NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for SMOKELESS TOBACCO

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of the NTP Board of Scientific Counselors

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NTP Report on Carcinogens for Smokeless Tobacco

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 ($37^\circ$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

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

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<tr>
<td>21 CFR 801.126—Sec. 801.126 Exemptions for cigarettes and smokeless tobacco. Promulgated: 61 FR 44615, Aug. 28, 1996.</td>
<td>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.</td>
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<td>21 CFR 897.1—Sec. 897.1 Scope.</td>
<td>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&amp;C Act.</td>
</tr>
<tr>
<td>21 CFR 897.2—Sec. 897.2 Purpose.</td>
<td>Restrictions on the sale, distribution, and use of cigarettes and smokeless tobacco are established &quot;to reduce the number of children and adolescents who use these products and to reduce the life-threatening consequences associated with tobacco use.</td>
</tr>
<tr>
<td>21 CFR 897.3—Sec. 897.3 Definitions.</td>
<td>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.</td>
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<td>21 CFR 897.10—Sec. 897.10 General responsibilities of manufacturers, distributors, and retailers.</td>
<td>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.</td>
</tr>
<tr>
<td>21 CFR 897.12—Sec. 897.12 Additional responsibilities of manufacturers.</td>
<td>Manufacturers shall remove self-service displays, advertising, labeling, and other items that do not comply.</td>
</tr>
<tr>
<td>21 CFR 897.14—Sec. 897.14 Additional responsibilities of retailers.</td>
<td>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.</td>
</tr>
<tr>
<td>21 CFR 897.16—Sec. 897.16 Conditions of manufacture, sale, and distribution.</td>
<td>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.</td>
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<tr>
<td>21 CFR 897—Subpart C—Labels.</td>
<td>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.</td>
</tr>
<tr>
<td>21 CFR 897.24—Sec. 897.24 Established names for cigarettes and smokeless tobacco.</td>
<td>Each package shall bear the statement &quot;Nicotine-Delivery Device for Persons 18 or Older.&quot;</td>
</tr>
<tr>
<td>21 CFR 897.25—Sec. 897.25 Statement of intended use and age restriction.</td>
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<tr>
<td>21 CFR 897—Subpart D—Labeling and Advertising.</td>
<td>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.</td>
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<td>21 CFR 897.30—Sec. 897.30 Scope of permissible forms of labeling and advertising.</td>
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<td><strong>FDA</strong> 21 CFR 897.32—Sec. 897.32 Format and content requirements for labeling and advertising.</td>
<td>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 &quot;Nicotine-Delivery Device for Persons 18 or Older&quot; after the appropriate product name as specified in 21 CFR 897.24.</td>
</tr>
<tr>
<td>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.</td>
<td>&quot;No manufacturer and no distributor of imported cigarettes and smokeless tobacco may market, license, distribute, or sell items or services&quot; (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.</td>
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<td>FTC 16 CFR CHAPTER I—FEDERAL TRADE COMMISSION.</td>
<td>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)].</td>
</tr>
<tr>
<td>16 CFR 307.3—Sec. 307.3 Terms defined.</td>
<td>No other statements shall be required by Federal, state, or local statute or regulation. 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.</td>
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<td>16 CFR 307.4—Sec. 307.4 Prohibited acts.</td>
<td>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.</td>
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<tr>
<td>16 CFR 307.5—Sec. 307.5 Language requirements.</td>
<td>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.</td>
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<tr>
<td>16 CFR 307.6—Sec. 307.6 Requirements for disclosure on the label.</td>
<td>This section stipulates warning statement placement and point size, depending on package type so that the warning will be prominent and conspicuous.</td>
</tr>
<tr>
<td>16 CFR 307.7—Sec. 307.7 Requirements for disclosure in print advertising.</td>
<td>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 &quot;circle and arrow format.&quot;</td>
</tr>
<tr>
<td>16 CFR 307.8—Sec. 307.8 Requirements for disclosure in audiovisual and audio advertising.</td>
<td>Audiovisual advertisements must display the warning statement conspicuously in a &quot;circle and arrow format&quot; 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.</td>
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<tr>
<td>16 CFR 307.9—Sec. 307.9 Requirements for disclosure on utilitarian objects.</td>
<td>On objects such as t-shirts, the warning statement must be conspicuous displayed and its permanence must be equivalent to that of the smokeless tobacco product brand name, logo, or selling message.</td>
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<td>16 CFR 307.10—Sec. 307.10 Cooperative advertising.</td>
<td>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.</td>
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<tr>
<td>16 CFR 307.11—Sec. 307.11 Rotation, display, and distribution of warning statements on smokeless tobacco packages.</td>
<td>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.</td>
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<tr>
<td>16 CFR 307.12—Sec. 307.12 Rotation, display, and distribution of warning statements on smokeless tobacco advertising.</td>
<td>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.</td>
</tr>
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<td>45 CFR—TITLE 45—PUBLIC WELFARE. SUBTITLE A—DEPARTMENT OF HEALTH AND HUMAN RESOURCES.</td>
<td>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.</td>
</tr>
<tr>
<td>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.</td>
<td>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.</td>
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<td>45 CFR 96.122—Sec. 96.122 Application content and procedures.</td>
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<td><strong>H H H H S</strong> 45 CFR 96.123—Sec. 96.123 Assurances.</td>
<td>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.</td>
</tr>
<tr>
<td>45 CFR 96.130—Sec. 96.130 State law regarding the sale of tobacco products to individuals under age of 18.</td>
<td>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.</td>
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<td><strong>OTHER</strong> 41 CFR 101-20—MANAGEMENT OF BUILDINGS AND GROUNDS.</td>
<td>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.</td>
</tr>
<tr>
<td>31 CFR—TITLE 31—MONEY AND FINANCE: TREASURY.</td>
<td>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.</td>
</tr>
<tr>
<td>32 CFR—TITLE 32—NATIONAL DEFENSE. CHAPTER I—OFFICE OF THE SECRETARY OF DEFENSE. PART 85—HEALTH PROMOTION.</td>
<td>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.</td>
</tr>
<tr>
<td>32 CFR85.6—Sec. 85.6 Procedures.</td>
<td></td>
</tr>
</tbody>
</table>

