch (Rizio, 1984).

Twist or roll tobacco is made of cured leaf which has been treated with flavouring materials. The processed leaves are then twisted into strands and allowed to dry.

Fine-cut tobacco: Some types of fine-cut smokeless tobacco, which had been classified as chewing tobacco prior to 1982, have been recategorized as moist/fine-cut snuff (see below).

In other regions of the world where tobacco is chewed, the sun-cured leaf is cut into strips of various sizes, followed by a short period of fermentation. For example, in some parts of India, the leaf strips may be over 5 mm in width, while in Indonesia the strips are finely cut.

The habit of chewing tobacco alone is practised predominantly in the USA and South-East Asia with some use in Europe and other Asian countries. Specific information on tobacco

chewing practices was limited for countries other than the USA, the UK, the USSR and India. Sales and production figures reported in section 1.5 give an indication of the extent of usage in other countries.

(i) *USA*

Data for the period 1880 to 1960 show a peak in the consumption of chewing tobacco in the USA between 1910 and 1920 (Heimann, 1960). More recent figures from the US Department of Agriculture (1984a) show a gradual decline in production from 1931 (the earliest year listed in the report) to the 1960s, at which time production began to increase again; this trend continued through the 1970s, and recent figures show an apparent plateau in the early 1980s. Since the upward trend began, production has increased by approximately 60%.

Total US consumption of smokeless-tobacco products was approximately 132 million pounds (60 million kg) per year during the period 1980-1982 (Tobacco Institute, 1981, 1982, 1983). On 1 January 1982, some types of chewing-tobacco products were reclassified as snuff (US Department of Agriculture, 1983). Under this classification, consumption of chewing tobacco in 1982 was 88 million pounds (40 million kg) (Tobacco Institute, 1983).

Part of the recent increase in the use of smokeless-tobacco products may have occurred among adolescents (Christen *et al.*, 1982; Glover *et al.*, 1983). It has been estimated that there are now 22 million users of smokeless-tobacco products (Greer & Poulson, 1983). Holleb (1984) indicates that the increase in chewing was from 2-4% per year from 1972 to 1980 for adults, with a slight decrease from 1981 to 1983; he estimates that there are seven million smokeless-tobacco users in the USA. The 1979 Report of the Surgeon General (US Department of Health, Education, and Welfare, 1979) found the prevalence of tobacco chewing in the USA in 1970 to be 5.6% among men and 0.6% among women. The figure for men decreased to 4.9% in 1975 but remained the same for women.

Chewing usually consists of placing a 'chaw' or 'quid' of leaf or plug tobacco in the gingival buccal area where it is held or chewed. A 'chaw' is a wad of chewing tobacco the size of a golf-ball, and a 'quid' is a much smaller portion, which is usually held in the mouth rather than chewed. Many persons chew during most of their working hours, and some keep a quid in place for 24 h a day (Christen, 1980a). Tobacco chewers habitually spit out the liquid extract produced by chewing.

Some measure of how long a tobacco chewer exposes himself to chewing can be determined from studies on selected populations. Moderate chewers have been defined as individuals for whom the total length of exposure of the buccal mucosa to tobacco was up to 200 min per day, while, for heavy chewers, it was more than 200 min per day (Wahi *et al.*, 1970). Men in Texas reported that they used two to eight chaws per day (Christen *et al.*, 1979a).

A market research bureau undertook a study in 1981 to determine a profile of the smokeless-tobacco user. Those men most likely to chew tobacco were between the ages of 18 and 34 years, were married and/or a parent, and had a high-school education or less (Maxwell, 1982).

There are several regional and demographic variations in chewing-tobacco patterns. According to Rizio (1984), twist or roll chewing tobacco is sold mainly in the southern and southern border states of the USA. Chewing tobacco is preferred by many workers in heavy industries, such as steel, coal and petroleum, where smoking is prohibited due to inflam-

mable work environments. There is also an increase in its use by persons who enjoy leisure-time activities involving their hands, and who prefer to chew rather than smoke cigarettes.

The areas in the USA where chewing is practised and identification of the populations who chew are described in several recent studies, which give indications of the age at which the habit is acquired and the dosages for various groups.

In 1976-1977, as part of the Bogalusa Heart Study in Louisiana, USA, 3147 children aged 8-17 years were asked various questions concerning chewing tobacco. White boys far exceeded those of other races in tobacco usage other than tobacco smoking. Approximately 25% of white boys aged 12-15 years had tried chewing tobacco (Hunter *et al.*, 1980).

A teen-aged population of smokeless-tobacco users in Denver, CO, USA, was identified; of 1119 students, 117 used snuff or chewing tobacco (113 boys and 4 girls). The length of time tobacco was kept in the oral cavity ranged from 53 to 177 min per day, and duration of use of these products ranged from 2.19 and 3.25 years (Greer & Poulson, 1983).

In 1980, a sample population of 2616 students in Nebraska, USA, aged 11-17 years, were asked about their use of chewing tobacco. Only eight girls admitted to chewing tobacco, whereas 90 boys (7.1%) reported practising the habit. Approximately one-third of the tobacco chewers were also cigarette smokers. The product was used daily by 36% of the chewers, 32% chewed weekly and 32% said they chewed only once a month; 23% had chewed for more than three years, 47% for two to three years and only 30% had chewed for one year or less (Newman & Duryea, 1981).

A study of 500 boys aged 10-16 years in Atlanta, GA, USA, showed widespread use of smokeless tobacco. Several boys aged 8-10 mixed chewing tobacco with bubble gum; 15% reported regular use of chewing tobacco (Anon., 1983).

A survey of college athletes in Texas showed that many began chewing between the ages of 10 and 12 years (Christen *et al.*, 1979a).

(ii) *Canada*

A survey was conducted in 1982 in the Northwest Territories to determine the extent of use of chewing tobacco among 7300 school children, 5-19 years old. Approximately 17% (boys, 26%; girls, 8%) of the student population reported that they had used chewing tobacco, and 9% (boys, 13%; girls, 2.9%) were current users. Use was more prevalent among the Indian/Métis and Inuit youths than among the non-native youths (Millar & van Rensberg, 1984).

(iii) *UK*

Tobacco chewing is uncommon in the UK. However, a study of five coal mines in south Lancashire revealed different chewing habits based on the actual location of work at the pit. Among 1490 miners, only 1.7% of surface workers chewed tobacco, as compared to 34.3% of those who worked underground. A further analysis of 858 of these miners found that 91.2% of the 195 tobacco chewers also smoked cigarettes. The quantity of tobacco chewed was 3-15 g per day, with an average daily use of 4.5 g (Tyldesley, 1971).

(iv) *India*

Chewing tobacco in India is made from sun-dried, coarsely cut leaves. About 85% of the tobacco used is sold loosely and is consumed raw. The dried leaf is broken into smaller pieces before being consumed. It may be used alone, in combination with lime (*khaini*), as a component of betel quid (see this volume) or in other forms such as *mishri*, *zarda*, *kiwam* and *pills* (described in section 1.3(*c*)).

Tobacco chewing is prevalent among people of all castes, creeds and religions, except Sikhs and Parsees. Overall, more men than women chew tobacco. In rural and urban low-income groups, the habit of chewing begins at 10-12 years of age and continues until old age.

In the one available study on the prevalence of pure tobacco chewing, Pindborg *et al.* (1967) reported that 2.1% of a population sample of 10 000 chewed tobacco alone.

(*b*) *Snuff taking*

The types of tobacco and ingredients used in snuff differ in the countries where the habit is practised. Prior to the nineteenth century, in Europe and the USA, the most popular means of consuming tobacco was sniffing the finely-ground leaf tobacco through the nose. Presently, the major use of snuff is to place it in the mouth between the lip or buccal mucosa and gum. In South-East Asia and Africa, snuff is taken both orally and nasally.

In the USA and Europe, snuff is currently described either as moist snuff, which is placed in the oral cavity and has a relatively high moisture content (up to 50%), or as dry (Scotch) snuff, which is placed in the oral cavity or administered through the nasal passage. Dry snuff usually has a moisture-content of less than 10%. Originally, in the USA, 'fine-cut' was used to describe chewing tobacco; however, at one point it was also used to describe snuff which may have been made from fine-cut chewing tobacco. It is a term that still may be used by some persons to describe snuff, but has little meaning in terms of a comparison of tobacco particle size. Frequently, the moist snuff category is listed as moist/fine-cut snuff.

Snuff used in Asia and Africa differs in its preparation and constituents from that used in the USA and Europe, as described in subsequent sections.

Although production and usage data, as reported in section 1.5, suggest that snuff is widely used in a number of countries, little or no information was available on the products used and number of persons practising the habit in areas other than Europe (particularly in Scandinavia) and North America.

Since snuff, its tobacco content, flavour components, manufacturing processes, and the customs affecting its use vary throughout the world (Axéll *et al.*, 1976), habits associated with its use are described by country or area.

(i) *Scandinavia*

During the eighteenth century in Denmark and Sweden, snuff was used as a fine, dry powder that was inhaled through the nose (Zacho *et al.*, 1968). Since the end of the nineteenth century, snuff has been used orally in Sweden and is usually placed behind the upper lip (Frithiof *et al.*, 1983); in Denmark it is usually placed behind the lower lip (Pindborg

& Poulsen, 1962; Pindborg & Renstrup, 1963). Currently, wet snuff, which is highly alkaline (pH 8-9), is the preferred type in the Scandinavian countries (Pindborg & Axelsen, 1980; Hirsch *et al.*, 1982).

It is estimated that in 1978, there were 700 000 to 800 000 snuff users in Sweden (Frithiof *et al.*, 1983). Data from clinical studies in Sweden and Denmark indicate that the average snuff user is approximately 50 years old (mean of three investigations: Axéll *et al.*, 1976; Pindborg *et al.*, 1980; Hirsch *et al.*, 1982), uses snuff 10 h per day (mean of three investigations: Roed-Petersen & Pindborg, 1973; Axéll *et al.*, 1976; Hirsch *et al.*, 1982) and has been using snuff for 22 years (mean of two investigations: Axéll *et al.*, 1976; Hirsch *et al.*, 1982). The estimated consumption per day for an average user is about 15 g (mean of two investigations: Axéll *et al.*, 1976; Hirsch *et al.*, 1982). Average consumption is estimated to be approximately 100 g per week and 5.4 kg per year per user (Österdahl & Slorach, 1983).

Almost all snuff users in Sweden are men, and they constitute 17% of the population. The prevalence is 19% in the age group 15-30 years and 10% among those 65 years and older. Only 1% of Swedish women use snuff regularly (Axéll, 1979; Barroll & Ramström, 1979; Hirsch, 1983). Among school children aged 13-16 years, 11-15% of boys but no girls used snuff regularly (Modéer *et al.*, 1980; Hibell & Jonsson, 1982; Hirsch, 1983). The average consumption of snuff among school children aged 13 and 14 years was five pinches per day; the snuff was kept in the oral cavity for an average of 3.5 h (Modéer *et al.*, 1980).

There are geographical differences in the use of snuff in Sweden. The habit is more widespread in the northern parts of the country, where approximately 25% of the male population over the age of 15 years use snuff daily. Snuff usage is also more common in rural areas (20%) and in small towns (13%) than in large towns (7%). It was found that people with high consumption of snuff usually smoked fewer cigarettes than did those people who used moderate amounts of snuff. Of male snuff users, 43% did not smoke, 22% smoked occasionally and 35% were regular smokers (Hirsch, 1983); two-thirds of snuff users were social drinkers. Many different brands of snuff are available, but the majority of snuff takers use only one or two brands (Hirsch *et al.*, 1982).

(ii) *USA*

This section should be read in conjunction with that on p. 55, which describes trends in the production and use of smokeless-tobacco and chewing-tobacco products in the USA.

Data for the period 1880 to 1940 show a peak in snuff consumption in the USA between 1910 and 1920 (Schuman, 1977). More recent figures from the US Department of Agriculture (1984a) show that production was relatively stable from 1931 (the earliest year listed in the report) to the mid-1950s, at which time there was a slow decline in production that continued until the early 1980s.

On 1 January 1982, some chewing-tobacco products were reclassified as snuff (US Department of Agriculture, 1983); under this classification, 43.9 million pounds (20 million kg) of snuff were consumed in the USA in 1982 (Tobacco Institute, 1983). In 1983, sales of moist snuff had increased by 7.1% over the 1982 figures (Shelton *et al.*, 1984).

In 1970, 2.9% of men and 1.4% of women used snuff; by 1975, only 2.5% of men and 1.3% of women used snuff (US Department of Health, Education, and Welfare, 1979); a 1983 market research bureau reported that smokeless tobacco is used in 3.2% of US households in which a male is living (Maxwell, 1984). Christen and Glover (1981) reported that

approximately 30% of women in central North Carolina were snuff users as compared to 1.3% of women in the USA as a whole. A 1983 market research bureau report indicated that US snuff users were more likely to live in a rural area of eastern-central or southern USA, and to be in a lower-income, part-time employment category. In contrast to chewing-tobacco users, a significant number of snuff users had attended college (Anon., 1984).

In the USA, dry snuff is used mostly by middle-aged and older people, whereas moist snuff is used mainly by young adults. The average user of moist snuff consumes 1.5 tins (1.2 oz or 34 g) per week (Maxwell, 1980).

The usual method of taking snuff in the USA is to place a pinch in the gingival buccal area, between the buccal mucosa or lip and the gums, or beneath the tongue and to leave it in position for a few minutes or as long as desired. This is in contrast to the eighteenth-century practice of inhaling the snuff into the nasal cavity. Oral use of snuff is colloquially referred to as 'snuff dipping' in the USA, from the habit of dipping into snuff a stick that had previously been chewed to flatten the end, and applying the snuff to the gums. Some users of dry snuff measure out a small quantity in the lid of the container or into a special spoon and then place the snuff between the lower lip and the gum. Moist snuff is used by taking a 'pinch' of the slightly damp tobacco between the thumb and forefinger and tucking it between the lower lip and gum. Snuff has recently become available in 'tea-bag'-like packets, which are placed in the mouth.

In a study of snuff usage among middle and lower socioeconomic groups in south-eastern USA, it was found that many used snuff most of their waking hours and some slept with the quid in their mouths. In a study of 290 patients with oral-cavity lesions, it was not unusual to find that the persons had used snuff regularly before the age of 10 years, and 75% of the patients had used snuff for 40 years (McGuirt, 1983a).

In a study in south-eastern USA, of 255 women with oral and pharyngeal cancer, it was found that whites had used snuff for an average of 47.6 years *versus* 36.1 years for blacks, and that they used more tins (1.15 oz or 36 g) per week than did blacks (3.0 *versus* 2.4). The average duration of snuff use was less among cigarette smokers (33.0 years) than among nonsmokers (47.4 years) (Winn *et al.*, 1981a).

Of a population of 15 000 snuff users who resided primarily in one state in south-eastern USA, the average age of the subjects who were examined for 'snuff-dippers' lesions' was 55 years; 75% were women. This elevated number of users probably represents a regional population sample bias (Smith *et al.*, 1970; Smith, 1975).

In another south-eastern US state, 11% of 500 boys, 10-16 years of age, reported using snuff regularly (Anon., 1983).

In an urban school population of 565 boys (average age, 13.8 years), 200 were selected for a follow-up dental study. Of this selected group, 11% used snuff regularly (Weather & Offenbacher, 1983). In another study of college men in a southern state, as many as one-third were either tobacco chewers, snuff users, or both (Christen, 1980b).

(iii) *Canada*

A survey was conducted in 1982 in the Northwest Territories to determine the extent of snuff use among 7300 school children aged five to 19 years. Approximately 10% (boys, 16%; girls, 5%) were current users of snuff. Use was more prevalent among the Indian/Métis and Inuit youths than among non-native youths (Millar & van Rensberg, 1984).

(iv) *South-East Asia and Africa*

Unscented snuff is used in many parts of *India* as a dentifrice, while scented snuff is used for sniffing. The habit of sniffing is on the decline, but use of snuff as a dentifrice by men, women and children in the poorer sections of society is widely prevalent. It is used from twice to eight to ten times per day (Anon., 1953). In a study from Ahmedabad, of 57 518 industrial workers examined, 1316 (2.3%) used tobacco only by inhaling snuff (Smith *et al.*, 1975).

In *Thailand*, snuff ('a tan, dry powder') is taken with the aid of a U-shaped metal tube: one end of the tube is placed in the mouth and the other in the nasal passage. Air blown from the mouth scatters the powder into the nose, through which it is inhaled (Harrison, 1964).

In *South Africa*, snuff inhalation is widely practised by Bantu men and women, for whom its use has an important cultural and ritual history. The product typically contains tobacco leaves and ash from aloe plants or other species, with the occasional addition of oil, lemon juice and herbs; use is often one teaspoonful per day (Keen *et al.*, 1955; Harrison, 1964; Baumslag *et al.*, 1971). In a small survey in one town, 19% of Bantu men and 30% of the women used snuff orally or nasally (Higginson & Oettlé, 1960).

Among the Fingo and Xhosa tribes of *South Africa*, snuff is placed between the gingiva and lower lip or buccal area (Harrison, 1964). Among elderly Bantu men and women, snuff is usually retained in the front of the mouth. The saliva mixes with the snuff and is then swallowed. After the snuff has lost its flavour, the procedure is repeated (van Wyk, 1966).

(*c*) *Other smokeless-tobacco habits*

(i) *Tobacco and lime* (khaini)

Tobacco is sometimes chewed in the presence of lime. In certain parts of India, this is referred to as *khaini*. A pinch of raw powdered tobacco is taken in the palm and a small amount of slaked lime paste is added; the mixture is then rubbed thoroughly with the thumb and placed in the mouth — generally in one or both cheeks, or in the mandibular groove. The mixture is retained for 10-15 min, after which time it becomes bland; occasionally it is left in the groove during sleep (Bhonsle *et al.*, 1979). Pieces of areca nut are sometimes chewed with *khaini*.

Use of *khaini* is prevalent among the Munda and Santal tribes of Bihar, India; it is usually placed in the inner side of the lower lip in the gingivolabial groove. Among those examined, approximately 42% of *khaini* users held the mixture in the front of the oral cavity, whereas others placed it either in the left or right side, where it was generally retained with very little chewing (Stich *et al.*, 1982).

Pattiwala tobacco is sun-cured tobacco leaf and is used with or without lime (Wahi, 1968). Mainpuri tobacco is a mixture with slaked lime, areca nut and spices; its use is discussed in the monograph on betel-quid and areca-nut chewing, p. 141.

(ii) Mishri (misheri, masheri)

Mishri is a form of tobacco used in India as a substitute for chewing tobacco. It is a 'roasted or half-burnt' tobacco, prepared by baking tobacco on a hot metal plate until it becomes uniformly black. It is then powdered and used primarily for cleaning teeth (Mehta *et*

al., 1972). However, its use frequently becomes habitual, and a user may apply and retain *mishri* in the mouth (usually along the teeth and in the sulcus) several times a day. Rural Indian women place it in the oral cavity between the gum and buccal mucosa instead of chewing tobacco (Murdia *et al.*, 1982).

Mishri usage has been reported from a house-to-house survey of 101 761 individuals in the state of Maharashtra, where 38.9% of the women and only 0.8% of the men used it (Mehta *et al.*, 1972). In a house-to-house survey of 10 071 individuals in Gujarat (the Bhavnagar District), 7.1% (mostly women) used *mishri* (Mehta *et al.*, 1971). The use of burnt tobacco for cleaning teeth was reported by 0.24% of the population in the Mainpuri District (Wahi, 1968).

(iii) Zarda, kiwam *and* pills

Zarda, which is produced and used in India, is also exported to a number of Arab countries (Sinha, 1984). During the manufacture of *zarda*, tobacco leaf is first broken into small pieces and boiled in water with lime and spices until evaporation. The residual particles of tobacco are then dried and coloured with vegetable dyes. *Zarda* is usually chewed mixed with finely-cut areca nut and spices.

For *kiwam*, the stalks, midribs and veins of tobacco leaves are removed, and the remaining matter is soaked and boiled in water with added rose water and powdered spices, such as saffron, cardamom, aniseed and musk. The mixture is stirred and allowed to macerate. The pulp is strained to remove any stalk or rib remnants and allowed to dry. The product has the consistency of a thick, rough paste. Granules or *pills* are prepared using the same process, but the paste is dried further and pelleted (Anon., 1953).

(iv) Gudakhu

Gudakhu is a paste consisting of powdered tobacco, molasses and some other ingredients. It is used for cleaning teeth by populations in central and eastern states of India. In a house-to-house survey of a random sample in Singhbhum (Bihar district), 8.3% of the population were reported to use this product (Mehta *et al.*, 1971).

(v) Shammah

Shammah is the native name for a tobacco mixture used in some parts of southern Saudi Arabia. It is described by Yousef and Hashash (1983) as a quid of powdered tobacco leaf, carbonate of lime, and other substances, including ash. Salem *et al.* (1984), however, describe *shammah* as a snuff prepared by mixing powdered tobacco leaves with sodium carbonate. The material, a greenish-yellow powder, is placed in the buccal or lower labial vestibule of the mouth. Periodically, the user spits out insoluble debris that is freed from the *shammah* bolus. In a survey made on 661 individuals in various geographical locations of the Gizan district, it was reported that 24% practised this chewing habit (Salem *et al.*, 1984).

(vi) Nass

The habit of using *nass* is practised by the native populations of Iran and the Soviet Central Asian Republics (the Uzbek, Turkmenian, Kirghiz, Tadzhik and Kazakh SSRs) (Shilovtsev, 1941; Joint Iran-International Agency for Research on Cancer Study Group, 1977; Paches & Milievskaya, 1980). It is usually made with local tobacco (which is sometimes only partially cured), ash, cotton oil or sesame oil and lime (Paches & Milievskaya, 1980). How-

ever, the composition of *nass* varies in the different regions in which it is used, the primary difference being the content of lime (Table 1). *Nass* prepared in the Tadzhik SSR and most types of *nass* prepared in the Kazakh SSR do not contain lime (Paches & Djuliev, 1965; Aleksandrova, 1970), whereas that in the Bukhara, Samarkand and Kashka-darya regions of the Uzbek SSR contains the highest amounts of lime (Paches & Milievskaya, 1980).

Table 1. Variations in the constituents of *nass* in five regions of the USSR[a]

Region	Constituents of *nass* (%)						
	Tobacco	Ash	Cotton oil	Sesame oil	Gum	Water	Lime
Bukhara	55	18	-	20	-	2	10
Kashka-darya	50	30	10	-	-	3	7
Samarkand	50	25	15	-	-	3	7
Tashkent	50	20	-	-	2	17	3
Fergana	55	20	-	-	3	18	4

[a]From Paches and Milievskaya (1980)

In addition to variations in composition, there are regional differences in the anatomical site in the mouth where *nass* is put (Aleksandrova, 1970; Khasanov & Fasiev, 1970; Zaridze *et al.*, 1985a). Nugmanov and Baimakanov (1970) reported that 60% of *nass* users in the Chimkent region of the Kazakh SSR put *nass* under the tongue, and 40% between the gum and lower lip. According to Aleksandrova (1970), 96% of *nass* users in the Djambul region of the Kazakh SSR place the *nass* against the inside of the lower lip. In a survey of 1569 men carried out in the Samarkand region of the Uzbek SSR, all but one of the *nass* users reported placing the *nass* under the tongue (Zaridze *et al.*, 1985a).

The daily frequency of *nass* chewing also varies considerably. Surveys indicate that most users take *nass* about 10-15 times per day (Aleksandrova, 1970; Khasanov & Fasiev, 1970; Zaridze *et al.*, 1985b,c). The saliva produced during chewing is expectorated and the mouth rinsed with water when the chew is removed.

According to various surveys, 4-49% of the adult population in *nass*-using areas practise this habit. In a survey of 15 672 persons living in urban (5135) and rural (10 537) areas of the Chimkent region (Oblast) of the Kazakh SSR, 7.5% of the total population and 49% of the native Kazakhs used *nass*. The proportional age distribution and proportion of *nass* users in each age group are given in Table 2; 67.8% of the *nass* users were men and 32.2% women (Nugmanov & Baimakanov, 1970).

According to another survey (Aleksandrova, 1970), the proportion of *nass* users in the Chimkent region is 4.5% (364 out of 8123 persons surveyed). A survey of 2012 persons in

Table 2. Age distribution of *nass* users in the Chimkent region of the Kazakh SSR[a]

Age (years)	% surveyed	% *nass* users
35-39	20.5	6.9
40-49	28.5	17.3
50-59	21.7	21.6
60-69	17.1	31.3
70+	12.2	22.9

[a]From Nugmanov and Baimakanov (1970)

the Djambul region of the Kazakh SSR revealed that 14.3% (289) used *nass*. In the Chimkent region, 22% of *nass* users were women, while in the Djambul region the proportion was 52%.

A survey of 988 persons aged 25 years and above in a rural area of the Mary region of the Turkmenian SSR revealed that 14% of persons of both sexes and 36% of men were *nass* users (Saparov, 1965).

A survey carried out in three regions (Vakhsh, Zeravshan and Gorno-Badakhshan) of Tadzhikistan revealed that 20%, 27% and 17%, respectively, of the population over the age of 20 years were *nass* users. In Tadzhikistan as a whole, the proportion of *nass* users was estimated to be 20% (Paches & Milievskaya, 1980). Of 6520 men and women residents of the Bukhara region of the Uzbek SSR, 1479 (22.6%) chewed *nass* (Khazanov & Fasiev, 1970).

The proportion of *nass* users in surveys carried out in Kirghiz was estimated as 5% (Paches & Djuliev, 1965).

A survey of 1569 men aged between 55 and 69 years, performed in the Samarkand region of the Uzbek SSR revealed that 636 (41%) of the men interviewed were *nass* users (Zaridze *et al.*, 1985a) and 3% both used *nass* and smoked (Zaridze et *al.*, 1985b,c).