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 ≤ 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

<table>
<thead>
<tr>
<th>Design</th>
<th>Population Groups</th>
<th>Exposure</th>
<th>Effects</th>
<th>Potential Confounders</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort</td>
<td>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</td>
<td>Estimation: data from mailed questionnaire; followed prospectively for 26 years</td>
<td>Evaluation: Calculated relative risk (RR) of fatal colon and rectal cancer, after adjustment for potential confounders  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</td>
<td>age, calendar time, year of questionnaire response, physical activity</td>
<td>Heineman et al. (1995)</td>
</tr>
<tr>
<td>Case-control; multicenter hospital-based</td>
<td>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</td>
<td>Estimation: direct interview with structured questionnaire  Categories: never, ≤10 times/wk, &gt;10 times/wk</td>
<td>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</td>
<td>age, education</td>
<td>Mascat et al. (1995)</td>
</tr>
<tr>
<td>Case-control; international multicenter population-based</td>
<td>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</td>
<td>Estimation: direct interview with questionnaire and protocol; same protocol used at study centers in five countries (Australia, Denmark, Germany, Sweden, United States)</td>
<td>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</td>
<td>age, sex, center, and body-mass index</td>
<td>McLaughlin et al. (1995)</td>
</tr>
</tbody>
</table>
Table 3-1. Recent Human Studies of Effects of Exposure to Smokeless Tobacco (Continued)