(vii) Naswar

Naswar is widely used in Afghanistan and Pakistan. In Afghanistan, it is described as a mixture of powdered tobacco, slaked lime and indigo, and is available in ready-made form which is sold under licence from the government. Most people place it on the floor of the mouth; some put it in the labial groove behind the lower lip; and in rare cases it is placed on the dorsum of the tongue. It is kept in the mouth for about three to 10 min and then spat out; most people rinse their mouth with water after use (Mehta & Pindborg, 1968). To prepare *naswar*, water is poured into a cement-lined cavity; lime is added, followed by air-cured, dried, powdered tobacco and a colouring material, indigo. The ingredients are then thoroughly pounded and mixed. A large, heavy, wooden mallet (an ingenious contrivance raises the wooden mallet-head and brings it down heavily on the mixture) is used for thorough mixing. A number of people in rural areas make their own *naswar* in a manner similar to that used in commerce (Mehta & Pindborg, 1968).

In Pakistan, *naswar* is manufactured by mashing and blending high-nicotine, sun-cured *Nicotiana rustica* tobaccos with ashes of plants, slaked lime, water and flavouring essences, usually cardamom oil and menthol, and the product is formed into semi-dried pellets and powder. It is placed in the mouth behind the lower lip and chewed slowly. Its use dates back to the introduction of tobacco into this region (Ahmad, 1976).

Consumption in Pakistan in 1975 was estimated to be 5.0 million pounds (2.3 million kg) (Ahmad, 1976).

1.4 Manufacturing processes

(*a*) *Chewing tobacco*

Types of chewing tobacco produced by local techniques and those for which limited information was available on the manufacturing processes are described in the previous sections.

Complete details of current US manufacturing processes for plug, twist and loose-leaf tobacco were not available to the Working Group; however, a general description can be given on the basis of published reports. Usually, tobacco is aged for one to four years, during which time natural fermentation occurs (Shapiro, 1981; Rizio, 1984). Beyond this step, the techniques differ for specific products.

Loose-leaf chewing tobaccos are made with cigar leaf from Pennsylvania and Wisconsin. Following removal of the stem, the tobacco is cut into uniform strips and flavourings are added, which may include honey, liquorice and rum. The combination of ingredients and quantities vary with the brand. One sweet type contained 56% tobacco materials and 44% combined flavouring additives and moisture (Akehurst, 1981).

Plug chewing tobaccos are made with the same aged, cut and blended tobacco used in loose-leaf as well as with air- and fire-cured types (Rizio, 1984). Leaf strips may be immersed in a mixture of liquorice and some form of sugar before being pressed into the plug form. The pressed leaf blends are then wrapped in fine tobacco and moulded into flat bars. The consumer bites or cuts off a small portion of the plug and places it in the mouth.

Twist chewing tobaccos are made with air-cured types such as burley, as well as with fire-cured types; flavouring ingredients and sugar may be added. The twist is created by twisting the tobacco leaves into a shape that resembles a rope (Rizio, 1984).

In the UK, chewing tobacco is made only in plug form. It is unsweetened and usually made of fire-cured tobacco. The wrapper is a particularly fine, thin, well-textured leaf, and the moisture content of the manufactured plug is approximately 30% (Akehurst, 1981).

Chewing tobacco in India is made from *Nicotiana rustica* and *N. tabacum*. The tobacco leaves are harvested when they turn yellow and brown spots start appearing; the leaves remain in the field and are turned over occasionally to achieve uniform drying. They are then tied in bundles and moistened by sprinkling with water; the bundles are stacked for fermentation for a couple of weeks, separated and dried again. The leaves are cut into various sizes. About 85% of the tobacco harvested are consumed raw without further processing.

Nass is not produced commercially in the USSR but is produced and sold at local markets by peasants.

(*b*) *Snuff*

The quality of tobacco is dependent upon the soil and climate where it is grown, as well as the variety. The final snuff product is also influenced by such factors as harvesting and curing; and the drying process is critical to the ultimate chemical composition of the tobacco. Manufacturers may vary the composition of the final product through the use of additives, the identities of which are protected by rules of proprietary information.

(i) *USA*

In the USA, snuff is made primarily from Kentucky, Tennessee, and Virginia air- and fire-cured tobaccos. After the dark tobacco has been stalk-harvested, it may be air- or fire-cured, a process that requires several weeks. In the fire-curing process, the leaf undergoes a yellowing stage, after which brown spots start to appear; a small amount of heat is then created by lighting small fires, thus slowly reducing the humidity. When the stems have darkened, the tobacco is exposed to large volumes of heavy smoke for two to three weeks.

The number of fires is reduced, and the sawdust and floor are kept wet continuously. As soon as the firing is completed, the tobacco is gathered into piles to preserve the finish and flavour (Everette, 1958).

In general, the whole tobacco leaf is used. The leaves are aged for one to four years; snuff tobacco may be allowed to age for a greater number of years than chewing tobacco (Shapiro, 1981; Rizio, 1984). Following ageing, the tobacco is cut into strips, the size of which depends upon the product, and then undergoes fermentation for several weeks. The moisture is adjusted, and the tobacco is cut to meet product specifications. Casings (hygroscopic agents and flavouring constituents, including synthetic sweetening agents (see IARC, 1980)) may be added; however, the composition is proprietary information (Akehurst, 1981). Some products undergo a second fermentation, the temperature and duration of which are carefully monitored; this second fermentation may take several years. After these curing processes, the tobacco is ground into coarse-, medium- or fine-grained powder. The powdered tobacco is then moistened again with additives (Pöschl, 1983).

(ii) *Europe*

In Sweden, snuff is manufactured from dark Kentucky and Virginia tobaccos that are stronger than those used for cigarettes. Water and salt are added to keep the product fresh before it is heated. Various ingredients are then added, the exact composition remaining a trade secret (Hirsch, 1983).

In addition to the method used in the USA, three further processes are used in the manufacture of snuff in Europe.

In the 'Rapid' method, tobacco leaves and stems are pulverized in high-speed, blower-type crushers, sieved and then moistened with a brine solution. The tobacco is then fermented rapidly in hot rooms for six to eight weeks. It is sieved again and mixed with 5-8% fine salt and then fermented for a longer period of time. The Rapid method is the most widely used process today and produces the so-called 'green' snuffs known as *Kovno*, 'refreshment' tobacco, and Danzig types, as well as the modern English type of snuff. Menthol, peppermint oil, camphor and other aromatic additives, such as attar of roses and oil of cloves (see IARC, 1985), are blended with the tobacco. The grain size is small, much like powder. An important feature of these rapid-method snuffs is their high concentration of aromatics (Pöschl, 1983).

In the 'Paris' method, Virginia and Kentucky tobaccos are pounded in salt water and left to ferment for several years in cool storage rooms. The tobacco is then compressed into batches and subsequently crushed or pulverized by pounding machines, sieved, and remoistened with salt water. This method produces the so-called 'black' varieties, such as 'Paris' and 'Saarbrücken'. The Paris method is used in the Federal Republic of Germany and France (Pöschl, 1983).

The final type of processing is by the 'Schmalzler' method. The tobacco leaves, which come mainly from Brazil, are cut and moistened with sugar sauces prior to fermentation at high temperatures over a period of a few months. The tobacco is then dried and ground in special machines called 'grinding chairs', sieved, and then moistened with fine oils. At one time, clarified butter was used on Brazilian tobacco, leading to use of the term 'Schmalzler' (*schmalz* is the German word for lard or melted fat). The fragrance of Schmalzler tobacco results from the admixture of 'mangotes', which are ropes of tobacco that have been treated with special sugar sauces, fermented and then pressed and sewn into fresh cowhides (Pöschl, 1983).

Britain's largest snuff producer uses dark-fired tobacco leaves and stems, mostly from Malawi. The dried leaves are ground into a coarse powder in a motor-powered mortar before being reduced to a finer powder in a grinder. After potash, soda ash and pharmaceutical soda have been added to the sieved tobacco, ground menthol is blended in (Anon., 1981).

(iii) *Asia, Africa and other regions*

With the exception of India, Thailand and Turkey, almost no manufacture of snuff is carried out in Asia, eastern Europe or South America (Pöschl, 1983).

Oriental snuff is used in such countries as Thailand and differs in its preparation and constituents from that used in Europe and the USA. It consists of approximately 50% dry tobacco and 50% oriental gum with a small amount of pulverized cuttle bone. The gum is made by heating 'white earth', which contains calcium carbonate and phosphate, at high temperatures in a kiln. After the addition of water, the gum paste is mixed with tobacco and dried in the sun (Harrison, 1964).

In parts of Africa, snuff is prepared by mixing powdered tobacco leaves with ash from various incinerated plants, such as aloe, *Amaranthus spinosum* or *Turbinata oblongata*; oils and lemon juice are sometimes added (Keen *et al.*, 1955; Harrison, 1964; Baumslag *et al.*, 1971).

1.5 Production and use

(*a*) *Chewing tobacco*

(i) *Production*

US production data for the major categories of chewing-tobacco products have been reported by one source for the period 1931-1980 (US Department of Agriculture, 1984a). Selected data that show the trends of production for each category are shown in Table 3. Maxwell (1984) reported US production data under slightly different categories, as summarized in Table 4, in which certain types of fine-cut smokeless tobacco that had been classified as chewing tobacco prior to 1980 have been recategorized as moist/fine-cut snuff.

Table 3. US production of chewing tobacco by major category[a]

Year	Production (millions of kg)				
	Plug	Twist	Fine-cut	Loose-leaf	Total
1931	34.8	2.9	1.9	27.8	67.4
1942	24.6	2.7	2.3	21.9	51.7
1950	18.2	2.5	1.2	17.7	39.7
1960	12.0	1.5	1.4	14.5	29.5
1962	11.8	1.3	1.5	14.7	29.4
1974	8.3	1.0	2.8	23.3	35.5
1979	7.0	0.9	6.1	31.8	45.8
1980	7.6	0.9	6.7	32.8	48.1

[a]Data from US Department of Agriculture (1984a)

Table 4. US production of chewing tobacco by major category[a]

Year	Production (millions of kg)				
	Plug[b]	Moist plug[b]	Twist/ roll[c]	Loose-leaf	Total[d]
1981	5.1	2.9	0.9	32.0	40.9
1982	4.6	2.3	0.8	32.2	39.9
1983	4.4	2.0	0.8	32.2	39.3

[a]Data from Maxwell (1984)
[b]Data for plug plus moist plug correspond to data for plug in Table 3
[c]Data correspond to data for twist in Table 3
[d]Excludes data for moist/fine-cut tobacco, which is currently classified as snuff

Data on production of chewing tobacco in selected countries during the period 1974-1978 are given in Table 5.

Table 5. Production of chewing tobacco in selected countries[a]

Country	Production (thousands of kg)				
	1974	1975	1976	1977	1978
Algeria			4045.7	4220.2	
Austria			8.9	5.2	
Belgium	7.1	6.0	4.8	3.9	3.3
Canada			230.6	197.8	
Denmark	102.0	99.0	90.0	84.8	80.5
Egypt			52.0	50.0	
Finland			1.3	1.2	
France			133.6	131.0	
Libyan Arab Jamahiriya			80.3	79.3	
Mexico			1000.0	1050.0	
Netherlands	313.0	288.0	253.0	253.0	
Pakistan			2344.0	3954.9	
South Africa	158.5	185.3	181.1	158.7	139.9
Sweden	13.0	12.0	12.0	13.0	13.0
Tunisia			705.3	752.9	

[a]Data from Anon. (1978, 1979)

Available statistics on production and exports of chewing tobacco for India in recent years are shown in Table 6.

Table 6. Available statistics on production and exports of chewing tobacco for India (millions of kg)[a]

	1977-1978	1978-1979	1979-1980	1980-1981	1981-1982
Production	70.8	70	72	85.3	85
Export	4.0	8.9	9.2	7.0	-

[a]From Sinha (1984)

Although a major portion of the chewing tobacco produced in India is used internally, considerable quantities of scented tobacco and *zarda* are exported to a large number of countries, particularly to Arab countries; Saudi Arabia is the major importer of Indian chewing tobacco. It is also exported to Bahrain, Belgium, Dubai, Egypt, Israel, Kuwait, the Maldives, Nepal, Oman, Qatar, Singapore, Somalia, the UK and the USA (Sinha, 1984).

(ii) *Use*

In 1880, the earliest recorded year, US per-capita consumption of chewing tobacco was 3.15 pounds (1.43 kg) per person aged 14 years or over (Wynder *et al.*, 1957a). The highest US per-capita consumption for persons aged 15 years or over occurred in 1900, at 4.1 pounds (1.9 kg), followed by a gradual decrease to 0.5 pounds (0.23 kg) in 1962 (Schuman, 1977). Per-capita consumption by males 18 years and older was 1.05 pounds in 1966, increasing to 1.34 pounds (0.61 kg) in 1979 (US Department of Agriculture, 1980). In 1982, some chewing-tobacco products were reclassified as snuff. Under this new classification, male per-capita consumption was 1.06 pounds (0.48 kg) in 1983 (US Department of Agriculture, 1984b).

Data on total use of chewing tobacco have been reported for various countries for the period 1920-1973 (International Trade Centre, 1968; Wilson, 1975). Selected data on trends in individual countries are shown in Table 7. Additional data on sales in four countries during 1974-1978 are given in Table 8.

Estimated per-capita consumption of chewing tobacco in selected countries is summarized in Table 9.

(*b*) *Snuff*

(i) *Production*

Data for production of snuff in selected countries during the period 1974-1978 are given in Table 10 (Anon., 1978, 1979).

The production of snuff in the USA increased from 4 million pounds (1.8 million kg) to more than 40 million pounds (18 million kg) between 1880 and 1930 (Garner, 1951). US production of snuff for the period 1931-1980 has been reported by the US Department of Agriculture (1984a). Selected data to show the trends are shown in Table 11. Maxwell (1984) reported US production data under different categories, as summarized in Table 12, in which certain types of fine-cut smokeless tobacco that had been classified as chewing tobacco prior to 1982 have been recategorized as moist/fine-cut snuff.

The Federal Republic of Germany is the largest producer of nasal snuff (about 250 tonnes per year), followed by the UK (about 150 tonnes per year), and France and Italy (less than 100 tonnes per year) (Pöschl, 1980).

In the Federal Republic of Germany, manufacturers of snuff produce many varieties and flavours, of which the best known type is Bavarian 'Schmalzler'. Most of the snuff produced in Germany is flavoured with menthol. Snuff for nasal use is produced chiefly in Bavaria, particularly in Landshut, where some 70% of German snuff manufacturing is located (Pöschl, 1983).

Table 7. Tobacco use in various countries (millions of kg)[a]

Year	Austria	Brazil		Canada	Denmark	France	Germany (Fed. Rep.)[b]	India	Norway	Sweden[c]
	Chewing tobacco	Cut tobacco	Plug tobacco	Plug tobacco	Chewing tobacco	Chewing tobacco	Chewing tobacco	Chewing tobacco	Chewing tobacco	Chewing tobacco
1920	-	-	-	3	1.4	-	-	-	-	0.4
1922	-	-	-	4.6	0.9	-	-	-	-	0.3
1930	0.4	-	-	2.7	0.9	-	-	-	0.9	0.1
1940	0.3	-	-	1.4	0.6	0.5	-	-	0.5	0.1
1945	0.05	1.1	41	1.4	0.7	0.4	-	-	0.2	0.04
1948	0.05	1.1	41.3	1.0	0.4	0.7	-	56.6	0.4	0.04
1950	0.2	1.1	41.4	1.0	0.4	0.6	-	58.5	0.3	0.04
1955	0.1	0.9	41.6	0.7	0.3	0.5	-	53.7	0.2	0.04
1960	0.05	1.3	40.8	0.5	0.3	0.6	-	60.4	0.2	0.04
1962	0.05	1.5	39.2	0.5	0.2	0.5	1.5	64	0.2	0.04
1963	0.05	1.6	38.4	0.4	0.2	0.5	1.4	54.2	0.1	0
1964	0.05	1.7	37.6	0.4	0.2	0.5	1.2	53.8	0.1	0
1965	0.05	1.8	36.8	0.4	0.2	0.5	1.3	53.7	0.1	0
1966	0.05	1.6	35.9	0.4	0.2	0.5	1.3	53.3	0.1	0
1973	0	2.2	29.3	0.3	0.1	0.5	-	43.4	0.1	0

[a]Data from Wilson (1975), unless otherwise specified

[b]Data from International Trade Centre (1968)

[c]Svenska Tobaks Ab (1964, 1973) reported that use in Sweden was less than 50 tonnes in all years from 1947 on, and the Swedish Tobacco Co. (1983) reported that consumption of chewing tobacco in Sweden was 15 tonnes in 1973 and 23.3 tonnes in 1982.

Table 8. Sales of chewing tobacco in selected countries (thousands of kg)[a]

Country	1974	1975	1976	1977	1978
Netherlands	313.0	293.0	256.0	263.0	-
Norway	85.0	63.0	69.0	69.0	59.0
South Africa	152.5	185.3	181.1	158.7	139.9
Sweden	1.6	1.7	2.4	2.0	1.9

[a]Data from Anon. (1979)

Table 9. Estimated per-capita consumption (g) of chewing tobacco in selected countries[a]

Country	1962	1963	1964	1965	1966
Austria	8	8	8	8	8
Canada	41	36	35	32	31
Denmark	68	66	66	51	50
France	16	14	15	14	14
Germany, Federal Republic of	37	34	30	33	32
Norway	67	52	50	50	50
Sweden	8	-	-	-	-

[a]Data from International Trade Centre (1968)

Table 10. Production of snuff in selected countries (thousands of kg)[a]

Country	1974	1975	1976	1977	1978
Algeria	-	-	745.3	992.1	-
Austria	-	-	2.9	2.0	-
Belgium	6.4	5.2	3.6	3.0	2.9
Canada	594.2	553.6	585.5	570.5	565.9
Denmark	241.0	240.0	223.0	215.3	202.9
Egypt	-	-	22.0	9.0	-
Finland	-	-	13.2	14.2	-
France	-	-	80.0	70.0	-
Germany, Federal Republic of	-	-	292.0	294.0	-
Ireland	11.0	10.0	10.0	9.0	9.0
Israel	-	36.5	35.2	35.2	33.0
Italy	145.6	107.2	126.4	150.8	85.0
Libyan Arab Jamahiriya	-	-	11.6	15.0	-
Morocco	-	-	60.6	66.1	-
Pakistan	-	-	4501.0	4082.0	-
South Africa	1391.0	1474.1	1495.0	1363.8	1244.2
Sweden	2831.0	2917.0	3241.0	3523.0	3468.0
Switzerland	-	-	10.0	8.3	-
USA	-	-	11271.0	11164.0	-

[a]Data from Anon. (1978, 1979)

Table 11. US production of snuff[a]

Year	Production[b] (millions of kg)
1931	18.1
1935	16.4
1943	19.6
1945	19.9
1950	18.2
1960	15.7
1965	13.0
1975	11.4
1980	10.9

[a]Data from US Department of Agriculture (1984a)
[b]Prior to 1982, some moist/fine-cut snuff was classified as chewing tobacco.

Table 12. US production of snuff by major category[a]

Year	Production (millions of kg)		
	Dry snuff	Moist/fine-cut[b] snuff	Total
1981	5.3	13.8	19.2
1982	5.1	14.9	20.0
1983	4.9	15.9	20.7

[a]Data from Maxwell (1984)
[b]Prior to 1982, some moist/fine-cut snuff was classified as chewing tobacco.

World production of snuff is estimated to be 20 million kg per year. Production of snuff tobacco for nasal use amounts to only about one million kg per year at the most. The remainder is used for *snus* and *souffi* used in Scandinavia and North Africa, respectively, and 'moist snuff' used in the USA; these are not the same forms of snuff tobacco that are used nasally and are meant for oral use (Pöschl, 1983).

(ii) *Use*

Data on total use of snuff have been reported for various countries for the period 1920-1973 (Wilson, 1975) and for Sweden for the period 1973-1982 (Swedish Tobacco Co., 1980, 1981, 1982, 1983). Selected data on trends in individual countries are shown in Table 13. Additional data on sales in seven countries for the period 1974-1978 are given in Table 14.

In 1880, the earliest recorded year, US consumption of snuff was 0.12 pounds (0.05 kg) per person aged 14 years or over (Wynder *et al.*, 1957a). The highest US per-capita consumption of snuff for persons aged 15 years or over occurred in the period 1910-1920 at 0.50 pounds (0.2 kg). Consumption *per capita* decreased steadily but slowly from 1920 to 1962, to 0.26 pounds (0.12 kg) (Schuman, 1977). Per-capita consumption for persons aged 18 years and over was 0.23 pounds (0.10 kg) in 1966 and decreased to 0.15 pounds (0.07 kg) in 1979 (US Department of Agriculture, 1980). In 1982, some chewing tobacco products were reclassified as snuff. Under this new classification, US per-capita consumption (18 years and over, including overseas forces) of snuff was 0.26 pounds (0.12 kg) in 1982 and 1983 (US Department of Agriculture, 1984b).

Table 13. Snuff use in various countries (millions of kg)[a,b]

Year	Austria	Canada	Denmark	Finland	France	India	Ireland	Italy	Morocco	Norway	South Africa	Sweden[c]	United Kingdom
1920	-	0.3	0.2	0.04	-	-	0.1	-	-	-	0.04	6.5	-
1928	0.1	0.4	0.3	0.1	-	-	0.1	-	-	0.4	0.04	5.0	0.4
1932	0.1	0.4	0.4	0.04	2.5	-	0.1	-	-	0.4	-	4.9	0.4
1940	0.1	0.4	0.5	0.04	1.4	-	0.1	-	0.3	0.5	0.1	3.9	0.4
1945	0	0.4	0.5	0	0.9	-	0.1	-	0.4	0.3	0.1	3.5	0.5
1950	0.04	0.4	0.5	0.04	0.8	2.5	0.04	-	0.4	0.5	0.1	3.1	0.3
1955	0	0.4	0.4	0.04	0.6	4.4	0.04	-	0.4	0.5	0	2.9	0.3
1960	0	0.4	0.4	0	0.4	5.5	0.04	0.4	0.4	0.4	0	2.7	0.3
1965	0	0.4	0.4	0.04	0.3	4.3	0	0.3	0.4	0.4	0.1	2.5	0.2
1970	0	0.4	0.3	0.04	0.2	4.5	0	0.2	0.4	0.3	0.1	2.5	0.2
1973	0	0.4	0.2	0	0.1	4.3	0	0.1	-	0.3	0.1	2.7	0.2

[a]Data from Wilson (1975)

[b]Other data reported by Wilson (1975) include the following (millions of kg): Argentina, 0.1 in 1951, 0.0 in all other years (1940-1973); Barbados, 0.004 from 1964-1972, 0.0 in 1973; Iceland, 0.02-0.04 from 1932-1973; Portugal, 0.04 from 1940-1953 and in 1957, 0.0 in all other years (1954-1956, 1958-1972)

[c]The Swedish Tobacco Co. (1983) reported that snuff use in Sweden increased from 2.7 million kg in 1973 to 3.4 million kg in 1982.

Table 14. Sales of snuff (thousands of kg)[a]

Country	1974	1975	1976	1977	1978
Australia	0.8	1.2	0.8	1.0	1.4
Canada	660.0	579.4	534.3	560.8	570.2
Ireland	10.9	10.1	9.5	8.5	9.6
Italy	142.9	126.5	122.8	110.8	104.9
Norway	283.0	263.0	267.0	283.0	268.0
South Africa	1391.0	1474.1	1495.0	1363.8	1244.2
Sweden	2812.0	2943.0	3189.0	3361.0	3442.0

[a]Data from Anon. (1979)

Use of snuff in Pakistan is declining and amounted to only 0.6 million pounds (0.3 million kg) in 1975. It was estimated that there would be a further decline to 0.4 million pounds (0.18 million kg) by 1980 (Ahmad, 1976).

Estimated per-capita consumption of snuff in selected countries is summarized in Table 15.

Table 15. Estimated per-capita consumption (g) of snuff in selected countries[a]

Country	1962	1963	1964	1965	1966
Canada	33	29	32	32	27
Denmark	12	12	12	10	10
France	10	9	9	9	7
Ireland	21	20	-	-	-
Italy	11	10	9	8	7
Norway	167	167	161	146	146
Sweden	430	420	420	410	400
UK	9	9	9	9	8

[a]Data from International Trade Centre (1968)

Estimated annual usage of nasal snuff in various countries is as follows (thousands of kg): the Federal Republic of Germany, 300; UK, 200; France and Italy, less than 100 each. Other countries in which it is used include Belgium, the German Democratic Republic, Switzerland and South Africa (Pöschl, 1983). Use of snuff in Austria and the eastern European countries is comparatively low, and use is relatively rare in the Near East.

2. Chemical Data Relevant to the Evaluation of Carcinogenic Risk to Humans

There is a great deal of literature on the chemistry of tobacco, most of which refers to *Nicotiana tabacum*, utilized in western Europe and North America.

At least 2549 individual constituents have been identified in tobacco (Dube & Green, 1982). This number includes the tobacco constituents themselves as well as chemicals that

are applied to tobacco during cultivation, harvesting and processing. Major classes of compounds identified in tobacco are aliphatic and aromatic hydrocarbons, aldehydes, ketones, alcohols, phenols, ethers, carboxylic acids, esters, anhydrides, lactones, carbohydrates, amines, amides, imides, nitriles, *N*- and *O*-heterocyclic compounds and chlorinated organic compounds, and at least 35 metal compounds (Wynder & Hoffmann, 1967; Stedman, 1968; Tso, 1972; Enzell *et al.*, 1977; Schmeltz & Hoffmann, 1977; Davis *et al.*, 1981; Dube & Green, 1982).

2.1 Aliphatic hydrocarbons

Waxy leaf coatings are almost universal throughout the plant kingdom. The major wax constituents are alkanes, alkenes, alcohols, carboxylic acids, esters, aldehydes and ketones. In tobacco, the alkanes (non-volatile aliphatic hydrocarbons) consist primarily of compounds with chain lengths of C_{25}-C_{35}. They comprise a homologue series of normal (*n*), iso (*i*, 2-methyl) and ante-iso (*a*, 3-methyl) saturated hydrocarbons (Mold *et al.*, 1963). Table 16 presents the percentages of non-volatile hydrocarbons in various tobaccos.

Table 16. Aliphatic hydrocarbons in tobacco[a]

Hydrocarbon	Kentucky reference cigarette[b]	Flue-cured tobacco[c]	Cigarette tobacco blend[d]	Flue-cured tobacco[e]
n-C_{25}	0.76	0.10	1.71	0.80
n-C_{26}	0.33	0.42	0.83	0.44
n-C_{27}	5.67	6.56	7.73	5.67
a-C_{28}	0.27	0.40	0.13	1.29
n-C_{28}	0.61	0.74	0.89	
i-C_{29}	1.82	2.03	1.24	9.22
n-C_{29}	6.16	5.41	6.72	
a-C_{30}	6.46	7.30	5.65	9.79
n-C_{30}	2.17	2.49	3.16	
i-C_{31}	12.81	14.19	10.92	39.51
n-C_{31}	23.81	21.31	26.30	
a-C_{32}	12.89	15.25	13.02	16.93
n-C_{32}	3.85	4.23	4.88	
i-C_{33}	6.26	6.96	15.62	15.13
n-C_{33}	12.81	10.00	10.77	
a-C_{34}	1.14	1.21	1.15	1.09
n-C_{34}	0.33	0.28	-	
i-C_{35}	-	0.16	-	
n-C_{35}	0.43	0.28	-	

[a]The values in this table represent the percentile composition of the paraffin fraction.

[b]From Chortyk *et al.* (1975). Paraffin fraction, as its percentile composition of the tobacco blend, was not specified

[c]From Chortyk *et al.* (1975). Paraffin fraction represents 0.36% of the flue-cured tobacco

[d]From Mold *et al.* (1963). Paraffin fraction represents 0.20-0.28% of the cigarette blend

[e]From From Severson *et al.* (1981). Total *n*, *i*- and *a*-isomers represent 0.15% of the flue-cured tobacco

2.2 Isoprenoids

The typical aroma of the tobacco leaf is created during post-harvest treatment. The major contributors to the aroma are isoprenoids (Rowland & Roberts, 1963; Demole & Enggist, 1976; Enzell *et al.*, 1977; Enzell & Wahlberg, 1980). The most prevalent acyclic isoprenoids

are solanesol, solanesenes, solanone, phytone, neophytadiene and norphytene. In processed tobacco, the most abundant of these, neophytadiene, which originates from chlorophyll *via* phytol, can occur in amounts of up to 0.2% (Wynder & Hoffmann, 1967; Enzell & Wahlberg, 1980; Severson *et al.*, 1981). Solanesol occurs in tobacco in free form (0.4%) and as fatty acid esters, predominantly palmitate and linoleate (Wynder & Hoffmann, 1967). In addition to the isoprenoids, hundreds of acyclic and cyclic isoprenoids have been identified in processed tobacco (Enzell & Wahlberg, 1980), the most abundant being duvatrienediols, levantenolides and β-carotene (Wynder & Hoffmann, 1967; Enzell & Wahlberg, 1980).

2.3 Phytosterols

The most widely distributed sterols in higher plants are C_{29}-sterols with a 3-hydroxy group-$\Delta^{5,6}$ unsaturation, stigmasterol and sitosterols. The sterols in tobacco are free alcohols, esters and glycosides (Wynder & Hoffmann, 1967; Tso, 1972; Schmeltz *et al.*, 1975; Davis, 1976; Enzell *et al.*, 1977). Table 17 presents data on the four major phytosterols, which amount to 0.15-0.2% (dry weight) of some processed tobacco types.

Table 17. Phytosterols in processed tobacco (μg/g)[a]

Phytosterol	Tobacco type	Free alcohol	Esters and glycosides	Total steroids
Cholesterol	Cigarette blend	129	45	174
	Cigar filler	155	131	286
	Flue-cured L-1	151	25	176
	Flue-cured L-2	165	49	214
Campesterol	Cigarette blend	197	131	328
	Cigar filler	226	213	439
	Flue-cured L-1	192	36	228
	Flue-cured L-2	241	88	329
Stigmasterol	Cigarette blend	480	94	574
	Cigar filler	584	414	998
	Flue-cured L-1	677	43	720
	Flue-cured L-2	715	108	823
Sitosterol	Cigarette blend	288	181	469
	Cigar filler	329	310	639
	Flue-cured L-1	313	71	384
	Flue-cured L-2	662	278	940
Total sterols	Cigarette blend	1094	451	1545
	Cigar filler	1294	1068	2362
	Flue-cured L-1	1333	175	1508
	Flue cured L-2	1783	523	2306

[a]Data for cigarette blend and cigar filler (wet weight, 11.7%) from Schmeltz *et al.* (1975); data for flue-cured tobacco (dry weight) from Davis (1976)

2.4 Polynuclear aromatic hydrocarbons (PAHs)

Although it has been known for a long time that tobacco smoke contains naphthalene and PAHs (IARC, 1983a), it is less well known that traces of these aromatic hydrocarbons are also found in processed tobaccos (Wynder & Hoffmann, 1967). Whereas naphthalene and its alkyl derivatives may be formed from certain cyclic isoprenoids during tobacco processing, especially during flue-curing, the presence of PAHs in processed tobacco at the part-per-billion level is more likely to be due to contamination with ambient air pollutants or pollu-

tants from heating sources used for the curing process. One US cigarette blend contained 170 μg/kg naphthalene, 18 μg/kg 1-methylnaphthalene and 42 μg/kg 2-methylnaphthalene (Schmeltz *et al.*, 1976). Certain types of tobaccos are treated with wood smoke in order to enhance the flavour of the resulting tobacco smoke. For a processed Latakia tobacco, the following data were reported: 1.1 mg/kg naphthalene, 2.8 and 2.6 mg/kg 1- and 2-methylnaphthalene, respectively, 1.4 mg/kg 1- and 2-ethylnaphthalene, 28.6 mg/kg of 10 isomeric dimethylnaphthalenes, 7.8 mg/kg of 11 isomeric ethylmethylnaphthalenes and 24.4 mg/kg of seven isomeric trimethylnaphthalenes (Nicolaus & Elmenhorst, 1982).

Campbell and Lindsey (1956) reported up to 0.3 mg/kg acenaphthalene, 3 mg/kg phenanthrene, 1.1 mg/kg anthracene, 0.8 mg/kg pyrene, 0.15 mg/kg fluoranthene and 0.08 mg/kg benzo[*a*]pyrene in processed tobaccos. Significantly higher levels (6.6 mg/kg phenanthrene) were found in a dark pipe tobacco. Onishi *et al.* (1957) reported levels as high as 5 mg/kg phenanthrene, 4.2 mg/kg anthracene, 1.8 mg/kg pyrene and 1.4 mg/kg fluoranthene in a Japanese burley leaf.

In a UK snuff, Campbell and Lindsey (1977) found 260 μg/kg pyrene, 335 μg/kg fluoranthene and 7.2 μg/kg benzo[*a*]pyrene. Cooper and Campbell (1955) reported the following levels of PAHs in Zulu snuff: 50-70 μg/kg anthracene, 560-580 μg/kg pyrene, 800 μg/kg fluoranthene, 250-270 μg/kg benzo[*a*]pyrene and 140 μg/kg benzo[*ghi*]perylene; and in Vendu snuff, the following values were found: anthracene, 10 μg/kg; pyrene, 80-90 μg/kg; fluoranthene, 120-150 μg/kg; and benzo[*a*]pyrene was present. In Indian snuff and *mishri*, PAHs occurred in amounts similar to those reported in other studies (Bhide *et al.*, 1984a).

2.5 Alcohols

Tobacco contains isoprenoids with one or more alcoholic functional groups, such as solanesol, duvatriene diols and phytol (see p. 00) and a number of sterols. In addition, long-chain alcohols, eicosanol ($C_{20}H_{41}OH$), docosanol ($C_{22}H_{45}OH$) (Tso, 1972), tetracosanol ($C_{24}H_{49}OH$) and octacosanol ($C_{28}H_{57}OH$), were identified (Severson *et al.*, 1978). Levels of alcohols and sterols in four varieties of flue-cured tobacco are given in Table 18 (Higman *et al.*, 1979). Dark air-cured tobaccos, such as those used for snuff, were also found to contain 6-methyl-5-hepten-2-ol, 3,7-dimethyl-1,6-octadien-3-ol (linalool), 2-buten-2-ol, 2-methyl-1-penten-3-ol, 3-methyl-2-buten-1-ol, 3-methyl-1-penten-3-ol, 2,6-dimethylcyclohexanol, benzyl alcohol and phenylethanol (Davis *et al.*, 1981).

Table 18. Alcohols and sterols (mg/kg) in flue-cured varieties of tobacco[a]

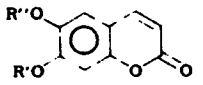
Compound	Concentration
Terpenes and alcohols	
Phytol	149-192
Docosanol	61-123
β-Amyrin	47-242
Cycloartenol	83-277
Solanesol and sterols	
Solanesol	0.88-22.4
Cholesterol	0.09-0.21
Campesterol, Stigmasterol	0.41-0.98
Sitosterol	0.23-0.71

[a]NC2326, SC1971, Coker 139 and Speight G-28 (Higman *et al.*, 1979)

2.6 Phenols and phenolic acid

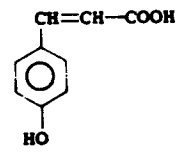
Tobacco-leaf phenols comprise coumarins, caffetannins and flavonoids (Fig. 1). The percentage contents of the two major tobacco polyphenols, chlorogenic acid and rutin (for information on the aglycone of rutin, quercetin, see IARC, 1983b) in a number of processed tobaccos are given in Table 19 (Häusermann & Waltz, 1962). In addition, simple phenols, derived partially from hydrolysis of the polyphenols, have been isolated from processed tobacco (Fig. 1). Furthermore, processed tobacco contains phenol, cresols, dimethyl-

Figure 1. Major polyphenols and precursors in tobacco[a]

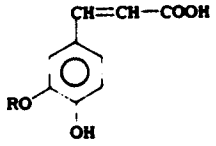


A. Coumarins

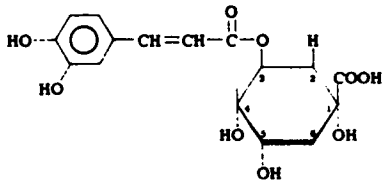
(I) Scopoletin	R' = H	R'' = CH_3
(II) Scopolin	R' = *d*-Glucosyl	R'' = CH_3
(III) Fabiatin	R' = Primverosyl (xylosido-*d*-glucosyl)	R'' = CH_3
(IV) Scopoletin-β-gentiobioside	R' = 6-Glucosido-*d*-glucosyl	R'' = CH_3
(V) Esculetin	R' = H	R'' = H
(VI) Cichoriin	R' = *d*-Glucosyl	R'' = H



Melilotic acid
(I)

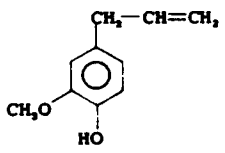


(II) Caffeic acid R = H
(III) Ferulic acid R = CH_3

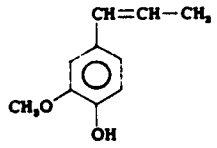


B. Caffetannins

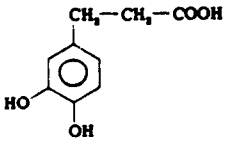
(VII) Chlorogenic acid (3-*O*-caffeoyl-*d*-quinic acid)
(VIII) Neochlorogenic acid (5-*O*-caffeoyl-*d*-quinic acid)



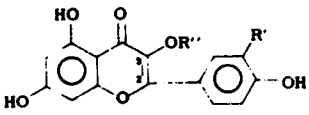
Eugenol
(IV)



Isoeugenol
(V)

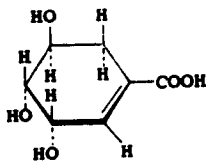


Dihydrocaffeic acid
(VI)

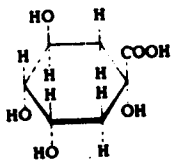


C. Flavonoids

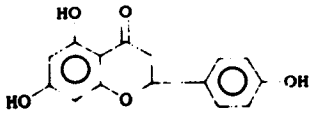
(IX) Rutin	R' = OH	R'' = Rutinosyl (*l*-rhamnosido-*d*-glucosyl
(X) Isoquercetin	R' = OH	R'' = *d*-Glucosyl
(XI) Kaempferol-3-rhamnoglucoside	R' = H	R'' = *l*-Rhamnosido-*d*-glucosyl
(XII) Naringin	R' = H	R'' = *l*-Rhamnosyl



Shikimic acid
(VII)



Quinic acid
(VIII)



Naringenin
(XIII)

[a]From Wynder and Hoffmann (1967)

phenols and other volatile phenols (up to 30 μg/g tobacco) (Lipp, 1965). These volatile phenols appear to be present in relatively high concentrations in fire-cured dark tobaccos that are used for snuff (Davis *et al.*, 1981); it is possible that they originate partially from pollution.

Table 19. Chlorogenic acid and rutin (%) in processed tobaccos[a]

Tobacco	Chlorogenic acid	Rutin
Air-cured tobaccos		
Maryland	0.15-0.28	0.08
Burley	0.0-0.46	
Bright and oriental tobaccos		
Virginia - USA	2.32-3.4	0.24
Virginia - Rhodesia	3.20	
Virginia - Italy	3.10	
Oriental	1.60	1.29
Tobacco blends		
Oriental - mixture	1.49-1.58	
Oriental - Virginia	1.85	
Virginia	2.38	
USA - cigarette blend	1.03	

[a]From Häusermann and Waltz (1962)

In addition to melilotic acid, caffeic acid, dihydrocaffeic acid, shikimic acid and quinic acid (Wynder & Hoffmann, 1967; Stedman, 1968; see Fig. 1), tobacco leaf contains 3,4-dihydroxybenzoic acid, 2-hydroxybenzoic acid (salicylic acid), 3,5-dimethoxy-4-hydroxycinnamic acid (sinapic acid) and hydroxynaphthoic acids (Snook *et al.*, 1981). These phenolic acids are genuine tobacco constituents and are, at least partially, biosynthetic intermediates of polyphenols.

2.7 Carboxylic acids

More than 80 organic acids have been identified in tobacco (Wynder & Hoffmann, 1967; Stedman, 1968; Tso, 1972; Spears & Jones, 1981). The concentrations of the major volatile carboxylic acids in bright, oriental (Ismir), burley and Japanese varieties are listed in Table 20 (Wynder & Hoffmann, 1967). The free fatty-acid content amounts to 0.08-0.4% of the leaf tobacco. The specific flavour of oriental tobacco leaves has been attributed to β-methylvaleric acid, which occurs in these tobacco types in significantly higher concentrations than in the others (Kaburaki *et al.*, 1969). Table 21 shows the composition of the fraction of non-volatile fatty acids, which constitute 0.09-0.43% of the leaf (Hoffmann & Woziwodzki, 1968). The major dicarboxylic acids in tobacco are malonic, oxalic, malic and citric acids. After processing, burley tobacco and cigar-type tobaccos, used for chewing tobaccos, have especially high levels of malic and citric acids (Jarboe & Quinn, 1960).

Table 20. Concentration of volatile carboxylic acids in tobacco leaves (mg/kg dry tobacco leaves)[a]

Volatile acid	Bright tobacco		Japanese tobacco types				Burley tobacco	Oriental tobacco	
	Japan	USA	Shifu L.	Matsukawa L.	Daruma L.[c]	Ibusuki L.	Mito No. 3[c]	Turkey	Greece
Formic	208	456	68.9	32.8	107	88.5	161	664	456
Acetic	1378	1552	51.0	36.1	70.1	191	75.5	1423	1075
Propionic	25.2	21.0	1.4	1.3	1.7	2.1	2.4	24.8	21.2
Isobutyric	2.4	2.5	-	-	-	-	-	13.9	11.4
n-Butyric	1.4	2.8	-	0.3	-	-	0.3	8.8	1.8
α-Methylbutyric	9.1	7.8	0.7	0.3	4.0	1.6	2.9	26.2	31.0
Isovaleric	7.7	6.6	0.5	0.3	0.5	5.8	1.2	24.8	33.7
Crotonic	6.2	5.4	-	-	0.6	-	0.4	8.3	6.0
n-Valeric	2.7	4.2	-	-	0.2	-	0.3	2.2	2.1
β-Methylvaleric	8.4	1.2	6.1	0.7	5.7	26.2	-	83.0	115.6
Caproic	1.7	12.2	0.7	0.7	0.7	0.2	0.9	5.3	6.6
α-Furoic	31.7	14.0	-	-	0.5	7.5	-	20.8	16.6
Benzoic	16.2	10.8	10.6	13.3	3.7	3.4	26.0	16.4	22.0
Phenylacetic	7.8	8.1	2.3	2.7	1.3	-	5.1	21.1	16.2
TOTAL	1706.5	2104.6	142.2	88.5	196.0	326.3	276.0	2342.6	1815.2

[a]From Wynder and Hoffmann (1967) unless otherwise specified

[b]From Kaburaki and Sato (1972)

Table 21. Concentration of free non-volatile fatty acids (μg/g) in tobacco[a]

Acid	Tobacco[b]					
	Turkish I	Turkish II	Bright	Maryland	Burley	Blend
Myristic	220	150	65	Trace	-	180
Palmitic	1480	530	930	420	220	530
Palmitoleic	300	160	Trace	-	-	Trace
Stearic	520	480	330	180	110	280
Oleic	480	220	230	110	70	220
Linoleic	880	320	610	250	180	420
Linolenic	2120	870	2130	420	360	1160
TOTAL	6000	2730	4300	1380	940	2790

[a]From Hoffmann and Woziwodzki (1968)

[b]Dried tobacco: moisture content between 0.5-1.0%

2.8 Amines and amides

Processed tobacco contains 27 volatile amines, 11 aromatic amines and more than 50 *N*-heterocyclic compounds, such as pyrroles, pyrrolidines, imidazoles, pyridines and pyrazines (Schmeltz & Hoffmann, 1977). Of special relevance to tobacco carcinogenesis are secondary amines, which can give rise to *N*-nitrosamines during curing, fermentation and ageing. This group includes dimethylamine, di-*n*-butylamine and pyrrolidine (Table 22). Of importance is the observation that nitrogen-containing compounds, including nitrates, amines, amides and proteins, comprise up to 24% of cured and fermented cigar tobaccos, from which many smokeless-tobacco products are made, while they make up only 15.5% of cigarette tobaccos (Wynder & Hoffmann, 1967; Tso, 1972). Some of these nitrogen-containing compounds are known precursors of *N*-nitrosamines.

Table 22. Secondary amines identified in tobacco[a]

Aliphatic amines
n-Butylisobutylamine
Di-*n*-butylamine
Di-*sec*-butylamine
Diethylamine
Dimethylamine
Di-*n*-propylamine
Ethylmethylamine
Methyl-*n*-butylamine
Methylisoamylamine
Methylisopropylamine
Methylpropylamine

Aromatic amines
N-Ethylaniline
N-Methylaniline (*ortho*-toluidine)
N-Methyl-2-toluidine
Tyramine

Pyrrolidines and pyrrolines
2-Methylpyrrolidine-3-carboxaldehyde
Pyrrolidine
Δ^3-Pyrroline

[a]From Schmeltz and Hoffmann (1977)

The acyclic and cyclic amides identified in tobacco include *N*,*N*-dimethylacetamide, maleic imide and *N*-methylnicotinic amide (Schmeltz & Hoffmann, 1977). Although these secondary amides could give rise to *N*-nitrosamides, none of them has as yet been detected in smokeless-tobacco products. It appears that the general instability of the *N*-nitrosamides in moist matrices and the lack of a highly sensitive analytical method for nitrosamides are the major reasons for the scarcity of information on the presence of these compounds in chewing tobacco and snuff.

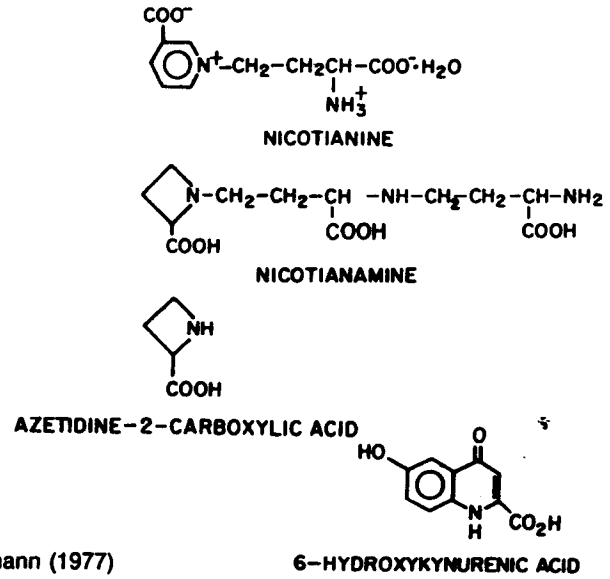
Tobacco contains many free amino acids, which are listed in Table 23 and Figure 2.

Table 23. Free amino acids identified in tobacco[a]

α-Alanine	6-Hydroxykynurenic acid
β-Alanine	Hydroxyproline
D-Alanyl-*D*-alanine	Isoleucine
α-Aminoadipic acid	Leucine
α-Aminobutyric acid	Lysine
γ-Aminobutyric acid	Methionine
Arginine	Methionine sulphone (methionine *S*-oxide)
Asparagine	1-Methylhistidine
Aspartic acid	Nicotianamine
Azetidine-2-carboxylic acid	Nicotianine
Betaine	Nicotinamide
Choline	Nicotinic acid
Citrulline	Norleucine
Cysteic acid	Ornithine
Cysteine	Phenylalanine
Cystine	Pipecolic acid
Glutamic acid	Proline
Glutamine	Pyrrolidine-2-acetic acid
α-L-Glutamyl-L-glutamic acid	Serine
Glutathione	Taurine
Glycine	Threonine
Histidine	Tryptophan
Homocystine	Tyrosine
Homoserine	Valine

[a]From Schmeltz and Hoffmann (1977)

Figure 2. Free amino acids identified in tobacco[a]

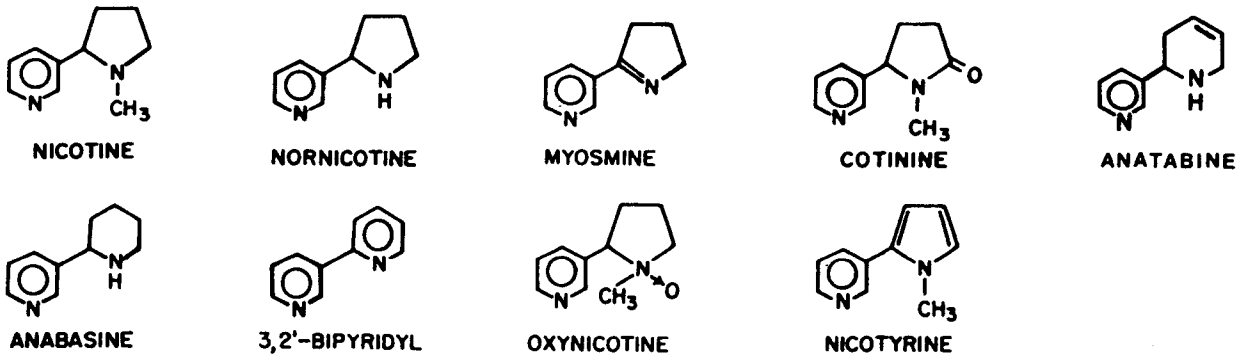


[a]From Schmeltz and Hoffmann (1977)

2.9 Alkaloids

Nicotine dependence is now widely held to be the prime factor in the worldwide popularity of tobacco products, including the acceptance of tobacco chewing and oral or nasal use of snuff. Tobaccos contain 0.5-5% of alkaloids (Schmeltz & Hoffmann, 1977), depending on regional customs and preferences for smokeless-tobacco products. At least 85% of the total *Nicotiana* alkaloids are nicotine (Piade & Hoffmann, 1980), almost exclusively present in the L(-) form (Schmeltz & Hoffmann, 1977), which is the pharmacologically active isomer. (The asymmetric centre is the C-2'-carbon of the pyrrolidine ring; Fig. 3.) The remainder of the alkaloid portion of tobacco consists of the minor *Nicotiana* alkaloids, some of which are also presented in Figure 3. A number of studies have shown, in recent years, that the methyl group on the pyrrolidine-ring nitrogen can be replaced by a formyl, acetyl or other acyl group with six or eight carbons (Enzell *et al.*, 1977). Nicotine also occurs as nicotine-*N'*-oxide in chewing tobaccos. Most secondary amines, such as anabasine and anatabine, can be methylated to tertiary amines (Schmeltz & Hoffmann, 1977); however, methylanabasine and methylanatabine rarely amount to more than 0.1% of smokeless-tobacco products. *Nicotiana rustica* differs from *N. tabacum* in the quantitative composition of its alkaloid fraction, in that, generally, nicotine, anabasine and nornicotine are present in higher concentrations (Shmuk, 1953; Sisson & Severson, 1984). The contents of the major alkaloids in four tobacco types in comparison to those in a tobacco blend, the reference cigarette IR1 of the University of Kentucky, are given in Table 24 (Piade & Hoffmann, 1980).