<table>
<thead>
<tr>
<th>Design</th>
<th>Population Groups</th>
<th>Exposure</th>
<th>Effects</th>
<th>Potential Confounders/Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case-control; population-based</td>
<td>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</td>
<td>Estimation: Direct interview of cases and controls Categories: former user or current user</td>
<td>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</td>
<td>age, race, study site</td>
<td>Hayes et al. (1994)</td>
</tr>
<tr>
<td>Case-control hospital-based</td>
<td>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</td>
<td>Estimation: Direct interview with structured questionnaire</td>
<td>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</td>
<td>age, education, cigarette smoking</td>
<td>Muscat et al. (1997)</td>
</tr>
<tr>
<td>Cohort</td>
<td>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</td>
<td>Estimation: mailed questionnaire; 20-year follow-up Categories: ex-users, occasional, regular</td>
<td>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</td>
<td>cigarette smoking (some smokeless tobacco users also smoked cigarettes); age</td>
<td>Hsing et al. (1990)</td>
</tr>
</tbody>
</table>

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 \( [\text{NNN} = \text{N-nitrosonornicotine}; \text{NNK} = \text{4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone}] \), 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

<table>
<thead>
<tr>
<th>Study Design</th>
<th>No. of rats Exposed</th>
<th>Cell Type</th>
<th>Solutions and Dosing</th>
<th>Route of Administration</th>
<th>Endpoint</th>
<th>Summary of Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>F344 male rats; age 10 wk</td>
<td>Groups of 30</td>
<td>Group of 21 given only water on swab</td>
<td>1) aqueous snuff extract 2) snuff extract enriched with NNN (N-nitrosonornicotine) and NNK [4-(methyleneimino)-1-(3-pyridyl)-1-butanone] 3) solution of NNN and NNK; purity not reported</td>
<td>0.5 mL applied by swab to oral cavity</td>
<td>Daily for 131 wk</td>
<td>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</td>
<td>Hecht et al. (1986)</td>
</tr>
<tr>
<td>F344 male rats; age 13 wk</td>
<td>Groups of 32 rats for snuff and enriched snuff; group of 21 rats for extracted snuff</td>
<td>Group of 10 rats that received no application to test canals</td>
<td>1) snuff 2) snuff after aqueous extraction 3) snuff enriched with an aqueous snuff extract</td>
<td>Preparation inserted into surgically prepared test canals in lower lip 5 times weekly</td>
<td>5 times/wk for 116 wk</td>
<td>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</td>
<td>Hecht et al. (1986)</td>
</tr>
</tbody>
</table>
### Table 4-1. Recent Experimental Carcinogenicity Studies of Smokeless Tobacco (Continued)

<table>
<thead>
<tr>
<th>Species, Strain, Age</th>
<th>Number/sex Exposed</th>
<th>Chemicals</th>
<th>Chemical Form and Purity</th>
<th>Route Exposed</th>
<th>Exposure Duration</th>
<th>Result/Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Sprague-Dawley rats; age 3 mo</td>
<td>Groups of 30</td>
<td>Two groups of 30; 1) received cotton pellet dipped in saline; 2) propylene glycol applied to palate with sable hair brush</td>
<td>Snuff (commercially available U.S. brand) 4-Nitroquinoline N-oxide (4-NQO); purity unspecified</td>
<td>Three treatments applied to surgically-created canal in lower lip</td>
<td>Snuff applied daily 5 d/wk for 104 wk 4-NQO applied 3 times/wk for 4 wk 4-NQO for first 4 weeks; then snuff</td>
<td>No tumors in rats of either control group</td>
<td>Johansson et al. (1989)</td>
</tr>
</tbody>
</table>

- 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
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 hydroxylation 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 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 methylation 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).
8.0 REFERENCES


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
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
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:

<table>
<thead>
<tr>
<th>Review Committee</th>
<th>Recommendation</th>
<th>Vote</th>
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</thead>
<tbody>
<tr>
<td>NIEHS (RG1)</td>
<td>list as known human carcinogen</td>
<td>11 yes/0 no</td>
</tr>
<tr>
<td>NTP EC Working Group (RG2)</td>
<td>list as known human carcinogen</td>
<td>8 yes/0 no</td>
</tr>
<tr>
<td>NTP Board RoC Subcommittee</td>
<td>list as known human carcinogen</td>
<td>6 yes/0 no</td>
</tr>
</tbody>
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Public Comments Received
One comment was received which was opposed to listing Smokeless Tobacco as a known to be human carcinogen.