Figure 3. Major tobacco alkaloids[a]



[a]From Schmeltz and Hoffmann (1977)

Table 24. Alkaloid content of various tobacco brands (mg/kg, dry basis)[a]

Alkaloid	Dark commercial tobacco		Burley	Bright	Kentucky ref. (IRI)[b]
	A	B			
Nicotine	11 500	10 000	15 400	12 900	21 100
Nornicotine	550	200	630	210	630
Anatabine	360	380	570	600	930
Anabasine	140	150	90	150	190
Cotinine	195	140	90	40	80
Myosmine	45	50	60	30	85
2,3'-Dipyridyl	100	110	30	10	30
N'-Formyl-nornicotine	175	210	140	40	100

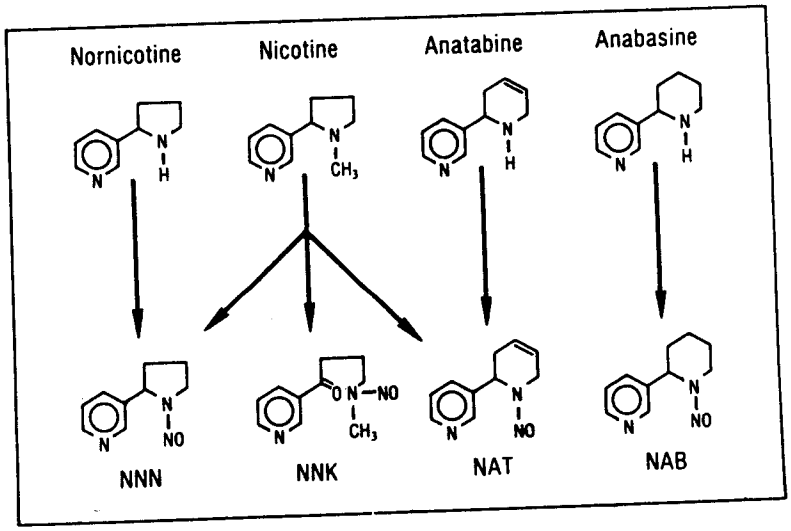
[a]From Piade and Hoffmann (1980)

[b]The tobacco blend used in the reference cigarette IR1 of Kentucky University

2.10 *N*-Nitrosamines

A large number of studies have shown that, during the ageing, curing, fermentation and processing of tobacco, nicotine and other alkaloids give rise to carcinogenic, tobacco-specific *N*-nitrosamines (Hoffmann *et al.*, 1984; Fig. 4; see also monographs on pp. 205-261 of this volume). The concentration of these nitrosamines in tobacco exceeds by at least 100-fold the concentrations found so far in other consumer products (Table 25). It has been calculated (National Research Council, 1981) that, in the USA, cigarette smoking gives rise to at least a 20-fold greater daily exposure to *N*-nitroso compounds than any other consumer product; however, since the relative concentration of tobacco-specific nitrosamines *N'*-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N'*-nitrosoanatabine (NAT) in chewing tobacco is much higher than in cigarette smoke (see monographs pp. 209-223, 233-261) and since the average chewer consumes 10 g of tobacco (Hecht *et al.*, 1983) *versus* <1 g tar inhaled by the smoker, tobacco chewing appears to be the greatest exogenous source of exposure to *N*-nitrosamines (Hoffmann & Hecht, 1985). In certain products marketed in 1980 in the USA and in Sweden, the concentration of nitrosamines was significantly lower than that measured earlier (Table 26). Statistical models for making correlations between tobacco components in commercial tobacco products (Brunnemann *et al.*, 1983) show that this can be achieved by selecting tobaccos with low nitrate levels or by reducing the nitrate content and sealing the smokeless-tobacco products in airtight packages (Brunnemann *et al.*, 1982).

Figure 4. Formation of tobacco-specific nitrosamines (TSNA)[a]



[a]From Hoffmann *et al.* (1984)

Table 25 shows that, in addition to the alkaloid-derived nitrosamines, processed tobacco can also contain volatile nitrosamines, e.g., *N*-nitrosomorpholine (NMOR), *N*-nitrosodiethanolamine (NDELA) and/or *N*-nitrosoproline (NPRO) (Hoffmann *et al.*, 1984; Fig. 5). The morpholine, which is the precursor to NMOR, in the tobacco derives either from the container waxes used in packaging materials or from flavour additives employed in product formulation (Brunnemann *et al.*, 1982). NDELA is formed from residual diethanolamine in those tobaccos that were treated with the sucker-growth inhibitor, maleic hydrazide-diethanolamine (Brunnemann & Hoffmann, 1981). NPRO is formed during the processing of tobacco and can serve as an indicator of the concentration of other non-volatile nitrosamines in tobacco products (Brunnemann *et al.*, 1983).

Table 25. *N*-Nitrosamines in commercial tobacco products (μg/g, dry basis)[a,b]

Tobacco products	Nitrosamines[b]							
	NDMA	NPYR	NMOR	NDELA	NPRO	NNN	NNK	NAT
Cigarette, USA	nd[c]-28	nd-10	0-10	100-200	900-2300	600-6600	100-700	500-1600
Cigarette, UK	nd-10	nd-10	nd	nd-80	600-1000	300	100	200
Cigarette, France	40-180	nd-10	nd-1	nd	1400-1600	600-11900	500-1100	1800-2000
Cigar, little	20	20	nd	420	2000	11200	4500	13000
Cigar	10	10	nd	110	1100	3000-10700	1100-3500	2500-3300
Chewing tobacco, USA	30	20	30	200-300	450	3500-8200	100-3000	500-7000
Chewing tobacco, India	10	10	nd	nd	-	2400	-	-
Snuff, USA	nd-215	nd-360	30-690	29-6900	3500-22000	800-89000	200-8300	200-4000
Snuff, Sweden	nd-60	nd-210	0-44	200-390	-	2000-6700	600-1500	900-2400
Snuff, FRG[d]	10-50	10-300	nd	nd	-	6000-6800	1500-1600	3900-4400
Snuff, Denmark	20-50	20-60	nd	nd	-	4500-8000	1400-7000	2600-6200

[a]From Hoffmann *et al.* (1984)

[b]*N*-Nitrosoanabasine: in cigarette tobacco, ⩽ 20 μg/kg; in snuff, 10-1900 μg/kg. NDMA, *N*-nitrosodimethylamine; NPYR, *N*-nitrosopyrrolidine; NMOR, *N*-nitrosomorpholine; NDELA, *N*-nitrosodiethanolamine; NPRO, *N*-nitrosoproline; NNN, *N'*-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT, *N'*-nitrosoanatabine

[c]nd, not detected

[d]FRG, Federal Republic of Germany

Table 26. *N*-Nitrosamines in snuff (μg/kg, dry basis)[a,b]

Brand of snuff		Volatile *N*-nitrosamines[c]				NDELA	Tobacco-specific *N*-nitrosamines				
		NDMA	NPYR	NMOR	Total		NNN	NNK	NAT	NAB	Total
USA	I	215	(-)	24	240	760	2200	600	1700	100	4600
	II	37	120	690	850[d]	1700	19000	2400	19000	800	41200
	III	100	360	690	1150	3300	33000	4600	40000	1900	79500
	IV	92	110	630	830[e]	290	20000	8300	9100	500	37900
	V[f]	(-)	(-)	31	<50	600	830	210	240	10	1290
Sweden	I	22	(-)	44	70	240	5700	1700	900	140	8440
	II	60	(-)	(-)	60	225	6100	1000	2200	80	9380
	III	14	210	(-)	230	390	5300	1400	2400	70	9170
	IV	30	50	10	90	310	4000	610	1400	80	6310
	V	(-)	(-)	(-)	(-)	290	2000	800	1400	40	4240

[a]From Brunnemann *et al.* (1982)

[b]NDMA, *N*-nitrosodimethylamine; NPYR, *N*-nitrosopyrrolidine; NMOR, *N*-nitrosomorpholine; NDELA, *N*-nitrosodiethanolamine; NNN, *N'*-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT, *N'*-nitrosoanatabine; NAB, *N'*-nitrosoanabasine

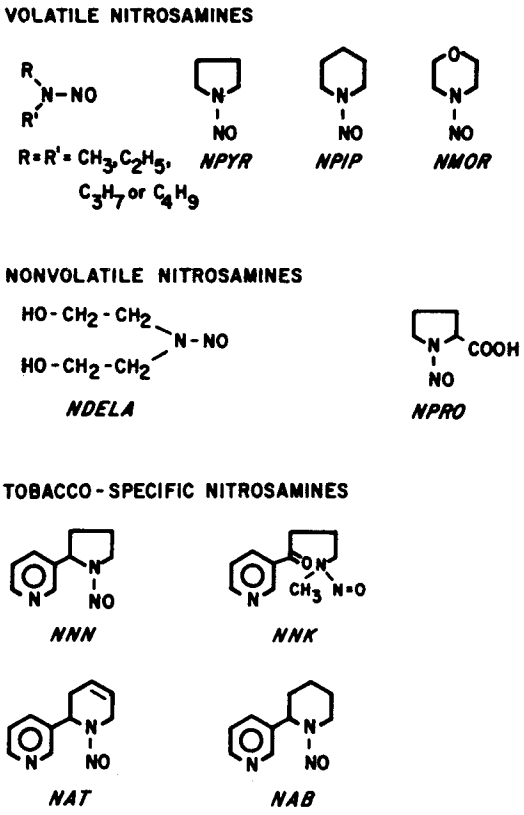
[c](-), below detection limits; values for *N*-nitrosodiethylamine in snuff were below detection limit (<2 μg/kg, except for Sweden I, II, III and IV, which had values of 6, 4, 12 and 5 μg/kg, respectively)

[d]Contained 44 μg/kg *N*-nitrosopiperidine and 21 800 μg/kg *N*-nitrosoproline (Hoffmann *et al.*, 1984)

[e]Contained 13 μg/kg *N*-nitrosopiperidine and 350 μg/kg *N*-nitrosoproline

[f]Introduced on the market in 1982

Figure 5. *N*-Nitrosamines in tobacco products



[a]From Hoffmann *et al.* (1984)

Four *nass* samples from a local authority district in Samarkand Oblast of the Uzbek SSR, were analysed for volatile *N*-nitrosamines, tobacco-specific *N*-nitrosamines, nitrate and nitrite (Table 27) (Zaridze *et al.*, 1985a,b). The relatively low levels of tobacco-specific nitrosamines in *nass*, compared to US and Swedish snuff brands, is at least partially explained by the short ageing process of the tobacco used.

Table 27. *N*-Nitrosamines[a] in samples of *nass*[b]

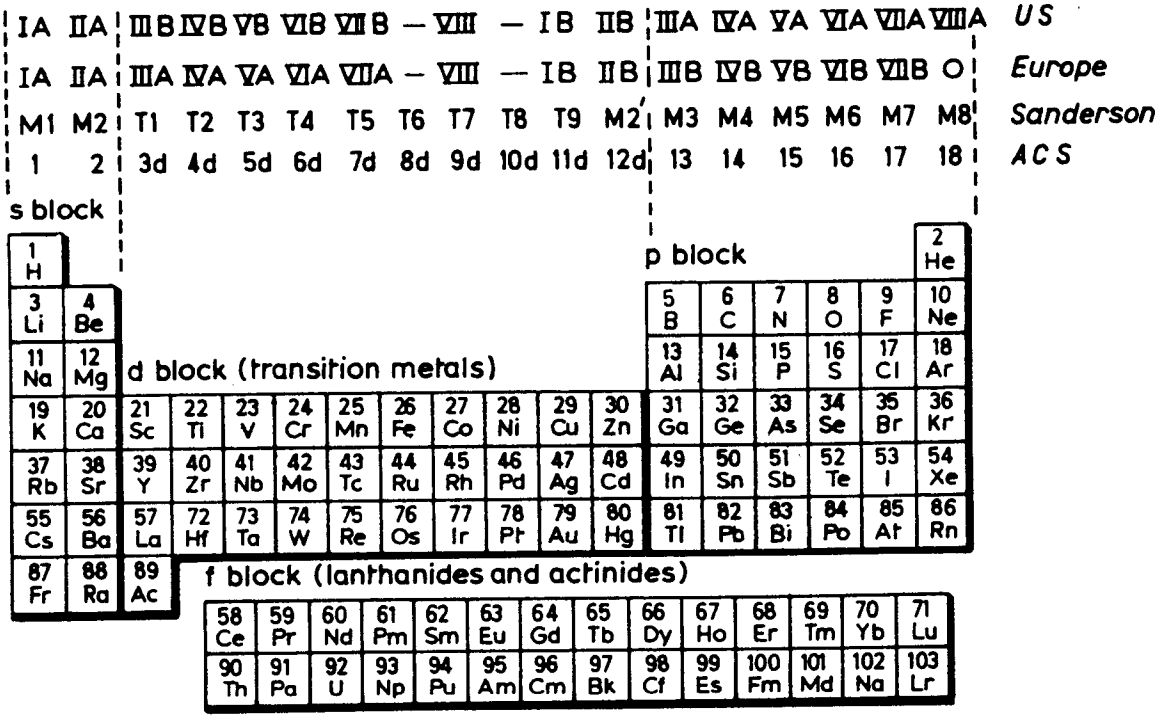
N-Nitrosamine (ng/g)	A	B	C	D
N-Nitrosopiperidine	9.0	7.7	8.0	6.0
N-Nitrosopyrrolidine	8.8	1.8	1.7	4.3
N'-Nitrosonornicotine	519	143	119	516
N'-Nitrosoanatabine	289	39	39	167
N-Nitrosoanabasine	34	3.0	4.0	17
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone	108	16	29	126
Moisture (%)	7.3	8.6	10.1	6.4

[a]*N*-Nitrosodimethylamine and other volatile nitrosamines were not detected (detection limit, 1 ng/g)
[b]From Zaridze *et al.* (1985a,b)

2.11 Metals

Tobacco leaf contains compounds of at least 35 metallic elements (Tso, 1972; Wynder & Hoffmann, 1977; Fig. 6). The most abundant of these metals in cured tobacco leaf are potassium (300-3500 mg/kg), calcium (5000-90 000 mg/kg), magnesium (500-13 000 mg/kg), sodium (150-8500 mg/kg), iron (80-900 mg/kg), copper (4-100 mg/kg) and zinc (0.8-7 mg/kg). The highest concentrations of potassium and calcium in processed tobacco accumulate in the middle vein of the leaves (Wynder & Hoffmann, 1967; Tso, 1972; Tso *et al.*, 1980).

Figure 6. Metals in tobacco[a]



[a]From Tso (1972); Wynder and Hoffmann (1977)

Of special concern in tobacco carcinogenesis are arsenic, lead, cadmium and nickel. Arsenic (as its trioxide, As_2O_3) has been reported to occur in processed tobacco at concentrations of up to 50 mg/kg. However, since the use of arsenic products as pesticides was suspended in most tobacco-producing countries, the arsenic content of leaf tobacco has decreased drastically during the last decades. The latest available data show a level of 0.5-0.9 mg/kg arsenic trioxide in cured tobacco (Guthrie & Bowery, 1967). Lead has been reported to occur in tobacco at concentrations of 5-80 mg/kg; the earlier levels cited appear to be rather high, and levels of lead do not now generally exceed 10 mg/kg (Cogbill & Hobbs, 1957; Voss & Nicol, 1960; Perinelli & Carugno, 1978). Cadmium levels of between 1-2 mg/kg have been reported (IARC, 1973; Perinelli & Carugno, 1978).

Since a number of nickel compounds are carcinogenic to laboratory animals and since some forms of nickel are probably human carcinogens (IARC, 1982), a large number of studies have been concerned with the nickel content of tobacco products (National Academy of Sciences, 1975; IARC, 1976). While concentrations of nickel in tobacco leaf

generally do not exceed 4 mg/kg, higher concentrations may occur in special settings (National Academy of Sciences, 1975). In Swaziland, a snuff product placed in the nostrils is made of powdered local tobaccos mixed with the ashes of incinerated plants or herbs, such as the plant *Aloe marlothii* (80% of all ashes), the roots of *Turbinata oblongata* and leaves and stems of *Amaranthus spinosum*. Metal analyses of these snuff products, as used by the consumer, revealed, among other metals, the presence of 43, 87 and 25 mg/kg nickel, respectively (Baumslag *et al.*, 1971). As seen in Table 28, the levels of copper, chromium and nickel in Swazi snuff are relatively high in comparison to those in commercial snuffs produced in the USA.

Table 28. Trace metal content of Swazi and commercial US snuffs (mg/kg)[a]

Type of snuff	Copper	Chromium	Lead	Zinc	Cadmium	Nickel
Aloe	25	9	8	65	1.4	43
Ubhoco	63	84	8	50	1.5	87
Amaranthus	16	13	6	47	1.1	25
US Brand I	10	1	4	41	0.8	3
US Brand II	12	1	4	27	0.9	2
US Brand III	9	2	4	40	0.7	2

[a]From Baumslag *et al.* (1971).

2.12 Radioelements

α- and β-Radioactivity have been reported to occur in leaf tobaccos. Naturally-occurring ^{40}K is the major contributor to the minute β-radioactivity in tobacco (Tso, 1972).

It was suggested (Radford & Hunt, 1964) that α-emitting ^{210}Po in tobacco smoke is a contributory factor in bronchogenic carcinoma in cigarette smokers. Since that report, many others have indicated that 1 g tobacco contains between 0.1-1.0 pCi of ^{210}Po. It appears that phosphate fertilizers that contain ^{226}Ra, ^{210}Pb and ^{210}Po and soils derived from rock rich in ^{226}Ra are the major source of the α-radioactivity of tobacco (Harley *et al.*, 1980). A 1-g sample of tobacco was found to contain about 0.021 pCi of ^{238}U (Chakarvarti *et al.*, 1981).

2.13 Agricultural chemicals

During the last three to four decades, many organic chemicals have been marketed for the cultivation and post-harvest treatment of tobacco (Tso, 1972). In the past, the sucker-growth inhibitor, maleic hydrazide, was dissolved in diethanolamine, since it is insoluble in water. More recently, maleic hydrazide has been formulated as its potassium salt (US Environmental Protection Agency, 1981). It has been reported to occur in US tobaccos at concentrations of between 17-178 mg/kg (Liu & Hoffmann, 1973; Chopra *et al.*, 1982). The concentrations of chlorinated hydrocarbon pesticides in a tobacco blend in 1977 are given in Table 29 (Reif & Moser, 1977); these are significantly lower than those reported earlier (Tso, 1972).

Table 29. Organochlorine pesticide residues on one sample of US tobacco analysed in the 1970s[a]

Pesticide	Concentration (mg/kg)
α-Hexachlorobenzene	0.12
γ-Hexachlorobenzene (Lindane)	0.18
β-Hexachlorobenzene	0.22
Heptachlor	0.02
δ-Hexachlorobenzene	0.03
α-Endosulfan	0.03
p,p'-DDE [1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene]	0.15
Dieldrin	0.05
o,p'-TDE [1,1-dichloro-2(2-chlorophenyl)-2(4-chlorophenyl)ethane]	0.05
o,p'-DDT [1,1,1-trichloro-2(2-chlorophenyl)-2(4-chlorophenyl)ethane]	0.38
p,p'-TDE [1,1-dichloro-2,2-bis(4-chlorophenyl)ethane]	0.43
β-Endosulfan	0.19
p,p'-DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane]	2.71
Endosulfan sulphate	0.27

[a]From Reif and Moser (1977)

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

Tobacco

The Working Group noted that the majority of the published studies evaluated below had various deficiencies, such as lack of quantitative and qualitative information on the nature of tobacco extracts and the degree of extraction, insufficient length of treatment, small group sizes and, in some cases, lack of appropriate controls.

(*a*) *Oral administration*

Mouse: Groups [numbers unspecified] of male Swiss mice, six to eight weeks of age, were administered a tobacco extract (ethanol extract from 50 g tobacco diluted in 10 ml distilled water) from a commercially-available Indian chewing tobacco (*Nicotiana tabacum*) at a dilution of 1:25 or 1:50 [actual dose unspecified] by oral intubation for 15-20 months. A further group of mice was fed a diet containing 10 g of an extract of tobacco per 5 kg diet for up to 25 months. A group of 20 mice received distilled water only by intubation and served as controls. Administration of the 1:25 dilution was terminated at 18 weeks because of high mortality. Tumour incidences at 15-20 months were 0/4, 8/15 and 4/10 in the control, 1:50 dilution and 1:25 dilution groups, respectively. At 21-25 months, 1/20 controls and 8/10 animals fed tobacco extract in the diet had tumours. The types of tumour observed were lung adenocarcinomas or hepatocellular carcinomas (Bhide *et al.*, 1984b). [The Working Group noted the incomplete reporting of the distribution of different types of neoplasm among the various groups.]

(*b*) *Skin application*

Mouse: Groups of 40 CAF_1 (Jackson) and 40 Swiss (Millerton) mice [sex and age unspecified] received topical applications of a 50% methanol extract of unburnt cigarette tobacco on the skin three times a week for 24 months. Groups of 30 CAF_1 and 30 Swiss mice, which received whole-tar extract in the same way for 21 to 24 months, served as controls. Among the CAF_1 mice exposed to the tobacco extract, 11 developed papillomas, and among the Swiss mice, three developed papillomas, compared to 16 papillomas in each of the control groups. One papilloma later developed into cancer in the Swiss mice test group, compared to three in Swiss and eight in CAF_1 controls (Wynder & Wright, 1957). [The Working Group noted that there was no statistical evidence for the carcinogenic effect of this tobacco extract.]

Groups of eight to 17 male and female strain A (Strong) and Swiss mice, two to three months old, received skin applications of five different extracts (petroleum ether, benzene, chloroform, chloroform ether and ethanol) of an Indian chewing tobacco (*N. tabacum*; Vadakkan, Meenampalayam variety) up to 18 months of age; no tumour was observed at the site of application, and no excess incidence was reported at other sites (Mody & Ranadive, 1959). [The Working Group noted the small numbers of animals used.]

A group of 10 male and six female C17 mice, two to three months old, received thrice-weekly applications of a dimethyl sulphoxide extract of an Indian chewing tobacco (Vadakkan variety) on the skin of the interscapular region for life (24 months of age). No skin tumour was observed (Ranadive *et al.*, 1976). [The Working Group noted the small number of animals used.]

Groups of 11-36 inbred Swiss or Paris albino XVII x C_{57} black (hybrid) mice [sex and age unspecified] received twice-weekly skin applications of E8 ('total') plus E9 ('partially alkaloid-free'), E9 or E10 ('totally alkaloid-free') tobacco extracts or acetone for 95 weeks followed by weekly applications of croton oil. Between 61 and 95 weeks after the start of treatment, the incidences of papillomas and of squamous-cell carcinomas at the site of application were 10/21 and 6/21 (E8 plus E9), 9/25 and 2/25 (E9) and 22/35 and 10/35 (E10) in the hybrid mice, respectively. Papillomas occurred in 3/19 acetone/croton oil-treated controls; no carcinoma was observed. [The increases in the incidences of papillomas and carcinomas were statistically significant, except in the E9-treated group.] The incidences of papillomas in the Swiss mice were 2/9, 2/4 and 3/10, respectively; no carcinoma was observed (Ranadive *et al.*, 1963). [The Working Group noted that no control group of Swiss mice was included.]

The cocarcinogenic [promoting] effect of the E10 tobacco extract was tested in a group of 16 Swiss albino and 13 Swiss (Baldy) mice, which received a single topical application of benzo[*a*]pyrene [dose unspecified] followed by twice-weekly applications of E10 for 80 weeks. A group of seven Swiss albino and 10 Swiss (Baldy) mice received the benzo[*a*]pyrene treatment only and served as controls. Two carcinomas and four papillomas were observed in Swiss (Baldy) mice treated with E10 and benzo[*a*]pyrene; no tumour was observed in benzo[*a*]pyrene-treated controls (Ranadive *et al.*, 1963). [The Working Group noted the small number of animals and incomplete information concerning the initiating dose of benzo[*a*]pyrene.]

Groups of 30 female ICR Swiss mice, 57 days old, received a single topical application of 125 µg 7,12-dimethylbenz[*a*]anthracene (DMBA) in 0.25 ml acetone, followed 21 days later by applications of 0.25 ml of an acetone or barium hydroxide extract of unburned commer-

cial tobacco five times a week for 36 weeks. The amount of acetone extract was equivalent to 2.5 cigarettes per day. The barium hydroxide extract was prepared using two different extraction procedures, designated 'concentrated' and 'dilute', according to the yield: the 'concentrated' was equivalent to 0.5 cigarette per day and the 'dilute' was about one quarter as concentrated as the 'concentrated' extract. Two groups of 30 mice received DMBA treatment alone or no treatment and served as controls. The incidences of tumours, all of which were small papillomas, were: acetone extract, 16 tumours in 7/30 (2.3 tumours/mouse); concentrated barium hydroxide extract, 18 tumours in 8/30 (2.2 tumours/mouse); and dilute barium hydroxide extract, six tumours in 2/30 (three tumours/mouse). No tumour was observed in either of the control groups (Bock *et al.*, 1964).

Groups of 30 female ICR Swiss mice, 55-60 days old, received a single topical application of 125 μg DMBA in 0.25 ml acetone, followed three weeks later by applications of different aqueous extracts (crude, acidic, neutral and basic components) of an unprocessed, commercial, flue-cured tobacco five times per week for 26 weeks. A total of 12 papillomas developed in 6/30 mice treated with crude tobacco extract (equivalent to 0.5 g tobacco daily) following DMBA initiation. One mouse developed a papilloma after treatment with the acidic fraction and DMBA. No skin tumour was found in animals treated with DMBA alone or with the various fractions of tobacco alone. With half the concentration (0.25 g tobacco), one mouse developed a papilloma after application of the crude extract and one mouse developed three papillomas with the neutral extract following DMBA initiation. Additional studies demonstrated that the tumour-promoting components of the tobacco extract were stable and non-volatile (Bock *et al.*, 1965).

Groups of 20 female Swiss ICR/Ha mice, eight weeks of age, received a single topical application of 150 μg DMBA in 0.1 ml acetone, followed two to three weeks later by thrice-weekly applications of solvent extracts [ether (25 mg), chloroform (1 mg), methanol (25 mg) or a reconstituted sample (25 mg)] of a flue-cured cigarette variety of tobacco leaf for 52 weeks. Groups of 20 mice receiving DMBA alone or tobacco extracts alone served as controls. Two of 13 survivors in the DMBA/methanol extract group developed 'cancers'. The numbers of mice with papillomas in the various groups were: 4/12 (ether extract), 1/10 (chloroform extract), 2/13 (methanol extract) and 5/14 (reconstituted extract). No tumour was observed in mice treated with DMBA or extracts alone (Van Duuren *et al.*, 1966).

(*c*) *Inhalation*

Mouse: A group of 80 male strain A mice, three months old, were exposed by inhalation to powdered tobacco leaf on alternate days for 30 months. A further group of 80 animals served as controls. The incidences of 'lung cancer', leukaemia and hepatocellular carcinoma in animals surviving to 30 months were 12/75 and 1/80, 11/75 and 2/80, and 3/75 and 0/80 in the treated and control groups, respectively (Hamazaki & Murao, 1969). [The Working Group noted that the incidences of lung and liver tumours in the untreated mice were unusually low.]

(*d*) *Subcutaneous administration*

Mouse: Groups of 17 Paris albino XVII x C_{57} black mice received s.c. injections of 0.1 ml of a 2% solution of 'partially or completely alkaloid-free' extract of tobacco (Vadakkan, Meenampalayam variety) once a month for 41-95 weeks. One squamous-cell carcinoma [site not specified] developed in an animal that received the partially alkaloid-free extract (Ranadive *et al.*, 1963). [The Working Group could not draw any conclusion from this report.]

(e) *Application to the oral mucosa or cheek pouch*

Mouse: Groups of nine to 16 male and female strain A (Strong) and Swiss mice, two to three months old, were administered different alkaloid-free extracts of an Indian chewing tobacco of the Vadakkan type (*N. tabacum*; Meenampalayam variety). The extracts, a benzene extract and its neutral fraction, a water extract and four successive extracts (petroleum ether, benzene, chloroform and ethanol), were applied by daily application to the oral mucosa for up to 18 months of age. No excess incidence of tumours was observed (Mody & Ranadive, 1959). [The Working Group noted the small number of animals used.]

Rat: A group of 22 Wistar rats, five months of age, were painted on the oral mucosa with a 2% alkaloid-free extract of Vadakkan tobacco of the Meenampalayam variety in acetone twice a week for life; 12 of these animals were also painted with 20% lime in distilled water the day after each treatment. Control groups of 10-14 rats received no treatment or lime only. No tumour was observed at the application site (Gothoskar *et al.*, 1975).

Hamster: A group of 50 young golden hamsters received implantation of a 2-cm^3 plug of chewing tobacco [unspecified] in the cheek pouch. The opening in the cheek pouch was ligated and the animals were followed for up to 30 months. Survival after 13 months was 21/50; and eight were alive at 24 months, but none at 30 months. No tumour was observed in any of the animals (Peacock & Brawley, 1959; Peacock *et al.*, 1960).

Philippine leaf tobacco with 10% lime was mixed with beeswax, and pellets were implanted into the cheek pouch of 34 male and female Syrian golden hamsters, one to two months old. Animals were allowed to live their lifespan and were killed when moribund. No tumour at the implantation site was reported (Dunham & Herrold, 1962).

Groups of 11-12 male Syrian golden hamsters, nine weeks old, received topical applications on the cheek-pouch mucosa of a dimethyl sulphoxide (DMSO) extract of cured Banarsi chewing tobacco or DMSO alone thrice weekly for 21 weeks, at which time all animals were killed. No tumour was seen in treated or control hamsters, but 8/12 treated animals had leukoplakia (Suri *et al.*, 1971).

A group of 12 male inbred Syrian golden hamsters, two to three months old, received topical applications to the cheek-pouch mucosa of DMSO extracts of an Indian chewing tobacco (Vadakkan) thrice weekly for life. A control group of seven animals received applications of DMSO alone. No local tumour but moderate hyperkeratosis was observed (Ranadive *et al.*, 1976).

Groups of 30-41 Syrian golden hamsters [sex unspecified], weighing 40-50 g, received 60 g tobacco (Jada Jarda) alone, in combination with lime, or in combination with lime plus vitamin A in the cheek pouch thrice weekly for 100-110 weeks, at which time 24-32 animals were alive. Moderate to severe keratotic and dysplastic changes in the mucosa developed, but no neoplastic change was observed (Kandarkar *et al.*, 1981).

A group of 20 female Syrian golden hamsters, six to seven weeks of age, received topical applications to the cheek-pouch mucosa of 1 mg lyophilized aqueous tobacco extract in 0.05 ml water twice daily for six months. Animals were observed for a further six months then killed. Squamous-cell papillomas/carcinomas occurred in 3/17 animals, compared to none in 10 untreated and in 10 vehicle (water) controls (Rao, 1984).

(*f*) *Other experimental systems*

Groups of 5-12 male and female hybrid (inbred C_{17}) or Swiss mice, two to three months of age, received a single *intravesicular implantation* of paraffin pellets containing tobacco (Jarda variety of chewing tobacco), a mixture of tobacco and lime or an alkaloid-free tobacco extract or paraffin pellets alone and were observed for 10-30 months of age. Among the hybrid mice receiving the alkaloid-free tobacco implantation, 2/12 developed transitional-cell tumours of the bladder and one female developed a tumour described as a 'myosarcoma of the cervix with metastasis to the kidney'. No tumour was observed in the controls or in the other treated groups (Randeria, 1972). [The Working Group noted the small group size and the potential carcinogenic effect of intravesicular foreign bodies in mice.]

A group of four female hybrid (inbred C_{57}) mice and four female Swiss mice, two to three months of age, received daily *vaginal applications* of a fine mixture of Jarda tobacco dust containing lime derived from sea shells for 10-30 months; no vaginal tumour was observed (Randeria, 1972). [The Working Group noted that no control group was used in this study.]

Snuff

(*a*) *Oral administration*

Hamster: Groups of 50 male BIO 15.16 and BIO 87.20 (carcinogen-susceptible) strain Syrian hamsters, two to three months old, were fed one of the following five experimental diets for two years: 20% damp, fresh US snuff mixed with the diet; cellulose mixed with diet, such that the caloric content was reduced by 20% (negative control); control diet plus 50 treatments with 5 mg 20-methylcholanthrene (MC) per animal by stomach tube (positive control); cellulose diet plus 50 treatments with 0.5 mg MC per animal by stomach tube; and snuff diet plus 50 treatments with 0.5 mg MC per animal by stomach tube. The animals fed snuff diet alone showed a nearly identical tumour spectrum to that of controls. No increased incidence of tumours was noted in animals administered snuff with MC (Homburger *et al.*, 1976).

A total of 13 male and female Syrian golden hamsters, 1.5 months of age, were fed three different test substances for 16 months: group 1 (two males and two females) was fed 0.75 g aromatic snuff [type unspecified] per week; group 2 (two males and two females) was fed 0.75 g aromatic snuff [type unspecified] and 0.75 g calcium hydroxide per week; and group 3 (five animals) [sex distribution not specified] received calcium hydroxide only. One male hamster in group 2, estimated to have consumed 52 g snuff and 52 g calcium hydroxide during the 16-month period, developed a pancreatic carcinoid 4.5 months after the termination of treatment. The tumour incidence in the remaining groups and at other sites was not reported; however, the authors stated that carcinoids had been found previously in only 700 animals in that laboratory (Dunham *et al.*, 1975). [The Working Group noted the relatively small groups used.]

(*b*) *Subcutaneous administration*

Rat: A group of 82 male and female albino (Händler) rats (100 days old) was given s.c. injections of 0.15 ml (50 mg) of an ethanol extract of Swedish snuff (Ettan) in tri-*n*-caprylin once a week for 84 weeks. A group of 81 male and female rats received the same schedule

of injections of ethanol and tri-*n*-caprylin and served as controls. Malignant tumours developed in equal numbers in both test and control rats. These were 'retothelsarcomas' (one in each group), one uterine carcinoma (in a test animal) and one ovarian carcinoma (in a control animal) (Schmähl, 1965).

(*c*) *Application to the oral mucosa or cheek pouch*

Rat: A group of 21 male and 21 female Sprague-Dawley rats, three months of age, was administered snuff into a surgically-created canal in the lower lip. Approximately 0.2 g of a standard Swedish snuff (Röda Lacket), pH 8.3, was injected morning and night on five days per week for up to 22 months. [The calculated daily dose was 1 g/kg bw and the mean retention time after each administration was 6 h (range, 5-8 h) (Hirsch & Thilander, 1981).] The rats were killed at nine, 12, and 18-22 months. A second group of five male and five female rats was treated similarly with the same snuff but at pH 9.3 [produced by addition of 50% more sodium carbonate (1% of the total weight)] and sacrificed between 18 and 22 months. Of 42 animals administered the snuff, one developed a squamous-cell carcinoma of the oral mucosa at 8.5 months. No tumour was seen in rats exposed to the alkaline snuff or in 15 rats with surgically-created canals but not given snuff. Benign tumours outside the oral cavity were observed in roughly equal frequency in control and treated groups in both experiments (Hirsch & Johansson, 1983).

Four groups of 10 female Sprague-Dawley rats with surgically-created canals in the lower lip received the following treatments beginning at three months of age: group 1 was infected with herpes simplex type 1 (HSV-1) virus by scarification and topical application on the inside of the lower lip, followed, ten days later, by administration of a standard Swedish (Röda Lacket) snuff into the canal, morning and night on five days per week; group 2 was infected with virus and received no other treatment; group 3 was sham-infected with sterile saline followed by snuff treatment; and group 4 was given neither virus nor snuff and served as controls. The HSV-1 infection was repeated once after a one-month interval, and snuff was injected 10 days later as before. Snuff treatment was continued for 18 months, after which time all animals were killed. Three animals in each of groups 1 and 2 died from encephalitis shortly after the second infection with HSV-1. Squamous-cell carcinomas of the oral cavity developed in 2/7 rats, and a retroperitoneal sarcoma occurred in 1/7 rats exposed to HSV-1 and snuff. In the group exposed to snuff alone, 1/10 animals developed a squamous-cell carcinoma of the anus and 1/10 a retroperitoneal sarcoma (Hirsch *et al.*, 1984a). [The presence of two oral cancers in animals in group 1 does not constitute a statistically significant result. The Working Group noted, however, that these two tumours were located near the site of application of the snuff.]

Hamster: Groups of 50 young golden hamsters received an instillation into the left cheek pouch of 10 ml of a thick paste of snuff. The opening of the pouch was ligated, and the animals were followed for up to 30 months. The contralateral pouches of 25 of these animals were filled with sand and gum and served as controls. After 13 months, 21/50 were still alive; 10 were alive at 24 months, but none at 30 months. No tumour was observed in control or treated pouches (Peacock & Brawley, 1959; Peacock *et al.*, 1960).

A group of 35 male and female Syrian golden hamsters, one to two months of age, received snuff and lime in the cheek pouch as single depositions. A positive control group of 71 hamsters was exposed to the two carcinogenic hydrocarbons, 7,12-dimethylbenz[*a*]anthracene and 3-methylcholanthrene; and a negative-control group of 36 animals was exposed to beeswax, which was used as a vehicle to prolong the retention time of the test substances. The animals were killed after 15-20 months or when moribund. Two of the 35 animals exposed to 20% snuff and lime and 2/36 exposed to beeswax developed inflam-

matory lesions; among the positive controls, 23/56 developed malignant tumours, including carcinomas (20) and sarcomas (three) (Dunham & Herrold, 1962).

Groups of four to seven male and female weanling Syrian golden hamsters received twice-daily applications of 50 mg of a commercial US 'Scotch' (dry type) snuff, snuff and calcium hydroxide, or calcium hydroxide alone into the cheek pouch on five days per week for up to 99 weeks. No local tumour was observed in any group (Dunham *et al.*, 1966).

A group of 84 male and female Syrian golden hamsters (BIO hamsters of the RB strain), aged three to four months, were exposed to 0.5 g of snuff placed in a stainless-steel webbing cartridge attached to the lower incisors for 30 min daily for 51 weeks. A group of 84 hamsters exposed to dry cotton served as negative controls and further groups, one of 84 animals exposed to benzo[*a*]pyrene and one of 24 animals exposed to 7,12-dimethylbenz[*a*]anthracene, served as positive controls. No tumour was found in the oral mucosa, except in the positive controls (Homburger, 1971). [The Working Group noted the short duration of this study.]

Nass

A series of experiments were reported in two papers (Kiseleva *et al.*, 1976; Milievskaja & Kiseleva, 1976).

(*a*) *Skin application*

Hamster: A group of 19 female and 31 male Syrian hamsters received topical applications of a suspension of *nass* (45% tobacco, 8% lime, 30% ash, 12% plant oil and 5% water) on the dorsal skin. The average lifespan was 44.4 weeks. Three out of nine animals still alive at the time of appearance of the first tumours (53 weeks) developed neoplasms: one liver 'lymphangioendothelioma', one adrenal-gland tumour and one forestomach papilloma. No local tumour occurred. In the control group (either untreated or treated orally with sunflower oil), 2/57 hamsters that survived to the appearance of the first tumour (59 weeks) developed tumours: one adrenal-cortex neoplasm and one forestomach papilloma (Kiseleva *et al.*, 1976).

(*b*) *Application to the cheek pouch*

Hamster: A group of 28 female and 33 male Syrian hamsters, one to three months of age, received applications of *nass* (same composition as described above) as a dry powder into the left cheek pouch for life; another group of 13 females and 24 males received *nass* as a 50% suspension in refined sunflower oil in the cheek pouch (total dose per animal, 6.2-147.5 g, mean 53.8 $\pm$ 2.5 g). The animals were followed until death. No tumour was found at the site of *nass* application. The average lifespan of animals receiving *nass* (50.8 weeks) was slightly shorter than that of untreated animals (57.3 weeks) or that of hamsters receiving sunflower oil alone (57.6 weeks). Of 64 treated hamsters in both groups still alive at the time of appearance of the first tumour (17 and 37 weeks), 13 developed tumours: seven liver-cell tumours and one liver tumour of 'mixed structure', three tumours of the adrenal glands (described as a 'carcinoma of adrenal cortex' and as 'adenoma, chromaffinoma type' or 'carcinoma of adrenal cortex'), one forestomach papilloma, three uterine tumours (leiomyoma and/or fibromyoma and/or cysts), one skin melanoma and one unspecified tumour of the large intestine. Among 110 untreated and 10 animals treated with sunflower oil, 45 survived to the appearance of the first tumour (59 weeks), and two developed tumours (one

adrenal-cortex neoplasm and one forestomach papilloma) (Kiseleva *et al.*, 1976; Milievskaja & Kiseleva, 1976).

In another experiment described in these reports, *nass* was introduced as a suspension in refined sunflower oil into the cheek pouch of male and female hamsters comprising a total of 40 females and 46 males belonging to six generations. *Nass* was administered throughout life, including periods of pregnancy and lactation. No tumour was found at the site of application. Of 36 (36.1%) hamsters that survived to the appearance of the first tumour (54 weeks), 13 developed neoplasms at various sites: two liver-cell tumours, one haemangioendothelioma, one cholangioma and one liver tumour of 'mixed structure', three in the adrenal glands, four papillomas of the forestomach, one of the uterus or ovaries, one benign skin tumour and one pancreatic tumour. The average lifespan of the animals was 51.6 weeks (Kiseleva *et al.*, 1976; Milievskaja & Kiseleva, 1976). [The Working Group noted deficiencies in reporting the results of this series: the number of animals and incidence of tumours in each generation, the number of newborns and neonatal mortality are not indicated, and no multigeneration controls were available.]

[In consideration of the whole study, the Working Group noted that the effective number, i.e., the number of animals surviving to observation of the first tumour, was calculated separately for treated (number of survivors at 17 weeks with the dry powder) and control (59 weeks) animals. Therefore, the effective number of control animals should have been higher in the first experiment. High mortality of animals was noted, even in control groups, in the period preceding observation of the first tumour; average lifespan of untreated control animals was 57.3 weeks. The sex of animals in which liver tumours were found was not indicated.]

A group of 30 Syrian hamsters received a single application of 0.1 mg 7,12-dimethylbenz[*a*]anthracene (DMBA) as a 0.1% solution in benzene into the cheek pouch. Another group of 30 hamsters received the same treatment, followed seven weeks later by daily applications of *nass* (composition as described above) as a dry powder into the cheek pouch; the total dose ranged from 11.2 to 102.5 g (mean, 38.9 $\pm$ 5.2 g). Three out of 11 survivors at the time of appearance of the first tumour (23 weeks) receiving DMBA alone developed tumours: one rhabdomyoblastoma of the cheek pouch, and two papillomas of the forestomach. Six of 11 animals still alive at 50 weeks that received DMBA plus *nass* had tumours: five papillomas of the forestomach and one cystic epithelioma of the skin of the jaw (Milievskaja & Kiseleva, 1976). [The Working Group noted the small number of animals that survived to the time of observation of the first tumour.]

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

Application of *nass* to the cheek pouch of Syrian hamsters induced degenerative and proliferative changes in the epithelium, and an inflammatory response and fibrosis in the submucosal layer. The same changes were observed in the oesophageal mucosa of animals receiving *nass* either in the cheek pouch or by gavage. *Nass* administered into the cheek pouch or percutaneously induced foci of hepatocyte proliferation, bile-duct proliferation, cholangiofibrosis and severe vascular disturbances (Kiseleva *et al.*, 1976).

Two to four months after the beginning of daily *nass* administration by gavage to rats, basal-cell proliferation with cell polymorphism and 'signs of invasion' into the submucosa in the oesophagus were observed (Rahmatian *et al.*, 1965).

Snuff (0.2 g commercial brand) was inserted twice daily into a surgically-created canal in the lower lip of rats, on five days a week. Measurement showed that the snuff was retained in the canal after each insertion for 5-8 h. Exposure for nine or 12 months produced mild to severe hyperplasia, hyperorthokeratosis and acanthosis. After longer exposure, vacuolated cells were found in basal layers of the epithelium, with hyperplastic, atrophic, ulcerated lesions, slight dysplastic lesions, and, in a few rats, severe dysplastic changes in the epithelium of the crevice containing the snuff. Squamous-cell hyperplasia was also found in the forestomach of two rats exposed to snuff for 18-22 months (Hirsch & Johansson, 1983). A preliminary study suggests that these lesions, particularly the dysplasia of the squamous epithelium in the canal, may be exacerbated by concurrent herpes simplex virus infection (Hirsch *et al.*, 1984a).

No histological change specifically related to snuff exposure was found in the oral mucosa of Syrian golden hamsters alive at the end of an experiment in which they had been forced to chew on a stainless steel-gauze pouch containing 0.5 g snuff for 30 min per day, on five days a week for 30 (60 animals) or 52 weeks (24 animals). During treatment, mortality occurred in 30% of controls and 34% of the snuff-treated animals in the 30-week group, and 52% of controls and 60% of treated animals in the 52-week group, mostly as a result of trauma (Homburger, 1971). No toxic effect on the cheek pouch or oesophagus was seen in four hamsters fed 6 g of diet containing 2.5% American snuff, on five days per week, for 16 months, or in four hamsters fed diets containing 2.5% snuff and 2.5% calcium hydroxide (Dunham *et al.*, 1974).

Aqueous extracts of snuff inhibit the replication of herpes simplex virus-1 by cultured kidney cells from green monkeys. Greater inhibition was produced by a brand of snuff containing a high concentration of nitrosamines than by a brand with a low nitrosamine content (Hirsch *et al.*, 1984b).

Extracts of chewing tobacco, snuff and tobacco leaf did not inhibit the growth of the oral cariogenic bacteria *Streptococcus mutans* and *S. sanguis* when tested *in vitro* (Lindemeyer *et al.*, 1981).

Effects on reproduction and prenatal toxicity

Anabasine, a tobacco alkaloid, was tested for teratogenicity in pigs. After ingestion by dams of 2.6 mg/kg bw anabasine twice daily between days 43-53 of gestation, defects were induced in all of three litters (21/26 offspring), including cleft palate, fixed, excessive flexure of the front or rear pasterns, fixed, excessive flexure of the carpal joints, and rotation or bowing of limbs. Similar defects were induced by *Nicotiana tabacum* and *N. glauca* (Crowe, 1978; Keeler *et al.*, 1984). Anabasine was teratogenic to chicks (Landauer, 1960; Upshall, 1972). *N. glauca* was also teratogenic to cows (Keeler, 1979).

Nicotine failed to induce defects in pigs, sheep or cows (Keeler, 1979), but it has been shown to be teratogenic in rabbits (Vara & Kinnunen, 1951), mice (Nishimura & Nakai, 1958) and chicks (Landauer, 1960).

Addition of 0.1 mg nicotine/ml to the drinking-water of pregnant mice reduced the weight of 17-day-old foetuses by 12% (Rowell & Clark, 1982).

Absorption, distribution, excretion and metabolism

Nicotine (80 and 250 ng/ml of blood) was detected in two rats 30 min after insertion of snuff (0.2 g) into a surgically-created canal in the lower lip (Hirsch & Thilander, 1981).

Mutagenicity and other short-term tests

Ethanol extracts of a chewing variety of *Nicotiana tabacum*, known locally in India as the Pandharpuri variety, induced mutations in *Salmonella typhimurium* TA98 in the presence but not in the absence of phenobarbital-induced rat-liver 9000 x *g* supernatant (S9). No mutation was induced in *S. typhimurium* TA100, TA1535 or TA1538 in the presence or absence of S9 (Bhide *et al.*, 1984b). Ethanol extracts of this tobacco also induced mutations in Chinese hamster V79 cells; the presence of Aroclor-induced rat-liver S9 enhanced this effect. The same extracts induced micronuclei in bone-marrow cells of Swiss mice (Shirname *et al.*, 1984).

An ethyl acetate extract of Jaffna tobacco (used with betel quid) induced sister chromatid exchanges in cultured human lymphocytes and in a human lymphoblastoid cell line; in the latter case, rat-liver homogenate enhanced the effect. This extract, tested only in the absence of exogenous metabolic activation, did not induce ouabain-resistance in Chinese hamster V79 cells. The same extract, another ethyl acetate extract and an ethanol extract induced cell transformation in Syrian hamster embryo cells (Umezawa *et al.*, 1978; 1981).

Tobacco powder added to the feed of *Drosophila melanogaster* larvae did not induce sex-chromosome loss, sex-linked recessive lethal mutations or autosomal translocations (Abraham *et al.*, 1979).

Aqueous extracts of two *nass* samples induced a dose-related increase in the number of chromosomal aberrations in Chinese hamster ovary (CHO) cells. The frequency of these effects was not altered by the addition of rat-liver S9, catalase or superoxide dismutase (Zaridze *et al.*, 1985a,b). Aqueous extracts of *khaini* also induced chromosomal aberrations in CHO cells (Stich *et al.*, 1982).

The tobacco alkaloids, anatabine, nicotine and nornicotine, induced sister chromatid exchanges in CHO cells in the absence of S9. With anatabine (125-500 μg/ml), the effect was dose-related. The presence of Aroclor-induced rat-liver S9 inhibited the induction of sister chromatid exchanges (Riebe & Westphal, 1983).

(*b*) *Humans*

Toxic and pharmacological effects

Precancerous lesions occurring in users of smokeless-tobacco products are discussed in section 3.3 of this monograph, p. 89.

Few studies were available on the toxicology and pharmacology of smokeless-tobacco products. A significant increase in pulse rate and blood pressure after tobacco chewing has been observed which is presumably due to nicotine (Simon & Iglauer, 1960; Bordia *et al.*, 1977). The pharmacological effects of nicotine have been reviewed extensively (see, for example, Goodman & Gilman, 1982; Balfour *et al.*, 1984).

Studies in Tunisia (Ben Khedher *et al.*, 1984; Ben Miled *et al.*, 1984; Malej *et al.*, 1984) have suggested an increased frequency of bronchitis with the use of snuff (powdered Tunis-

ian tobacco, known as *neffa*). Gingival recession has been associated with the oral use of snuff (Christen *et al.*, 1979a). A case of periodontal bone destruction has been reported in a snuff user (Christen *et al.*, 1979b).

A study of Swedish school children aged 13-14 years, of whom 13 of the boys (11%) used snuff orally, has shown that the use of snuff was associated with an increased intensity of gingivitis (Modéer *et al.*, 1980).

Effects on reproduction and prenatal toxicity

The still-birth rate to Indian women who chewed tobacco was 50 per 1000 live births (11/220) compared with only 17 per 1000 live births (20/1168) in women who did not chew tobacco. The mean birth weight of the offspring of tobacco chewers was approximately 500 g less than that of controls. This change was associated with a decrease in the mean gestation period. The sex ratio (male:female) of the offspring was 80:100 in the chewers in comparison to 108.5:100 in the controls (Krishna, 1978).

The mean weight of the placenta from 48 Indian mothers who took tobacco (in 85% of the cases as a mixture of tobacco and lime) was 15% greater than that from 48 controls (Agrawal *et al.*, 1983). The mean weight of newborn babies of 70 Indian tobacco users (the tobacco was either chewed or ingested alone or mixed with betel leaf or with lime) was 14% less than the weight of the babies of 70 matched controls (Verma *et al.*, 1983).

Absorption, distribution, excretion and metabolism

Gaede (1941) found that during chewing about one-third of the nicotine present in tobacco is extracted each hour. Nicotine is readily absorbed from the mouth (Gritz *et al.*, 1981). The saliva of snuff users contains *N'*-nitrosonornicotine, *N'*-nitrosoanatabine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (Hoffmann & Adams, 1981; Hoffmann *et al.*, 1982). Three samples of the saliva of two healthy men chewing Indian tobacco contained *N'*-nitrosonornicotine (17-60 ng/ml), *N'*-nitrosoanatabine (14-52 ng/ml), *N*-nitrosoproline (0.5-10 ng/ml), nicotine (120-179 μg/ml), nitrite (10-36 μg/ml) and thiocyanate (9-40 μg/ml). Urine collected from a man from commencement of chewing contained a total of 80 ng *N*-nitrososarcosine per 6-h urine, 860 ng *N*-nitrosoproline, 950 μg nicotine and 795 μg cotinine (Nair *et al.*, 1985).

The concentration of nicotine in the plasma of 11 young adult male users of oral snuff rose from a morning level of 2.9 ng/ml (after overnight abstinence) to 21.6 ng/ml after consuming an average of 11 g snuff over a period of 6-8 h. Plasma cotinine levels rose from a mean level of 137 ng/ml to 197 ng/ml. Subjects fell into two groups, with two-thirds absorbing substantial amounts of nicotine and one-third appearing to have almost no absorption (Gritz *et al.*, 1981).

After inhalation of a single pinch of snuff, blood nicotine concentration rose within minutes to 40 ng/ml — about twice the peak concentration found after smoking a cigar and comparable with the concentration found in heavy cigarette smokers (Russell *et al.*, 1980). The amount of nicotine and the relative proportion of its metabolites in the urine of nasal snuff takers was similar to that in smokers (Temple, 1976).

Nitrite levels were higher in the saliva of *mishri* users from two locations (urban and rural) and of tobacco chewers than in that of control groups. Subjects who chewed tobacco or used *mishri* had higher levels of nitrate reductase activity than controls (Murdia *et al.*, 1982).

Mutagenicity and chromosomal effects

The proportion of exfoliated micronucleated cells from the mucosa of the inner lip of 27 *khaini* users was 2.2%, ranging from 0.8-4.9%. In 15 non-users of *khaini*, the proportion of micronucleated cells was 0.5%, ranging from 0.3-0.8% (Stich *et al.*, 1982; Stich & Rosin, 1984).

The proportion of sublingual exfoliated micronucleated cells in 45 Uzbekis using *nass* was 4.3%, ranging from 1.6-6.3%. In 12 non-users of *nass*, the proportion of micronucleated cells was 0.4%, ranging from 0.0-0.5% (Zaridze *et al.*, 1985b,c).

The saliva collected from subjects during the chewing of Indian tobacco (Parijat Zafrani Patti) enhanced the frequency of chromatid breaks and exchanges in Chinese hamster ovary (CHO) cells. No such increase was observed with saliva produced during the chewing of a western-type tobacco (Stich & Stich, 1982).

3.3 Studies of precancerous lesions and conditions in humans

A precancerous lesion is defined as a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart. A precancerous condition is a generalized state associated with a significantly increased risk of cancer (WHO Collaborating Centre for Oral Precancerous Lesions, 1978; Axéll *et al.*, 1984). Examples of oral precancerous lesions are leukoplakia and erythroplakia; oral precancerous conditions include sideropenic dysphagia, submucous fibrosis and, possibly, lichen planus.

The concept of leukoplakia as a precancerous lesion is based on the findings: (1) of a significant number of oral carcinomas associated with a pre-existing area of leukoplakia; and (2) that some leukoplakias appear to undergo malignant tranformation (for reviews, see Pindborg *et al.*, 1975; Silverman *et al.*, 1984). However, studies of oral leukoplakia are often difficult to compare owing to lack of uniformity in the histological definition of leukoplakia. This problem was discussed recently (Axéll *et al.*, 1984).

The precancerous conditions submucous fibrosis (Pindborg, 1972) and lichen planus have been observed mainly in Indians with a variety of chewing habits (Pindborg *et al.*, 1972). Lichen planus is a subacute or chronic idiopathic skin disease characterized by small, flat violaceous papules, often combining to form plaques. It is often pruritic and chiefly affects the flexor surface of the wrist, legs, penis and buccal cavity (Gennaro *et al.*, 1979).

Submucous fibrosis is an insidious, chronic disease affecting the oral mucosa and sometimes the pharynx and oesophagus, and occurs almost exclusively among Indians. Subepithelial changes lead to the presence of palpable fibrous bands, especially in the buccal mucosa, palate and labial mucosa. Diffuse blanching of the oral mucosa and especially of the soft palate may be another characteristic sign.

In the majority of studies carried out in Asia on oral precancerous lesions and conditions, the chewing habits of the subjects are not precisely defined, particularly in reference to inclusion of areca nut in the quid. These studies are therefore summarized in the monograph on betel-quid and areca-nut chewing. This section includes only reports of studies conducted in tobacco chewers and oral-snuff takers in North America, Europe and Africa, and on users of *shammah* and *nass*, which do not contain areca nut.

(*a*) *Prevalence of oral leukoplakia*

In a sample of 1490 British coal miners, Tyldesley (1971) found oral leukoplakia in 3.6% of 280 tobacco chewers and in none of 122 non-chewers. Roed-Petersen and Pindborg (1973) reported that of 450 Danish patients with oral leukoplakia, 32 (7.1%) used snuff. Axéll (1976) examined 20 333 Swedes aged 15 years and over: 14.2% of men and fewer than 0.1% of women took snuff. Of the 1444 snuff users, 116 (8%) had 'snuff dipper's lesion' (oral leukoplakia) (15.9% in men and 0.2% in women). The prevalence of oral leukoplakia among the total population examined was 3.6%.

Christen *et al.* (1979a,b) found leukoplakia in nine of 14 US university students who had been chewing tobacco or using snuff or both for two to nine years. Of 1119 US high-school students, 117 (11%) used 'smokeless tobacco' and 43% had oral-mucosal lesions in the labial groove in the form of hyperkeratotic or erythroplakic areas (Greer & Poulson, 1983).

In a study of 585 elderly coloured people resident in homes for the aged in the Cape Peninsula of South Africa, van Wyk *et al.* (1977) found that 119 (20.4%) had oral leukoplakia (excluding the tongue). Of these, eight (6.7%) chewed tobacco and four (3.4%) used snuff orally.

Of 661 individuals examined in Saudi Arabia, 187 used *shammah*; oral leukoplakia was found in 129 (69%) users but was not seen in non-users (Salem *et al.*, 1984).

Khasanov and Fasiev (1970) reported that 217 (14.7%) of 1479 *nass* users had pathological changes of the oral mucosa, such as atrophy, hypertrophy and chronic ulcer, while only 21 (0.5%) of 4674 non-users of *nass* and nonsmokers had the above changes. According to Aleksandrova (1970), 127 (44%) of 289 persons using *nass* and only one (0.4%) non-user had atrophic oral mucosa.

(*b*) *Histology of tobacco-related leukoplakia*

The first histological study of snuff-induced leukoplakia was carried out on seven patients by Pindborg and Poulsen (1962), who reported finding a band of homogeneous, eosinophilic periodic-acid-Schiff (PAS)-positive material in the connective tissue close to the salivary glands in the lower labial mucosa in four of the cases. In 1964, Lyon *et al.* concluded, on the basis of histochemical studies of these same Danish snuff users, that the PAS-positive material was amyloid. With regard to the epithelial changes, Pindborg and Renstrup (1963) studied biopsies from 12 snuff users and found a marked hyperplasia of the epithelium with a thickened surface layer of large and vacuolated cells; the surface epithelium was not keratinized, but streaks of focal parakeratosis were noted. Roed-Petersen and Pindborg (1973) found the streaks of parakeratosis most often in the lower labial mucosa in 31 biopsies; only one biopsy showed signs of epithelial dysplasia. Pindborg *et al.* (1980) compared the snuff-induced epithelial changes with similar changes caused by smoking. Clinically, both types of lesions present with a pumice-like appearance, and histologically they reveal a chevron-like (previously called 'streaks') keratinization of the epithelium. These changes are considered specific for tobacco usage.

Archard and Tarpley (1972) studied three oral biopsies from patients in the USA using snuff and found a homogeneous, eosinophilic deposit in the submucosa similar to that described by Pindborg and Poulsen (1962).

Axéll *et al.* (1976), examining 114 biopsies of snuff users in Sweden, described increased epithelial thickness with a vacuolated surface layer having wavelike, eosinophilic spikes directed toward the surface and a narrow, eosinophilic band demarcating the prickle-cell layer and acanthosis as characteristic features of the leukoplakia lesions associated with use of snuff. They also noted some slight inflammatory reaction.

Hirsch *et al.* (1982) examined clinically, histomorphologically and histochemically lesions of varying severity associated with snuff use. They found a high frequency of keratinized lesions, sialoadenitis and degenerative changes of the salivary glands (42%), and mild epithelial dysplasia (18%). These changes were seen to occur at a higher frequency than had been reported previously by others. Exposure to snuff was shown to be related to superficial as well as to deeply located histopathological cell changes. The most marked degenerative changes in the salivary glands were seen among patients with the most extensive exposure to snuff.

Van Wyk (1965) studied 25 biopsies from Bantus with snuff-induced lesions and characterized a typical snuff lesion as one with a hyperplastic, acanthotic and parakeratotic epithelial layer overlaying a chronically inflamed lamina propria. In four biopsies he found a 'disquiet epithelium'.

(*c*) *Malignant transformation of leukoplakia related to chewing habits*

In specimens from 12 oral-snuff users (Pindborg & Renstrup, 1963) and in 157 biopsies taken from clinically severe cases of leukoplakia among 15 000 oral-snuff users in the USA (Smith, 1975), no epithelial dysplasia was found.

Of 450 patients in Denmark with leukoplakia diagnosed from 1956-1970, 394 were followed for one to 14 years to ascertain oral cancer incidence and mortality (Roed-Petersen & Pindborg, 1973). This group included 32 oral-snuff users, with a mean exposure time of 22 years (exposure-time estimation was based on years of use and the duration of time a quid was retained in the mouth). One of the 32 had dysplasia at first examination, and another individual developed oral cancer during the follow-up period [not specified]. This corresponds to a rate of premalignant or malignant transformation of 6.2% for either dysplasia or carcinoma. In contrast, 19.5% of patients with leukoplakia not associated with snuff use developed carcinoma or showed dysplasia at first or later examination. [The Working Group noted that the follow-up period was not specified; in addition, the sample of snuff users was relatively small.]

In his study of British coal miners, Tyldesley (1976) followed up eight of 22 tobacco chewers with oral leukoplakia for five years. He found one case of malignant transformation to a squamous-cell carcinoma at the site at which the tobacco chew had been held for 30 years. In five other men there was no change, and in two, regression of the lesion was seen. [The Working Group noted the small sample size.]

Brown *et al.* (1965), in an investigation of 394 cases of oral cancer in Georgia, USA, noted that users of snuff had a significantly higher incidence of co-existing leukoplakia (60% *versus* 26% for non-users); 'extensive' leukoplakia was seen in 32% of users *versus* 6% of non-users. Both Landy and White (1961), in a study in Arkansas, USA, and Rosenfeld and Callaway (1963), in Tennessee, USA, noted that in cases of buccogingival cancer either there was concomitant leukoplakia or an evolution from leukoplakia had been observed.

3.4 Case reports and epidemiological studies of carcinogenicity to humans

Oral use

Although most of the studies of the relationship between the use of chewing tobacco or snuff and cancer have focused on risks to the oral cavity and pharynx, some evidence is also available concerning cancer at other anatomical sites. In many of the studies reported, chewing tobacco or snuff use was often only one of many potential risk factors considered.

(*a*) *Tobacco*

(i) *Descriptive studies and case series*

Many reports of case series have emphasized the relatively high frequency of chewing tobacco and snuff use among oral cancer patients (Table 30). [Early reports are not included if chewing tobacco or snuff habits were mentioned only in combination with other tobacco habits from which they were not distinguished; in many studies, neither smoking habits nor alcohol consumption was described.] The clinical characteristics of cancer patients who use smokeless-tobacco products have also been described (Table 31), especially the propensity of these cancers to occur in the presence of leukoplakia, to have often a verrucous appearance, and to be slow-growing, well-differentiated, squamous-cell carcinomas. Patients with cancer and with a chewing-tobacco or snuff habit are frequently described as having cancer at the site or on the side where the quid is most frequently placed.

In a study from Tennessee described more fully below (see Smith *et al.*, 1970; Smith, 1975), no oral cancer was observed in a group of 15 000 oral-snuff users; two carcinomas of the oral cavity were observed in another group of 500 oral-snuff users. [The Working Group noted that there is an absence of documentation on the source and characteristics of the study population.]

(ii) *Case-control studies*

Chewing tobacco or oral-snuff use (not specified) (Tables 32 and 35)

In Minnesota, Moore *et al.* (1952, 1953) studied the tobacco use histories of 40 white male patients, aged 50 years or more, with oral carcinoma (alveolar ridge, floor of mouth and buccal mucosa), 23 oral leukoplakia patients, 72 lip-cancer patients, and 93 with carcinoma of the 'face', in comparison with those of 38 control patients with hernias and other benign diseases. Although there was no difference between the case groups and the benign controls in the frequency of a 20-year history of pipe smoking or of cigar and cigarette smoking, there were statistically significant differences (all $p < 0.05$) for long-term use (more than 20 years) of chewing tobacco or snuff for each of the case groups. Within this population of primarily Scandinavian descent, more than half of the members in each case group and a third of the control group had used chewing tobacco or snuff for a minimum of 20 years. Tobacco histories were obtained by personal interviews conducted by the hospital personnel. [The composition of the case and control groups was not clearly defined. The relative risks associated with exposure of more than 20 years in contrast to none or 20 years or less were estimated by the Working Group from the numbers given. The relative risks were 2.4 for carcinoma of the face, 2.6 for lip cancer, 4.0 for oral carcinoma and 7.8 for oral leukoplakia.]

Peacock *et al.* (1960) identified persons with carcinoma of the buccal mucosa, alveolar ridge and floor of the mouth at a North Carolina, USA, hospital. Cases were included in the study only if information on tobacco use had been ascertained. These cases (staff and private patients) were compared to in-patient (staff and private patients) and out-patient (staff only) controls. It was found that 25/45 (55.6%) of the cases had used snuff or chewing tobacco for more than 20 years, whereas only 49/146 in-patient controls (32.6%) and 94/217 out-patient controls (43.3%) had done so. The association between oral cancer and use of chewing tobacco or snuff reached statistical significance only among older (60 years and over) staff patients in comparison with the in-patient control group. [The Working Group noted that controls whose habits were not known were apparently labelled non-users. Any such misclassification bias would have overestimated the strength of the association.]

The cases in a study by Vincent and Marchetta (1963) were 89 male and 17 female patients admitted successively to a New York State, USA, hospital with cancer of the oral cavity (tongue, floor of mouth, palate, gingiva, buccal mucosa), larynx, pyriform sinus or pharynx seen at the head-and-neck clinic. Controls comprised 100 male and 50 female patients of comparable age seen at the gastrointestinal clinic of the same hospital. Heavy alcohol consumption and smoking were more frequent in each case group than among controls, as determined by systematic history-taking from all subjects. The use of snuff or chewing tobacco (both designated 'snuff' by the authors in their Table 5) was also more common among the cases than in the controls. Only 5/100 male control patients (5%) had a chewing tobacco or snuff habit, whereas 9/33 (27.2%) oral-cavity cases, 3/33 (9.1%) pharynx cases, and 2/23 (8.7%) larynx cases chewed tobacco or took snuff. Cases were more likely to have smoked than controls. [Relative risks associated with use of chewing tobacco or snuff among men, calculated by the Working Group, were 7.1 for oral-cavity cancer, 1.9 for pharyngeal cancer and 1.8 for laryngeal cancer. Smoking was not controlled for.]

Williams and Horm (1977) conducted a population-based case-control study of the etiology of cancer at many different sites based on the interview responses of random-sample patients from the Third National Cancer Survey (1969-1971). Controls for the oral-cancer case group comprised patients with other cancers, excluding lung, larynx and bladder. Among men, use of chewing tobacco and snuff was strongly associated with cancer of the gum or mouth, but not with cancer of the lip or tongue; controlling for age, race and smoking habits, relative risks were 3.9 ($p < 0.01$) for moderate and 6.7 (non-significant) for heavy use of chewing tobacco or snuff. Among women, use of chewing tobacco or snuff was associated with cervical cancer; controls were patients with any other cancer. The relative risks, controlling for smoking, age and race, were 4.7 ($p < 0.05$) for moderate and 3.6 (non-significant) for high use. Suggestive associations (not statistically significant) were also found for laryngeal cancer (in men only, for whom risks were 1.8 and 2.6 for moderate and high use, respectively). [The Working Group noted that in this study multiple comparisons were made of many risk factors and many cancer sites, so that some positive findings may have been due to chance alone.]

Chewing tobacco (Tables 33 and 35)

Wynder *et al.* (1957a) compared 659 cases of lip, oral-cavity and pharyngeal cancer identified in a hospital in New York with 439 hospital-based controls with other benign and malignant conditions. Data on tobacco use and other factors were obtained from personal interviews. Cigarette, cigar and pipe smoking and alcohol use were associated with oral cancer in this study. A total of 17% of male cases had chewed tobacco in contrast to 8% of the controls, indicating a moderate association between tobacco chewing and cancer of the lip, oral cavity and pharynx. Some variation in the proportion of tobacco chewers was evident

Table 30. Proportions of smokeless-tobacco users in series of patients with upperdigestive/respiratory cancers[a]

Reference	US state or country	Series description	Habit	Proportion of smokeless tobacco users	Cancer at site where quid placed	Smoking habit
Abbe (1915)	New York	100 (90 men, 10 women) patients with oral cancer	Chew	13% of cases chewed tobacco	Yes, or near site	89/90 men
Ahlbom (1937)	Sweden	545 men with cancer of the oral cavity, pharynx, larynx or oesophagus, 1931-1936	Snuff, chew, NOS	70% of patients with buccal/-gingival/mandibular (outer oral cavity) cancer, 37% with cancer of the lip, 26% with cancer of the inner oral cavity, 16% with cancer of the pharynx/larynx/-oesophagus	NM	7, 6, 39, 64% cigarette + cigar smokers, respectively; 23, 57, 35, 20% pipe smokers, respectively
Ackerman (1948)	Missouri	31 (26 men, 5 women) patients with verrucous carcinoma of the oral cavity	Chew	61% of 18 patients with buccal-mucosal cancer were 'inveterate' tobacco chewers	NM	NM
Wilkins & Vogler (1957)	Georgia	81 (37 men, 44 women) patients with gingival cancer, 1937-1956	Snuff, chew	50% of women used snuff orally, 32% of men chewed tobacco	NM	1/44 women who used snuff orally also smoked, 3 other women smoked only; 9/37 men smoked only, the 12 who chewed also smoked
Dunn & Farrior (1962)	Florida	112 patients with cancer of the oral cavity, larynx or nasopharynx	Chew, snuff	3% of cases chewed tobacco (2/4 with buccal-mucosal cancer, 1/11 with tonsillar cancer). 5% of cases used snuff orally (1/3 with cancer in palate and upper alveolar ridge, 5/16 with cancer in floor of mouth and lower alveolar ridge)	NM	2 chewers smoked; oral snuff users did not smoke; 90% of cases smoked
Goethals *et al.* (1963)	Minnesota	55 (45 men, 10 women) patients with histologically-confirmed verrucous cancer of oral cavity, 1935-1957	Chew	13% of cases chewed tobacco	NM	21/55 smoked
Coleman (1965)	Virginia	23 patients with buccal-mucosal or gingival cancer, 1956-1964	Snuff, chew, NOS	100% used snuff orally or chewed tobacco	Yes	NM
Leffall & White (1965)	Washington DC	107 (77 men, 20 women) black patients with oral-cavity cancers, 1948-1963	Chew	4/7 (57%) patients with buccal-mucosal cancer chewed tobacco	NM	NM
Kraus & Perez-Mesa (1966)	Missouri	77 (68 men, 9 women) patients with verrucous carcinoma of the oral cavity, 1942-1962	Chew, snuff	47% chewed tobacco, 4% used snuff orally, 22% denied tobacco use (5/9 women chewed or used snuff)	Often	9/68 men and 1/9 women were heavy smokers

Dunn & Dykstra (1967)	Florida	427 patients with cancer of the upper respiratory tract	Chew, snuff	4/45 (9%) of patients with cancer of the floor of mouth and lower alveolar-ridge, 2/18 (11%) with cancer of palate and alveolar ridge and 4/26 (15%) with buccal-mucosal cancer used snuff orally; 7/26 (27%) with buccal-mucosal cancer chewed tobacco; 0/388 patients with other cancers chewed tobacco or used snuff		40/45, 15/18 and 13/26 smoked, respectively
Zacho *et al.* (1968)	Denmark	535 (385 men, 150 women) patients with gastric cancer and medical record notation on tobacco use	Chew, snuff, NOS	25% of men and no women used smokeless tobacco. Use especially common in patients with cancer of the pylorus	NA	87% of smokeless tobacco users were also smokers
Fonts *et al.* (1969)	Kentucky	10 (5 men, 5 women) patients with verrucous carcinoma of the oral cavity, 1961-1966	Chew, snuff	60% were tobacco chewers, 10% oral snuff users, 20% chewers and oral snuff users	NM	NM
Zacho *et al.* (1975)	Denmark	953 patients with gastric cancer (493 with tobacco-use data available in records), 1948-1973	Chew, snuff, NOS	No women used smokeless tobacco and 30 and 34% male smokers and nonsmokers used smokeless tobacco, respectively. Use common in patients with cancer of the body of the stomach	NA	In men, 36% smoked pipes, 29% cheroots, 13% cigars, 22% cigarettes
Hartselle (1977)	Alabama	289 patients with squamous-cell carcinoma of the oral cavity (39 indicated use of tobacco) compared with 248 cases in tumour registry (206 indicated use of tobacco), 1955-1975	Snuff, chew	Of the 39 pathology patients with tobacco use mentioned, 51% used snuff orally, 5% chewed tobacco. Of 206 patients from tumour-registry records, 31% used snuff orally, 6% chewed tobacco	NM	44% of 39 pathology patients smoked. 63% of 206 tumour registry patients smoked
McGuirt (1983b)	North Carolina	169 women with 'mucosal head and neck cancer', 1975-1980	Snuff, chew, NOS	33% used snuff orally or chewed tobacco	NM	40% smoked
Brown *et al.* (1965)	Georgia	394 (231 men, 163 women) patients with cancer in the mucous membrane of the mouth from the anterior tonsillar pillar anteriorly to the lips, but not including lip or tonsillar pillar, 1937-1957	Snuff, chew	In women, 44% used snuff orally, 2% chewed tobacco; in men, 3% used snuff orally, 9% chewed tobacco	NM	NM
Rosenfeld & Callaway (1963); Rosenfeld & Green (1969)	Tennessee	300 (125 men, 175 women) patients with oral or gingival cancer; 225 patients (154 men, 71 women) with cancer of the tongue or floor of mouth (accurate data regarding usage of snuff were obtained from 214 of the women)	Snuff	Among 159 women with buccal/gingival cancer, 90% used snuff orally; among 55 women with cancer of the tongue or floor of mouth, 22 used snuff orally	NM	NM

[a]NOS, not otherwise specified; NM, not mentioned; NA, not applicable

Table 31. Case reports and case series of smokeless-tobacco users with cancer

Reference	US state or country	Cancer site (no. of cases)	Special clinical/histological characteristics, other comments	Cancer at site of quid placement	Years of smokeless tobacco use	Smoking	Alcohol	Leukoplakia
Chewing tobacco								
Wynder (1976) [Warren (1837)]	Massachusetts	Tongue (1)	Men	Yes	NM	NM	Yes, 'ardent spirits'	NM
Friedell & Rosenthal (1941)	Illinois	Mouth (8)	All men, cancers generally well-differentiated, slow-growing	6/8 yes, 1 no, 1 NM	One for 12 years; the others, 35-65 years	4/8	NM	7/8
Moertel & Foss (1958)	Minnesota	Buccal mucosa (1)	Men, 2 separate squamous-cell carcinomas in the buccal mucosa	Yes	'Entire adult life'	NM	NM	Yes
Sorger & Myrden (1960)	Canada	Buccal mucosa (4)	Men with verrucous carcinoma of buccal mucosa. All over 70 years old	Yes for at least 3/4	One for 10 years; one, 'always'; 2, since teens	2/4	NM	2/4
Snuff								
Root *et al.* (1960)	Minnesota	Ear (1)	Farmer who placed snuff in ear	Yes	42 years	NM	NM	NM
Landy & White (1961)	Arkansas	Buccogingival (25)	Women, average age 68 years. Usually well-differentiated squamous-cell carcinoma. Metastases rare	Most	20->50 years	NM	NM	Concomitant
Stecker *et al.* (1964)	Minnesota	Gum (1)	71-year-old man who used snuff 10-12 h daily, verrucous carcinoma	Yes	40 years	NM	NM	Yes
Axéll *et al.* (1978)	Sweden	Oral cavity (49)	Older than other oral cancer cases, more likely to come from northern Sweden	67%	NM	NM for snuff users	NM	NM
Sundström *et al.* (1982)	Sweden	Anterior oral vestibule (23)	Men; *Candida* (58% of 19 cases), multiple carcinomas (26%). Verrucous carcinoma in some. Average age 76 years	Yes, by design	NM	NM	NM	At least 5
McGuirt (1983a)	North Carolina	Oral cavity (57)	13 men, 44 women. Persons with snuff use. Sites: 47% buccal mucosa, 32% alveolar ridge, 11% tonsillar trigone, 10% elsewhere in oral cavity. 24 verrucous-appearing, only 4 verrucous carcinomas, 58% well-differentiated histologically	89%	75% for >40 years	No, by design	No, by design	61%

[a]NM, not mentioned

Table 32. Case-control studies of smokeless-tobacco use (unspecified) and oral cancer

Reference	Source of patients	Cases	Controls	Smokeless tobacco use in cases and controls	RR (95 % confidence limit)[a]	Distribution of smoking habits
Moore *et al.* (1952, 1953)	Minneapolis, MN hospital, since 1951	40 men with oral cancer	38 patients with benign diseases	65% of cases, 32% of controls for >20 years	[4.0]	38% of cases, 53% of controls smoked cigarettes
Peacock *et al.* (1960)	Chapel Hill, NC hospital, 1952-1958	45 patients with oral cancer and known tobacco habits	146 patients over 40 years old, frequency matched on race, sex, economic status; 217 'randomly' sampled outpatients	55.6% of cases; 32.6% of inpatient controls; 43.3% of outpatient controls for >20 years	Significant among older staff cases	Not stated
Vincent & Marchetta (1963)	Buffalo, NY hospital	89 men and 17 women with cancer of the oral cavity, pharynx or larynx	100 men and 50 women, successive patients in same age group at gastrointestinal clinic	16% of male cases, 5% of male controls	Men (2.4-20.7) [7.1]	Among men, 85% of cases and 54% of controls smoked cigarettes
Williams & Horm (1977)	Third National Cancer Survey, 1969-1971	57 men and 27 women with cancer of the gum-mouth	2102 men and 3464 women with cancer at sites unrelated to smoking	Of cases, 11 men and 2 women; of controls, 164 men and 53 women	Men 3.9 ($p < 0.01$)	Smoking, age and race controlled for in analysis

[a]RR, relative risk; figures in square brackets were calculated by the Working Group.

by case type: patients with lip, buccal-mucosal and palate cancer were most likely to chew tobacco. However, all tobacco-chewing cases drank alcohol and all but one smoked.

Wynder and Bross (1961) reported that 21% of 150 male patients with squamous-cell carcinoma of the oesophagus were tobacco chewers, compared with 10% of the 150 male controls with other malignant cancers and benign conditions. Cases and controls were ascertained from hospitals in New York City and Brooklyn, USA, in 1956-1959, and tobacco use was ascertained through interviews with cases and controls. Smoking and alcohol consumption were associated with an increased risk of oesophageal cancer in this population and were more common habits than tobacco chewing, but were not controlled for in analyses related to tobacco chewing. [The Working Group noted that the actual number of tobacco chewers among cases could not be estimated.]

A case-control interview study in Atlanta, Georgia, USA, by Vogler *et al.* (1962) included four groups seen over a 19-month period (1956-1957): 333 patients with cancers of the oral cavity, pharynx and larynx, 214 patients with other diseases of the mouth, 584 patients with other cancers, and 787 patients without cancer whose mouths were not examined. Among rural men, the percentage of tobacco chewers was significantly higher in the oral, pharyngeal and laryngeal cancer group and in the mouth-disease controls (36%) than in other cancer and non-cancer controls (15% or less chewed). This association was also found for urban men: 17% of oral-cavity cancer patients chewed, compared to less than 10% in the other two groups. However, approximately 50% of rural male cases smoked cigarettes and approximately 70% of urban cases smoked (which was more common than in controls). Patients with cancer of the oral cavity were more likely to chew tobacco than patients with cancer at other oral and pharyngeal sites. [Percentages preceded by the word 'approximately' are derived from diagrams in the text. Smoking was not controlled for in the analysis.]

In a case-control study of bladder cancer (Wynder *et al.*, 1963), tobacco chewing was reported by interview in 33/300 male cases (11%) and 24/300 male controls (8%). Study subjects were ascertained over a five-year period (1957-1961) in hospitals in New York City, USA.

In Puerto Rico, Martinez (1969) conducted a population-based case-control study of oral, pharyngeal and oesophageal cancer to examine environmental, tobacco and dietary factors. Each of the 400 histologically-confirmed carcinoma cases was matched with three controls for age and sex: one from the hospital where the case was diagnosed and the other two from the neighbourhood in which the case lived. Overall, 3.7% of the cases (15 persons) chewed tobacco only, compared to 4.0% of controls (48 persons); however, the percentages varied considerably by cancer site and sex. For each of the three cancers studied, the percentage of male cases who chewed only exceeded that of the controls; the same was true for female cases of oesophageal cancer and controls. However, few women with oral (none) or pharyngeal (two in controls) cancer had this habit. The chewing tobacco was typically mixed with molasses. [The Working Group noted that if relative risks for those with only a chewing habit compared to those with no habit are calculated on the basis of the figures given, the risks for men are 11.9 for oral cavity cancer, 8.7 for cancer of the pharynx and 1.2 for cancer of the oesophagus. The relative risk for oesophageal cancer in women was 2.7. However, it was noted that the numbers of tobacco chewers in the site-specific tables do not add up to the total numbers of chewers in the study, and therefore these calculated relative risks may not be accurate.]

Cole *et al.* (1971) found no difference between the observed number of male lower-urinary-tract cancer patients who chewed tobacco (46) and that expected to have the habit (42.3), derived from the distribution of habits in the controls. This population-based study included 470 interviewed cases (men and women) from 111 hospitals in the Boston and

Brockton, Massachusetts, USA, statistical area over an 18-month period (1967-1968) and 500 controls drawn from 'residents lists', which enumerate almost all persons residing in the study area.

Browne *et al.* (1977) compared 75 cases of oral cavity cancer identified through a cancer registry between 1957-1971 with 150 controls matched for age, sex, primary occupation and residence drawn from a clinical practice in the UK in 1974-1976. The subjects or, in the event of death, next-of-kin were interviewed using a structured questionnaire. The authors found that controls were more likely to have used chewing tobacco (36/150) than cases (16/75). No difference between cases and controls was observed with regard to duration of tobacco chewing, and none of the subjects used snuff. Since there was matching in the design on primary occupation (but not on secondary occupation), it appears that some residual confounding by employment remained; indeed, the negative association with chewing tobacco disappeared when the data were stratified by occupation. [The Working Group noted that the discordance in time of ascertainment of the cases (1957-1971) and of controls (1974-1976), resulting in matching of attained age at different time intervals, and the necessity of interviewing primarily the next-of-kin of deceased cases raises concern about bias, especially from secular changes in tobacco habits and differential recall of habits. Overmatching may also have occurred by neighbourhood and occupation.]

Patients with cancers of the lung (1048 cases), oral cavity (591), larynx (387), oesophagus (183) and bladder (586) in 20 hospitals in eight major US cities were compared with 2560 matched hospital controls with diseases unrelated to tobacco use during 1969-1975 (Wynder & Stellman, 1977). Among men, 233 controls (9.0%) had used chewing tobacco at some time, whereas 61 (10.3%) of patients with oral-cavity cancer, 21% with lung cancer, 12% with laryngeal cancer, 11% with oesophageal cancer and 8% with bladder cancer chewed. Less than 0.5% of women chewed tobacco. [The authors estimated that the relative risks for cancer at each of these five sites in men who chewed tobacco included 1.0 within 99% confidence limits, and none attained statistical significance.] The smoking habits of chewers and non-chewers were similar. In this population, smoking was strongly related to cancer at each site studied, while alcohol consumption was linked to cancers of the oral cavity, larynx and oesophagus.

No association between chewing tobacco and bladder cancer was observed in a study of 480 male and 152 female pair-matched bladder-cancer cases and controls in Canada (Howe *et al.*, 1980); the estimated relative risk was 0.9, based on 61 discordant pairs. The relative risk estimate was unchanged after controlling for smoking.

In a study of etiological factors for oesophageal cancer, Pottern *et al.* (1981) noted that the proportion of tobacco chewers was slightly higher among matched controls than among oesophageal-cancer cases in their interview study with the next-of-kin of 120 black, male oesophageal-cancer decedents in Washington DC, USA, and 250 black men who had died of other causes. However, the authors commented that the number of subjects with a chewing habit was small: only 3.3% of subjects chewed.

In an examination of potential etiological factors related to the risk of cancer of the nasal cavity and paranasal sinuses, Brinton *et al.* (1984) found a relative risk of 0.7 associated with chewing tobacco; this was lower than those associated with oral-snuff use (1.5), cigarette smoking (1.2) or pipe smoking (1.2), and equal to that for cigar smoking (0.7), all of which were non-significant. Confidence intervals for all of these relative risks included the null value. This case-control study included 160 hospital-ascertained cases in North Carolina and Virginia, USA, and 290 hospital controls and controls ascertained from death certificate. Subjects or their next-of-kin were interviewed by telephone.

Table 33. Case-control studies of chewing tobacco use and oral cancer

Reference	Source of patients	Cases	Controls	Chewing tobacco use	Distribution of habits
Wynder *et al.* (1957a)	New York, NY hospital	659 (543 men, 116 women) white patients with cancer of the lip, oral cavity or pharynx	439 white patients with benign head and neck tumours, thoracic diseases, lymphomas or gastrointestinal cancers	17% of cases and 8% of controls	All except 1 chewer smoked as well
Vogler *et al.* (1962)	Atlanta, GA hospital, 1956-1957	333 (235 men, 98 women) patients with cancer of the lip, oral cavity, pharynx or larynx	3 groups: 214 with other mouth diseases, 584 with other cancers, 787 non-cancer patients	In urban men, 17% of cases and <10% of controls; in rural men, 36% of cases and other-mouth-disease controls, about 15% and 10% of other controls	At least 45% of male urban and rural cases and controls smoked
Martinez (1969)	Cancer Registry, Puerto Rico, 1966	400 (290 men, 110 women) histologically-confirmed cases of cancer of the lip and oral cavity, pharynx or oesophagus	1 hospital/clinic control per case, 2 neighbourhood controls matched for age and sex per case	3.7% of cases and 4.0% of controls [Among men, RR for oral cavity cancer, 11.9.][a] Few women chewed.	The most common mixed use of tobacco was cigarettes and cigars (50%), followed by cigarettes and chewing tobacco (15.2%), and cigars and chewing tobacco (11.4%)
Browne *et al.* (1977)	English regional cancer registry, 1957-1971	75 patients with cancer of the oral cavity	150 living residents matched for age, sex, residence and primary occupation	9% of cases and 13% controls	All cases smoked. The proportion of controls with no tobacco habit not clear
Wynder & Stellman (1977)	20 hospitals in 8 US cities, 1969-1975	591 patients with cancer or the oral cavity, 1048 of the lung, 387 of the larynx, 183 of the oesophagus and 586 of the bladder	2560 patients without smoking-related diseases matched for age, sex, race and city	Among men, 10.3% of oral-cavity cases and 9.0% of controls; <0.5% of women	Smoking habits did not differ in chewers and non-chewers

[a]RR, relative risk; calculated by the Working Group

Hartge *et al.* (1985) reported that use of chewing tobacco was unrelated to bladder-cancer risk. Their study included 2982 patients with bladder cancer who were identified from records of 10 large population-based case registries throughout the USA (1977-1978) and who were interviewed for information about tobacco use and other factors. There was a total of 5782 population-based controls: controls 65 years of age and older were selected from records of the Health Care Financing Administration, and controls aged under 65 years were chosen by a random digit-dialling method. The analysis was restricted to men. Among men who never smoked cigarettes, the relative risk for bladder cancer was 1.0 for chewing tobacco, controlling for age, race, residence and other tobacco habits. The authors cautioned that the relative risk estimates were somewhat unstable in view of the small number of users (40 in cases and 133 in controls).

Snuff (Tables 34 and 35)

Wynder *et al.* (1957b) compared 477 [misprinted as 472 in the table in the original paper] patients with cancers of the lip, oral cavity, maxillary sinus, nasopharynx, hypopharynx, oesophagus and larynx to 333 patients with other malignancies seen in a hospital in Stockholm, Sweden, from 1952-1955. Interviews with patients and a review of the medical history were undertaken for all study subjects. More of the buccal- and gum-cancer patients used snuff than did controls (no women practised the habit). There was suggestive evidence by ridit analyses that snuff use was related to buccal-mucosal cancer in men, and the majority of cases with gum and buccal-mucosal cancers had their tumours in the area of the mouth where the quid was held. [The Working Group could not extract the number of snuff users.]

In the case-control interview study in Atlanta, Georgia, USA, by Vogler *et al.* (1962) described on p. 98, among 642 female urban subjects, 40% of the 38 oral-cavity cases, but only 2%, 3% and 1% of the 57 other-mouth-disease, 170 other-cancer and 377 non-cancer controls, respectively, had used snuff. Similar findings were observed for the 371 rural females: 75% of the 55 cases had used snuff orally in contrast to 11% of 37 other-mouth-disease patients, 20% of 129 other-cancer patients, and 11% of 150 non-cancer patients. Only 7% of female rural patients smoked. About 30-40% of urban women smoked cigarettes, but smoking habits were similar in each study group. The differences in snuff use between cases and controls were statistically significant for most of the age strata studied. One of 3 (33%) female lip-cancer patients used snuff, in contrast to 53/72 (74%) women with oral-cavity cancer and 2/18 (11%) patients with pharyngeal of laryngeal cancer. [The Working Group noted that the reportedly similar proportions of smoking habits among urban women and the low proportion of smokers in the rural sample indicate that the association between snuff and oral/pharyngeal/laryngeal cancer was not confounded by smoking.]

In a case-control study of bladder cancer (Wynder *et al.*, 1963), study subjects were ascertained over a five-year period (1957-1961) in hospitals in New York City, USA. Snuff use was reported by interview in 6/300 male cases (2%) and 9/300 controls (3%).

In the study described on p. 98, Cole *et al.* (1971) found no difference in the observed number of male lower-urinary-tract cancer patients who used snuff (3) compared to the number expected (2.9), derived from distribution of habits in the controls.

In the study by Wynder and Stellman (1977) described on p. 99, 8 patients with oesophageal cancer, 11 with bladder cancer, 15 with laryngeal cancer, 10 with oral-cavity cancer and 35 with lung cancer used snuff. The highest relative risk was 1.7 for oesophageal

cancer associated with snuff use [but none of the risks attained statistical significance]. Smoking was strongly related to the development of cancer at each site studied, while alcohol consumption was linked to cancers of the oral cavity, larynx and oesophagus.

Westbrook *et al.* (1980) identified 55 female patients with cancer of the alveolar ridge or buccal mucosa from 1955 to 1975 at a university clinic in Arkansas, USA. A random sample of 55 female controls of comparable age seen at the institution over the same time period constituted the comparison group. Of the 55 female patients, 50 (91%) were oral-snuff users whereas only one (2%) member of the control group was a user (RR = 540, highly significant). Only three cases smoked cigarettes and one chewed tobacco. The average duration of snuff use was 52 years. The snuff users, 44 (80%) of whom were white, averaged 66 years of age. In the 15 patients for whom sufficient data were available, 14 were found to have a tumour where the snuff had been typically placed. The cancers in snuff users were all squamous-cell carcinomas, most of them well-differentiated. [The Working Group considered that the apparent use of medical records as a source of information on tobacco and alcohol use may have led to misestimation of snuff use. If controls who used snuff were less likely than cases to be recorded as such, the magnitude of the association would have been overestimated.]

In the study of Pottern *et al.* (1981) reported on p. 99, the proportion of oral-snuff users was slightly lower in matched controls than in oesophageal-cancer cases; 1.7% of oesophageal-cancer cases used snuff.

Winn *et al.* (1981a) conducted a case-control study of oral-cavity and pharyngeal cancers among women in North Carolina in 1975-1978 to examine reasons for the exceptionally high mortality rates from these cancers among white women throughout the south-eastern USA. A total of 232 women hospitalized with or who had died from cancers of the tongue (International Classification of Diseases, 8th revision, code 141), gum (code 143), floor of mouth (code 144), other mouth (code 145), oropharynx (code 146), hypopharynx (code 148), and pharynx unspecified (code 149) were included in the case group. Two age-, race- and region of residence-matched controls were obtained for each case; interview was completed for 410. Subjects or their next-of-kin were interviewed in their homes. Tobacco-related risks were estimated by using a common reference group: women with no tobacco habit. The relative risk for white women who used only oral snuff was 4.2 (95% confidence limits, 2.6-6.7), while the relative risk associated with cigarette smoking among non-users of snuff was 2.9 (1.8-4.7). Among whites, the relative risk in those with both habits was 3.3 (1.4-7.8); these women had smoked fewer cigarettes and used snuff for fewer years than women with only one habit. Risks for black women were somewhat lower, but they had used snuff for fewer years and used fewer tins per week. Although 37 women had chewed tobacco, all except three were also oral-snuff users. One-third of all oral snuff users had developed the habit by the age of 10 years, and the average duration of use was 45 years. For cases of cancer of the gum and buccal mucosa, oral-snuff use among nonsmokers was related to years of use, with relative risks ranging from 13.8 (1.9-98.0) for 1-24 years, 12.6 (2.7-58.3) for 25-49 years, and 47.5 (9.1-249.5) for 50 or more years of use. For cases of cancer at other mouth sites and of the pharynx, the corresponding relative risks were 1.7, 3.8 and 1.3. The findings relating to oral-snuff use could not be explained by poor dentition (Winn *et al.*, 1981b) or by use of mouthwashes (Blot *et al.*, 1983). The consumption of fruits and vegetables was associated with a reduction in risk in the study population, primarily evident in cigarette smokers and in women without tobacco habits, and not among oral-snuff users (Winn *et al.*, 1984).

In the study of Brinton *et al.* (1984) on cancer of the nose and paranasal sinuses, described on p. 99, a slightly elevated, but not statistically significant, relative risk of 1.5 was

attributable to oral-snuff use. This was higher than any smoking-associated risk and higher than the relative risk for chewing tobacco. When analysed by histological type, it was found that both squamous-cell carcinomas and adenocarcinomas were related to snuff use; relative risks were 1.9 and 3.1, respectively, controlling for sex. Snuff use was more strongly related to squamous-cell nasal-cancer risk in men (3.7) than in women (1.4). When data were analysed by site of cancer, it was found that the relative risk for maxillary-sinus cancer was 2.8 (95% confidence interval, 1.2-6.3).

Hartge *et al.* (1985), in the study described on p. 101, reported that snuff use was unrelated to bladder-cancer risk. Among men who never smoked cigarettes, the relative risk for bladder cancer was 0.77 for snuff use, controlling for age, race, residence and other tobacco habits. The authors cautioned that the relative risk estimates were somewhat unstable in view of the small number of users (11 in cases, 50 in controls).

(iii) *Cohort studies*

Chewing tobacco or oral-snuff use (not specified) (Table 36)

Bjelke and Schuman (abstract, 1982) and Schuman *et al.* (1982) described results from a cohort study of 12 945 men in Norway who had been followed for more than 10 years (1967-1978). Relative risks for regular users of oral tobacco were 2.8 for buccal-cavity and pharyngeal cancer and 3.1 for oesophageal cancer; these were statistically significant. In addition, users experienced a relative risk of 2.2 for histologically-confirmed cases of pancreatic cancer (reported to be 'significant'). Prostatic-cancer risk was unrelated to tobacco chewing or snuff use.

Bjelke and Schuman (abstract 1982) and Schuman *et al.* (1982) described cancer risk in relation to use of chewing tobacco and use of snuff in a study of 16 930 US men, who had been policy holders of an insurance association, and were followed for more than 10 years for vital status (1966-1981). Tobacco use was assessed by postal questionnaire. Former snuff users/tobacco chewers had a relative risk of 3.3 (statistically significant) for pancreatic cancer (based on 33 total deaths and 7 deaths in former users), controlling for age and urban/rural residence. The relative risk associated with regular snuff use/chewing was elevated, but was not as high (2.1, based on 5 deaths in regular users) as for former chewers and was not statistically significant. Regular snuff use/chewing (but not former or occasional use) was linked to a 2.2 relative risk for prostatic cancer (91 total deaths, 21 deaths in regular users), which was statistically significant, adjusting for age and urban-rural residence. The authors also noted that tobacco chewing and snuff use were positively related to oesophageal cancer (relative risk, 2.6, non-significant), and that a multiplicative effect was associated with use of chewing tobacco and snuff and of alcohol.

Heuch *et al.* (1983) examined pancreatic cancer etiology using data from a cohort study of 11 959 men and 2519 women who responded to a questionnaire on lifestyle factors. The group consisted of adult residents in the 1960 Norwegian census, brothers living in Norway of migrants to the USA, and the spouses and siblings of subjects from a case-control study of gastrointestinal cancer. Cancer incidence and mortality were ascertained from record linkage with the Norwegian Cancer Registry and death files. Adjusting for region, urban/rural residence, age, sex, and cigarette and alcohol consumption, a marginally significant trend of increasing risk of pancreatic cancer with increasing use of chewing tobacco or snuff was evident. The relative risk of developing cancer in histologically-verified cases was 2.9 in regular users compared to persons who had never used the products.

Table 34. Case-control studies of oral use of snuff and oral-cavity cancer

Reference	Source of patients	Cases	Controls	Snuff use. RR (95 % confidence limit)[a]	Distribution of smoking habits
Wynder *et al.* (1957b)	Swedish hospital, 1952-1955	477 (265 men, 212 women) patients with cancer of lip, oral cavity, maxillary sinus, pharynx, larynx or oesophagus	333 patients with other cancers	In men, snuff use associated by ridit analysis with buccal and gum cancer. Cancers often where quid placed	Smoking more common in cases
Vogler *et al.* (1962)	Atlanta, GA hospital, 1956-1957	333 (235 men, 98 women) patients with cancer of the lip, oral cavity, pharynx or larynx	3 groups: 214 with other mouth diseases, 584 with other cancers, 787 non-cancer patients	Among women, 40% of urban cases, and 2%, 3% and 1% of controls, respectively; 75% of rural cases, and 11%, 20% and 11% of controls, respectively	40% of urban and 7% of rural women smoked
Wynder & Stellman (1977)	New York, NY hospital	591 patients with cancer of the oral cavity, 1047 of the lung, 587 of the larynx, 183 of the oesophagus, 587 of the bladder	2560 patients without smoking-related diseases matched for age, sex, race and city	Among men, 1.7% of oral-cavity cancer cases and 2.7% of controls; only 1% of women	Smoking habits of snuff users not stated
Westbrook *et al.* (1980)	Little Rock, AR hospital, 1955-1975	55 women with cancer of buccal mucosa or alveolar ridge	55 random age-stratified samples of women patients	91% of cases and 1.8% of controls [RR, 540 (143.9-2026.0)]	5% of cases smoked. Controls said not to have different smoking habits
Winn *et al.* (1981a)	5 NC hospitals and death certificates, 1975-1978	232 women with cancer of the oral cavity and pharynx	410 controls with other diseases/causes of death matched for age, race and residence	46% of cases and 30% of controls. RR in white nonsmokers, 4.2	43% of cases and 33% of controls smoked, but controlled for in analysis. Association with snuff remained strong with control of other factors (Winn *et al.*, 1981b; Blot *et al.*, 1983; Winn *et al.*, 1984)

[a]RR, relative risk; RR in square brackets calculated by the Working Group

Table 35. Case-control studies of non-oral cancer sites in which smokeless-tobacco use is described

Reference	Source of patients	Cases	Controls	Smokeless tobacco use. RR (95 % confidence limit)[a]	Distribution of smoking habits
Nasal cancer					
Brinton *et al.* (1984)	VA and NC hospitals and death certificates	160 patients with cancer of nasal cavity or para-nasal sinuses	290 matched controls from hospitals or death certificates	In oral snuff users, RR, 1.9 for squamous-cell carcinoma, 3.1 for adeno-carcinoma. Maxillary sinus RR, 2.8 (1.2-6.3)	Mentioned but not controlled
Oesophageal cancer					
Wynder & Bross (1961)	NY hospitals, 1956-1959	150 men and 37 women with carcinoma of the oesophagus	150 men and 37 women with other cancers and benign conditions	In men, 21% of cases and 10% of controls chewed tobacco. RR, 2.4 (statistically significant)	Smoking more common in cases
Martinez (1969)	Cancer Registry, Puerto Rico, 1966	400 histologically-confirmed cases of cancer of lip, oral cavity, pharynx and oesophagus	1 hospital/clinic control per case, 2 neighbourhood controls matched for age and sex per case	[Estimated RR for oesophageal cancer, 1.2 for men and 2.7 for women]	The majority of men smoked but tobacco chewers only examined separately
Wynder & Stellman (1977)	20 hospitals in 8 US cities, 1969-1975	183 oesophageal cancers	2560 matched hospital controls	In men, 11% of cases and 9% of controls chewed tobacco	Smoking habits similar in cases and controls
Pottern *et al.* (1981)	Washington DC	120 black men who died of oesophageal cancer	250 black men who died of other causes	3.3% of subjects chewed tobacco, 1.7% used snuff orally. Too few users for firm conclusions	Smoking more common in cases
Bladder cancer					
Wynder *et al.* (1963)	NY hospitals, 1957-1961	300 men and 70 women with bladder cancer	370 (300 men, 70 women) matched patients with other diseases	In men, 11% of cases and 8% of controls chewed tobacco; 2% of cases and 3% of controls used snuff orally. Too few users for firm conclusions	Smoking more common in cases
Cole (1971)	Boston/Brockton, MA, 1967-1968	470 population-based cases with bladder cancer	500 population-based controls	46 observed, 42.3 expected tobacco chewers; 3 observed, 2.9 expected oral snuff users. No difference	Smoking more common in cases
Howe *et al.* (1980)	3 Canadian provinces, 1974-1976	480 men and 152 women with bladder cancer	Pair-matched controls	RR, 0.9 for tobacco chewing (not significant)	Unchanged with adjustment for smoking
Hartge *et al.* (1985)	10 US population-based cancer registries, 1977-1978	2982 population-based cases with bladder cancer	5782 population-based controls	In men, RR, 0.77 for snuff and 1.0 for chewing tobacco (estimates unstable)	RRs controlled for age, race, residence and other tobacco habits
Other					
Williams & Horm (1977)	Third National Cancer Survey, 1969-1971	7518 cancer patients from population-based cancer registries	Persons with other cancers	Elevated risks for cervical cancer, RR, 4.7 (statistically significant) for moderate, 3.6 for heavy use. Excess of laryngeal cancer suggestive in smokeless-tobacco users	RRs adjusted for smoking, age, race

[a]RR, relative risk; estimated RR in square brackets calculated by the Working Group

Snuff

In Tennessee, USA, Smith *et al.* (1970) found that, among the 20 000 persons they examined in an unspecified number of clinics over an unspecified time interval, 15 000 were snuff users. This population included 1751 persons (1240 of them female) with mucosal changes warranting further study. Of the 1751, 157 were thought to require biopsy, but none were found to have a dyskeratotic or malignant lesion. Only 237 of the 1751 had cytological findings consistent with benign hyperkeratosis. [The Working Group noted that it is unclear whether patients with these 237 cytological abnormalities were in the group biopsied.] Repeated biopsies were made on over 75% of the 1751 with 'mucous membrane changes' at six-month intervals for 5.5 years. No cancer occurred.

In a subsequent follow-up of 1550 of the original study population of 1751 persons, including 128 of the original 157 biopsied patients (Smith, 1975), an additional 4.5 years of observation yielded no carcinoma or dyskeratosis. An additional group of 400 snuff users, apparently followed during the same 4.5 years, included 78 patients with mucosal change identified by biopsy. None of these patients developed dyskeratosis or carcinoma.

[The Working Group considered that, in these two papers, the consistent lack of clear specification as to which subset of the study group reference is being made makes it difficult to determine who was examined or followed up. While 15 000 persons appear to have been followed, only 1550 persons, selected on the basis of mucosal change present at outset and available for follow-up, received the 5.5-year follow-up. Perhaps as few as 128 received the full 10-year follow-up. The period of time during which the initial 20 000 persons were accrued is not specified, although at one point the authors state that the paper is a 'report of a 20-year study'. Since no tracing method is described, it is not clear whether the authors would have learned of any hospitalizations for oral cancer or deaths from all cancers in their study population.]

Table 36. Prevalence and follow-up studies of populations using smokeless tobacco

Reference	US state or country	Cohort description	Results[a]
Smith *et al.* (1970); Smith (1975)	Tennessee	128 oral snuff users with oral lesions (not dyskeratotic or malignant)	10-year follow-up yielded no malignancy. Not clear that follow-up methods would have detected new oral cancer patients
Bjelke & Schuman (1982); Schuman *et al.* (1982)	USA	16 930 men followed for 15 years	RR, 3.3 for pancreatic cancer for former snuff users/tobacco chewers (statistically significant). Also elevated RR for regular users (not significant). RR, 2.2 for prostatic cancer (statistically significant). Also association with oesophageal cancer
	Norway	12 945 men followed for 11 years	RR, 2.2 for pancreatic cancer, reported as significant. RR, 2.8 and 3.1 for cancer of the buccal cavity and pharynx and of the oesophagus (statistically significant)
Heuch *et al.* (1983)	Norway	11 959 men and 2519 women from several sources of Norwegians	RR, 2.9 for pancreatic cancer in smokeless-tobacco users, adjusted for cigarette smoking and alcohol consumption

[a]RR, relative risk

(*b*) Mishri, gudakhu *and* shammah

No oral cancer was observed in 22 606 persons using *mishri* in a prevalence study of 101 761 Indian villagers. In contrast, eight oral cancers were noted in 28 638 Indians with a chewing habit which included use of tobacco in the quid; and two cancers occurred in 1073 mostly male users with mixed habits. No oral cancer occurred in persons with no tobacco habit. *Mishri* use was much more common among women (38.9%) than among men (22.2%) (Mehta *et al.*, 1972). [The Working Group noted that women might be expected to have lower cancer rates regardless of tobacco use.]

In a house-to-house survey of a random sample in Singhbhum district, India, 8.3% of the population were reported to use *gudakhu*. No oral cancer or precancerous lesion was found among them (Mehta *et al.*, 1971).

In a survey of 661 persons from Saudi Arabia, of whom 28% used *shammah*, Salem *et al.* (1984) described seven patients with squamous-cell carcinoma of the mouth. Leukoplakia was present, and all had used *shammah* for 'many' years. Smoking and alcohol consumption were not mentioned.

(*c*) *Tobacco plus lime*

Jafarey *et al.* (1977) reported a hospital-based case-control study in Pakistan. The cases were 1192 histologically-diagnosed oral-cavity and oropharyngeal cancers. Controls (3562) were matched for age, sex and place of birth. Among men, 4% (27/683) of cases and 3% (60/1978) of controls, and among women, 7.7% (39/509) of cases and 3% (48/1584) of controls chewed tobacco, giving relative risks of 10.4 and 13.7, respectively, compared to those who neither chewed nor smoked. For further details of this study see p. 108. [The Working Group considered that although the habit in this study is reported as 'tobacco' chewing, in view of other publications by the same authors, it is likely to have been chewing of tobacco and lime.]

Chandra (1962) selected 450 cases of cancer of the buccal mucosa registered in a hospital in Calcutta, India, during 1955-1959, and used 500 of the friends or relatives who came to hospital with the patients as controls. Cases and controls were approximately age matched. Tobacco chewing was reported by 6.3% (18/287) of cases and 4.2% (17/410) of controls among men and 3.1% (5/163) of cases and 2.2% (2/90) of controls among women. For further details see the monograph on betel-quid and areca-nut chewing (p. 177). [Relative risks calculated by the Working Group from the data for tobacco chewing and for no chewing or smoking were 2.7 for males and 2.5 for females. The author did not clarify whether the chewing habit was tobacco only or tobacco plus lime.]

[In addition to these two studies, which directly examined the habit of 'tobacco-and-lime' chewing, some indirect epidemiological evidence is available from various studies detailed in the monograph on betel-quid and areca-nut chewing. In those studies (Wahi *et al.*, 1965; Wahi, 1968; Jussawalla & Deshpande, 1971; see also Tables 17-20, 23, 24 of the monograph on betel-quid and areca-nut chewing, pp. 175-176, 180) in which cancer risks were studied in relation to unspecified habits of betel-tobacco-lime chewing, it is almost certain that the predominant habit within the study populations was tobacco-lime chewing *without* betel. Therefore, at least part of the increased cancer risk reported in those studies is reasonably attributable to tobacco-lime chewing *per se*.]

(d) Tobacco plus lime plus other components

The first mention in the literature of a possible association between the use of *nass* and oral cancer goes back to 1910, by Petrov (quoted in Shilovtsev, 1941). In 1929, the cancer-notification form developed for the Samarkand region included a question on the *nass* habit, and in 1936 a campaign against the use of *nass* was organized (Shilovtsev, 1941).

(i) *Descriptive studies and case series*

Early reports on a possible association between the use of *nass* and oral cancer are based on clinical observations. Four cases of oral cancer were observed by Borovsky in 1924 among 11 cases of cancer in the Uzbek SSR; 50 cases of oral cancer in *nass* users were described by Kasansky in 1935 in the Turkmenian SSR (quoted in Shilovtsev, 1941). Of 59 cancer cases diagnosed in 1935-1938 in Samarkand among the native population, 39 (66%) were cancers of the mouth; none of the 139 cancer patients of other ethnic groups had oral cancer. *Nass* use is frequent among the native population in Samarkand (Shilovtsev, 1941).

Sharipov (1965) reported that, out of 250 patients with oral cancer diagnosed in Samarkand, 233 were Uzbeks, of whom 203 (87%) used *nass*. Khasanov (1965) reported that of 133 Uzbek patients with oral cancer, 94 (71%) used *nass*.

According to Khasanov (1965), the most frequent sites of oral cancer in people in the Samarkand region are the floor of the mouth (25%) and tongue (48%), sites which are in direct contact with *nass*. In people in the Kazakh SSR, where *nass* is more frequently placed in the lower lip groove, the sites found chiefly to be affected are the gum, buccal-mucous membrane, lip and anterior tongue (Paches & Milievskaya, 1980).

The age-standardized incidence rate for oral cancer in the Uzbek SSR (an area where *nass* use is common) is 2.3 per 100 000, whereas the same figures for two republics where *nass* is not used are 0.9 and 0.4, respectively (Paches & Milievskaya, 1980).

In Afghanistan, only 2.0% of all cancers were of the oral cavity, although the habit of chewing *naswar* is frequent (Sobin, 1969).

(ii) *Analytical studies*

One case of oral cancer was reported among 289 *nass* users in the Kazakh SSR who underwent oral examination; no oral cancer was seen in 243 smokers or in 1480 persons who neither smoked nor used *nass* (Aleksandrova, 1970).

Nugmanov and Bainakanov (1970) carried out a study in the Kazakh SSR in which the habits of oral-cancer patients were compared with the habits of controls in relation to use of *nass*. Of 93 oral-cancer patients, 30.1% used *nass* while only 6.7% of 247 controls did so. Further comparisons, involving 28 *nass* users with oral cancer and 19 *nass*-using controls, revealed that patients with oral cancer used *nass* more frequently and kept it in the mouth longer than controls (Table 37). [The Working Group noted that the sources of cases and controls were not reported; confounding due to other tobacco-related habits was not adjusted for; and no adequate statistical analysis was performed.]

Jafarey *et al.* (1977) found that 35 oral-cancer patients and 33 controls in Pakistan used *nass*, 84 patients and 114 controls used *naswar*, and 88 patients and 1690 controls had no tobacco habit. The relative risk for oral cancer associated with *nass* use was thus 20.4,

Table 37. Oral cancer in *nass* users[a]

Frequency of daily use of nass	Cases (28) %	Controls (19) %
<2 times	3.6	21.0
3-5 times	28.6	57.4
6-10 times	21.4	15.8
>10 times	46.4	15.8

[a]From Nugmanov and Bainakanov (1970)

and that associated with *naswar* use was 14.2. [The Working Group noted that confounding due to other tobacco-related habits was not adjusted for.]

Nasal use

The first reference to nasal use of tobacco as a cause of cancer comes from Hill in 1761, who described nasal cancer in two of his patients in London, which he ascribed to heavy snuff inhaling (Redmond, 1970).

(*a*) *Descriptive studies and case series*

Of the 86 Bantu patients with respiratory cancer seen from 1949 to 1954 in a radiation department in Johannesburg, South Africa, 46 (54%) had nasal-cavity and sinus cancers, which were predominantly well-differentiated squamous-cell carcinomas of the maxillary antrum. The cases were drawn from a population of about 2 000 000, and the authors estimated that about 25% of cases reached a hospital. The authors compared this proportion with the 5% frequency of nasal cancer in previously published series of Europeans with respiratory-tract cancer. [The Working Group noted that the rate of 86/2 000 000 or 4.3/100 000 compares with the age-adjusted incidence rates for 1956-1959 of 1.3/100 000 in male Bantus, 1.1/100 000 in whites and 2.0/100 000 in cape coloureds (Doll *et al.*, 1970.]. The authors noted that cigarette smoking is uncommon in the area, but snuff inhalation is widely practised by both Bantu men and women for whom its use has an important cultural and ritual history. The product typically contains tobacco leaves and an ash from aloe plants or other species, with the occasional addition of oil, lemon juice and herbs (Keen *et al.*, 1955); use is often 'one teaspoonful' per day (Baumslag *et al.*, 1971).

Higginson and Oettlé (1960) conducted a large incidence and mortality survey of cancer and a case-control study among South African Bantus. Cases were found through hospital records, death certificates and private doctors, and census data provided the denominators for the rates. They observed that the incidence of oral cavity cancer is similar for Bantus in South Africa and for US blacks, but that paranasal-sinus cancer occurs at far greater frequency in Africa than in the USA (31 observed, compared with <1 expected for US blacks or whites). Use of snuff both orally and nasally is common among Bantus (19% in men, 30% in women), on the basis of a small survey in one town. In rural hospitals, 21% of men and 37% of women used it nasally. In sinus cancer patients, 43% of seven men used snuff, a proportion higher than that found for patients with cancer of the mouth (0), lung (9%) or oesophagus (7%), or for hospital controls (6% young, 21% older men) and the surveyed population (4% young, 15% older men). [The Working Group noted that it is not always clear whether the snuff was taken nasally or orally.]

Hou-Jensen (1964) described 97 cases of cancer of the postnasal space (nasopharynx) ascertained from hospital and medical laboratory records in Kenya during 1957-1962. On the basis of these figures and census data, one tribe in particular, the Nandi, was found to have a moderately raised incidence of nasopharyngeal cancer, 1.43 per 100 000 per year, in contrast to an incidence of 0.52/100 000 or less for other tribes. The total incidence of other oral, nasal, pharyngeal and laryngeal cancers taken together did not show a high rate of occurrence in the Nandi. Six of 12 patients still alive had been snuff users and one had chewed tobacco. The author noted that snuff inhaling is common among many tribes in East Africa, but that the Nandis use 'liquid' snuff, which is not further described. [The Working Group noted that although the incidences in this report are high, they are based on small numbers and might have been due to over-representation of cases from the Nandi tribe.]

(*b*) *Analytical studies*

In a case-control study, Shapiro *et al.* (1955) reported that cancer of the paranasal sinuses (22 in men, five in women) accounts for a high proportion of respiratory-tract cancer (31 in men, six in women) (71% for men, 83% for women) in Bantu Africans, on the basis of radiation therapy department records from 1949-1951 of 37 Bantu cases from a group of hospitals in Johannesburg, South Africa. This was in sharp contrast to European cases seen in the Transvaal, where only 5% of respiratory-tract cancers occurred in the nasal sinuses. Most of the cancers were in the maxillary antrum (28/34 studied) and were described typically as well-differentiated squamous-cell 'epitheliomata'. The authors noted that 80% (22/28) of antral cancer cases reported 'prolonged and heavy' use of snuff [probably of the same composition as that described by Keen *et al.*, 1955] in contrast to only 34% in Bantu men with cancer at other sites. The authors stated that 'there was no obvious correlation between antral cancer and cigarette, pipe or *dagga* [marijuana] smoking.' [The Working Group noted that the source and nature of the control group is not described. There may be some overlap in the cases in this article with those in the study of Keen *et al.*, 1955.]

A case-control study of oral, pharyngeal and oesophageal cancer in South India was conducted by Shanta and Krishnamurthi (1963). Controls were drawn from a 'non-tumorous population' attending fairs and general health clinics. None of the controls used snuff, but some patients with cancers under study did (probably intranasally). This was especially evident for cancer of the oesophagus: 12.2% of male and 11.1% of female patients practised the habit; for those with cancer of the hypopharynx, 11.1% of men and 8.3% of women used snuff; and for cancer of the oropharynx, tonsils and epilarynx, 9% and 4.4% of men and women, respectively, took snuff. [The Working Group noted that this habit may have been spuriously related to cancer risk because of correlations with other risk factors for the diseases studied, namely, areca-nut and tobacco chewing, and smoking.]

Snuff inhalation was reported in a study from Ahmedabad, India. Out of 57 518 industrial workers examined, 1316 or (2.3%) used tobacco only in the form of snuff inhalation. No oral cancer was found in either a cross-sectional (Smith *et al.*, 1975) or follow-up study of this population (Bhargava *et al.*, 1975).

[The Working Group noted that the composition of the snuff used was not described in any of these studies.]

In three studies on wood-dust exposure (see IARC, 1981) and tumours of the nasal cavity and sinuses, enquiry was made into the use of snuff in order to assess its possible confounding role. Acheson *et al.* (1968) reported that three out of 11 furniture workers who had developed nasal adenocarcinomas and for whom the appropriate history could be elicited

had ever taken snuff, probably by inhalation. Andersen *et al.* (1977) reported that none of the cases of tumours of the nasal cavity and sinuses treated in a major Danish hospital during a 10-year period had used snuff.

Engzell *et al.* (1978) reported that the smoking and snuff habits of cases of carcinoma of the nose and paranasal sinuses reported to the cancer registry of the National Board of Health and Welfare in Sweden between 1961 and 1971 were no different from those found in a general survey. The report does not distinguish between the different ways of administering snuff.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

Smokeless-tobacco habits are practised by many millions of people, principally in Africa, Asia, Europe and North America, utilizing several techniques, products and dosage levels. In some countries, average consumption by users is estimated to be about 5 kg per year.

Among the thousands of compounds present in tobacco, the tobacco-specific nitrosamines are the only identified carcinogens that occur in mg/kg concentrations. Low levels (μg/kg) of carcinogenic polynuclear aromatic hydrocarbons and metals and of the α-emitting ^{210}Po (0.1-1.0 pCi/g) have also been detected. Use of smokeless tobacco entails extensive exposure to relatively high levels of tobacco-specific nitrosamines.

4.2 Experimental data

Various chewing tobaccos and unburnt cigarette tobaccos and their extracts were tested by oral administration in mice, by topical application to the oral mucosa of mice, rats and hamsters, and by subcutaneous administration, skin application, inhalation, intravesicular implantation and intravaginal application to mice. All of these studies suffered from certain deficiencies.

In a two-stage, mouse-skin assay, applications of tobaccc extract followed by promotion by croton oil induced papillomas and squamous-cell carcinomas of the skin. In further two-stage, mouse-skin assays, application of tobacco extracts following initiation by 7,12-dimethylbenz[*a*]anthracene resulted in papillomas.

A commercial Swedish snuff was tested for carcinogenicity in rats, by topical administration in a surgically-created oral canal, alone or in combination with herpes simplex virus type 1 infection. Two squamous-cell carcinomas of the oral cavity were observed in the group receiving both treatments, but this result was not statistically significant.

Snuff was tested by oral administration in hamsters, alone and in combination with calcium hydroxide, but the data were insufficient for evaluation. Several studies in hamsters in which snuff was administered as single or repeated applications into the cheek pouch or fed in the diet yielded insufficient data for evaluation.

Subcutaneous injection of ethanol extracts of snuff to rats did not produce an increase in tumour incidence.

Nass was tested for carcinogenicity in hamsters by administration into the cheek pouch or by skin application. No tumour was found at the site of application. Although *nass* was associated with an apparent excess of liver tumours in various groups receiving cheek-pouch administrations, which may be indicative of carcinogenic activity, deficiencies in reporting do not allow an evaluation to be made.

Ethanol extracts of chewing tobacco (*Nicotiana tabacum*) induce mutations in *Salmonella typhimurium* and in Chinese hamster V79 cells. They also induce micronuclei in bone-marrow cells of Swiss mice.

Ethyl acetate extracts of a chewing tobacco induce sister chromatid exhanges in cultured human lymphocytes and in a human lymphoblastoid cell line. Ethyl acetate and ethanol extracts of this tobacco induce transformation in Syrian hamster embryo cells.

Aqueous extracts of *nass* and *khaini* induce chromosomal aberrations in Chinese hamster ovary cells.

Saliva collected during the chewing of an Indian tobacco induced chromosomal aberrations in Chinese hamster ovary cells.

An increased proportion of micronucleated cells was found in exfoliated oral-mucosa cells from users of *khaini* and *nass*.

Sister chromatid exchanges are induced in Chinese hamster ovary cells by anatabine, nicotine and nornicotine.

Overall assessment of data from short-term tests: Ethanol extracts of (*Nicotiana tabacum*) chewing tobacco[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes		+		
Fungi/green plants				
Insects				
Mammalian cells (*in vitro*)		+		
Mammals (*in vivo*)			+	
Humans (*in vivo*)				
Degree of evidence in short-term tests for genetic activity: *Sufficient*				Cell transformation: No data

[a]The groups into which the table is divided and the symbols used are defined on pp. 16-17 of the Preamble; the degrees of evidence are defined on p. 18.

Overall assessment of data from short-term tests: Ethyl acetate extracts of a chewing tobacco[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes				
Fungi/green plants				
Insects				
Mammalian cells (*in vitro*)			+	+[b]
Mammals (*in vivo*)				
Humans (*in vivo*)				
Degree of evidence in short-term tests for genetic activity: *Inadequate*				Cell transformation: Positive

[a]The groups into which the table is divided and the symbols used are defined on pp. 16-17 of the Preamble; the degrees of evidence are defined on p. 18.

[b]An ethanol extract of this chewing tobacco also gave positive results.

4.3 Human data

Oral leukoplakia, a precancerous lesion, has been associated with oral-snuff use in a number of studies. One study of *shammah* users and several studies of *nass* users showed the same association.

Epidemiological studies of cancer and the oral use of smokeless tobacco in western populations have often not distinguished between tobacco chewing and snuff usage. Studies that have are summarized first.

Chewing tobacco

Reports of series of oral-cancer patients indicate that a high proportion were tobacco chewers and that the cancer often developed at the site at which the quid was placed habitually. However, data on chewing tobacco often came only from medical records; coexistent smoking habits often were not mentioned.

In two of five case-control studies in which data on tobacco use were appropriately obtained, the proportion of tobacco chewers among patients with cancer of the oral cavity, pharynx or larynx was two to three times higher than in control subjects; however, confounding by tobacco smoking or alcohol consumption could not be excluded. A large study of oral, pharyngeal and oesophageal cancer reported no difference in chewing-tobacco use between cases and controls; although the relative risk of having cancer of the oral cavity or pharynx was increased in tobacco chewers, this study is not convincing because of major

discrepancies in the tabulated data. Data on dose-response are lacking in all three studies. The other two case-control studies provide no clear evidence that tobacco chewing is associated with oral cancer: one study was very large but did not control for smoking, and one had serious methodological limitations.

Results from the four case-control studies of chewing-tobacco use and cancer of the oesophagus tend to show a slight increase in incidence. Nose and nasal-sinus cancers were found to be unrelated to tobacco chewing in one case-control study. No association between chewing tobacco and bladder cancer was observed in five case-control studies.

No cohort study of chewing tobacco alone and cancer has been reported.

Oral snuff

Reports of case series indicate that a high proportion of oral-cancer patients took snuff orally, and that the cancer frequently developed at the site of snuff application.

Four case-control studies, three from the south-eastern USA and one from Scandinavia, have implicated snuff use in the etiology of cancer of the oral cavity and, to a lesser extent, of the pharynx. In three of these studies, relative risks could not be computed; however, the differences in snuff usage between cases and controls were substantial, and confounding by cigarette smoking could be largely excluded. In the fourth study, in the south-eastern USA, the relative risk of oral and pharyngeal cancer for white women who used snuff but did not smoke was four times that for women with no tobacco habit; a strong dose-response relationship was observed; adjustment for other risk factors did not substantially reduce the relative risks.

In a cohort study of snuff users with non-malignant oral lesions, none developed cancer; however, the study was inadequately reported, had methodological limitations, and therefore could not be satisfactorily interpreted.

One case-control study has suggested that oral use of snuff may be associated with certain types of nasal-sinus cancer; in other case-control studies, no association was evident between snuff use and bladder cancer or between snuff use and cancer of the oesophagus.

Smokeless tobacco, unspecified

Studies that have not distinguished snuff from chewing tobacco are informative for four reasons when considered in conjunction with the habit-specific studies summarized above. First, reports of three case series confirm the high relative frequency of smokeless-tobacco use in oral-cancer patients. Four case-control studies have reported smokeless-tobacco use to be moderately to strongly associated with oral cancer, although smoking habits were not controlled for in three of the studies.

Second, a dose-response relationship was found in one large case-control study. The relative risks for oral cancer in men, after adjustment for other risk factors, ranged from four-fold for moderate smokeless-tobacco use to more than six-fold for heavy use.

Third, two cohort mortality studies, in which large numbers of persons with and without unspecified smokeless-tobacco habits were followed, provide evidence of a positive association with cancer. There was a two- to three-fold increased risk of death from oral, pharyngeal and oesophageal cancer in one study and from oesophageal cancer in the second.

Fourth, studies of unspecified smokeless-tobacco use provide some evidence of an increased risk of cancers at sites outside the upper digestive and respiratory tracts.

Whereas the data summmarized above all come from studies in North America and western Europe, the data below refer to studies of oral use of tobacco and nasal use of snuff in South-East Asia and in Africa.

Mishri/gudakhu

Oral cancer in users of *mishri* and *gudakhu* has been studied only in prevalence surveys; no case was found.

Shammah

Oral cancers were seen in users of *shammah*.

Tobacco plus lime (khaini)

Two large case control-studies, from Pakistan and India, reported two-fold to 14-fold increases in the risk of oral-cancer occurrence in tobacco (presumably tobacco-lime) users relative to non-users, in smokers and nonsmokers considered separately. Indirect evidence, deducible from various other studies of chewing and oral cancer in which the predominant habit entailed use of tobacco and lime without areca nut, corroborates the existence of this increased cancer risk.

Tobacco plus lime plus other components

In two case series, the majority of oral-cancer patients used *nass*; in another, the cancers were found to develop at the site at which the quid was placed habitually. Two case-control studies showed five-fold to 20-fold increases in the risk of oral cancer in association with *nass* use in the USSR; however, adjustment was not made for smoking habits and other potential confounders.

Use of *naswar*, examined in one case-control study in Pakistan, was associated with a marked increase in oral-cancer risk; however, positive confounding by tobacco smoking and betel-quid chewing could not be eliminated.

Nasal snuff

Two case-control studies among Bantu subpopulations in Africa, among whom nasal and oral use of indigenous snuff (containing tobacco and other ingredients, including aloe) are common, showed a moderately elevated risk of nasal-sinus cancer in relation to this habit; however, the studies had severe methodological limitations.

In India, two studies (one cross-sectional, one prospective) of oral cancer found no association between oral cancer and snuff inhaling. A case-control study reported snuff inhaling to be more common among patients with cancers of the oesophagus, hypopharynx or oropharynx than among controls; however, adjustment was not made for other risk factors for these cancers.

No study was available that specifically addressed the possible carcinogenicity of nasal use of snuff formulated in North America or western Europe.

4.4 Evaluation[1]

There is *sufficient evidence* that oral use of snuffs of the types commonly used in North America and western Europe is carcinogenic to humans. There is *limited evidence* that chewing tobacco of the types commonly used in these areas is carcinogenic.

Epidemiological studies that did not distinguish between chewing tobacco and snuff provide *sufficient evidence* for the carcinogenicity of oral use of smokeless-tobacco products, as reported in these studies.

In aggregate, there is *sufficient evidence* that oral use of smokeless tobacco of the above types is carcinogenic to humans.

There is *sufficient evidence* that oral use of tobacco mixed with lime (*khaini*) is carcinogenic to humans.

There is *inadequate evidence* that oral use of the other smokeless-tobacco preparations considered (*nass*, *naswar*, *mishri*, *gudakhu* and *shammah*) is carcinogenic to humans.

There is *inadequate evidence* that nasal use of snuff is carcinogenic to humans.

There is *inadequate evidence* to evaluate the carcinogenicity of chewing tobacco, snuff or *nass* to experimental animals.

5. References

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[1]For definitions of the italicized terms, see Preamble, pp. 15-16 and 19.

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APPENDIX B

Description of Online Searches for Smokeless Tobacco

DESCRIPTION OF ONLINE SEARCHES FOR SMOKELESS TOBACCO

Searches were limited to 1984 [the year before the IARC Monograph (1985), which has an extensive literature review] through September 1997.

Online searches for smokeless tobacco were performed in databases on the systems of the National Library of Medicine, STN International, DIALOG, and the Chemical Information System from 1984 to date. Toxicology information was sought in the NLM databases CANCERLIT, MEDLINE, and TOXLINE using the MESH heading for all neoplasms. Other searches were conducted in BIOSIS, EMBASE, AND EMIC. Animal studies were a particular focus of the BIOSIS searches.

Regulatory information was sought from the online full-text versions of the *Federal Register* and *Code of Federal Regulations* from the in-house FESA CD-ROM containing the latest *Code of Federal Regulations* and the *Federal Register* pertaining to CFR titles 21 (FDA), 29 (OSHA), and 40 (EPA).

Also, the review of 1200 life sciences journals was accomplished using Current Contents on Diskette® for current awareness.

APPENDIX C

Report on Carcinogens (RoC), 9th Edition
Review Summary

Report on Carcinogens (RoC), 9th Edition
Review Summary

Smokeless Tobacco

NOMINATION
Review based on letter from Dr. Hiroshi Yamasaki (IARC) recommending listing in the RoC based on IARC classification of Smokeless Tobacco as a known human carcinogens (IARC Vol. 37, 1985).

DISCUSSION
There is sufficient evidence of carcinogenicity in humans which demonstrates a causal relationship between the oral use of smokeless tobacco products and cancers of the oral cavity. Tumors often arise at the site of placement of the tobacco. Studies have also been published which report positive relative risks for tumors at sites including rectum, kidney, and most strongly, the prostate in humans who use oral smokeless tobacco products. The recommendations from the three NTP reviews of this nomination are as follows:

Review Committee	Recommendation	Vote
NIEHS (RG1)	list as known human carcinogen	11 yes/0 no
NTP EC Working Group (RG2)	list as known human carcinogen	8 yes/0 no
NTP Board RoC Subcommittee	list as known human carcinogen	6 yes/0 no

Public Comments Received
One comment was received which was opposed to listing Smokeless Tobacco as a known to be human carcinogen.