Chapter 3

A Global View of Smokeless Tobacco Products

Chapter Contents

Tables and Figures

Global Diversity of Smokeless Tobacco Products

Product Overview

Unlike smoked tobacco, which is burnt or heated and then inhaled in products such as cigarettes (both manufactured and roll-your-own) and cigars, or via hookahs, smokeless tobacco (ST) is predominantly used orally (chewed, sucked, dipped, held in the mouth, etc.) or nasally, which results in absorption of nicotine and other chemicals across mucus membranes.[1] Smokeless tobacco products are used worldwide[2–4] in forms that vary greatly in appearance and toxicant emissions and in their composition of tobacco and non-tobacco constituents (Figure 3-1).[2,5,6]

Worldwide, ST products range in complexity from simple cured tobacco to elaborate products with numerous chemical ingredients and, in some cases, non-tobacco plant material that may affect the attractiveness, addictiveness, and toxicity of the products[2,5,6] (see chapters 9–14). For certain products, preparation, ingredient selection (including non-tobacco plant materials), and mode of use (oral, nasal, etc.) can vary based on geographic locality, ingredient availability, cultural/societal norms, and personal preferences[1,2,5,6] (chapters 9–14).

Production and Preparation

In terms of production and preparation, ST can be broadly divided into premade and custom-made products (Table 3-1). Premade ST products, which are made for sale and generally consumed as purchased (i.e., "ready-to-use"), can be subdivided into: (1) commercial products (i.e., moist snuff, snus, khaini) that are made in traditional manufacturing settings such as factories or production facilities; and (2) cottage products (toombak, nasway, mainpuri, mawa) that are made in non-traditional production environments (market stalls, shops, houses, etc.) and often sold in non-commercial packaging (paper or plastic bags; wrapped in paper).[2,5,6]

Premade manufactured ST products are available in a wide variety of physical forms, including, but not limited to, twisted tobacco leaves, loose tobacco, ground tobacco, dry tobacco (dry snuff), tars (chimó), pastes (kiwam), dentifrices (creamy snuff, toothpowder), tobacco-containing chewing gums, and mixtures of tobacco and other materials (zarda, gutka).[2,5–7] Manufactured ST products, such as moist snuff and snus, are available as loose tobacco or tobacco sealed in porous teabag-like sachets (Figure 3-1), which are easily inserted and removed from the mouth. Release of nicotine and presumably other compounds is greater from loose tobacco than from sachets.[8]

Figure 3-1. Examples of global smokeless tobacco products

Note: Products by country or region are:
South-East Asia: kiwam, betel quid (paan), zarda, gutka
United States: moist snuff, dry snuff, moist snuff (caffeinated), plug, twist tobaccos, dissolvables (Orbs, Strips, Sticks, tobacco-coated toothpicks)
Sweden: snus (pouch)
Venezuela: chimó
Uzbekistan: nasway
Sudan: toombak
India: red toothpowder, mawa
Saudi Arabia: shammah
Brazil: rapé.

Sources: All images except for betel quid (paan) courtesy of Clifford Watson, Centers for Disease Control and Prevention. Image of betel quid (paan) courtesy of World Health Organization South-East Asia Regional Office and Dhirendra N. Sinha.

Table 3-1. Characteristics and product examples of premade and custom-made smokeless tobacco products

Premade manufactured	Premade cottage industry	Custom-made vendor/individual
• Made in advance for sale • Made in a manufacturing environment • Sealed in labeled commercial packaging	• Made in advance for sale • Usually handmade in non-traditional environments • Often sold in non-commercial packaging	• Made by a vendor or individual according to user preferences, generally for immediate consumption • Involves mixing two or more components (including premade products) together by hand to form a final product
Product examples: • Chewing tobacco (plug/twist/ loose leaf) • Creamy snuff • Dissolvables • Dry snuff • Gudahku/Gudahka • Gutka • Khaini • Moist snuff • Kiwam • Rapé • Red toothpowder	Product examples: • Dohra • Gutka • Mainpuri • Nass/Naswar • Nasway • Betel quid with tobacco (paan) • Rapé • Shammah • Toombak • Tuibur	Product examples: • Gudahku/Gudahka • Iqmik • Nass/Naswar • Nasway • Betel quid with tobacco (paan) • Rapé • Shammah • Tapkeer • Tobacco leaf • Tombol • Toombak Some premade ingredients are used to make custom-made products: twist, zarda, toombak, gudahku/gudahka, and kiwam.

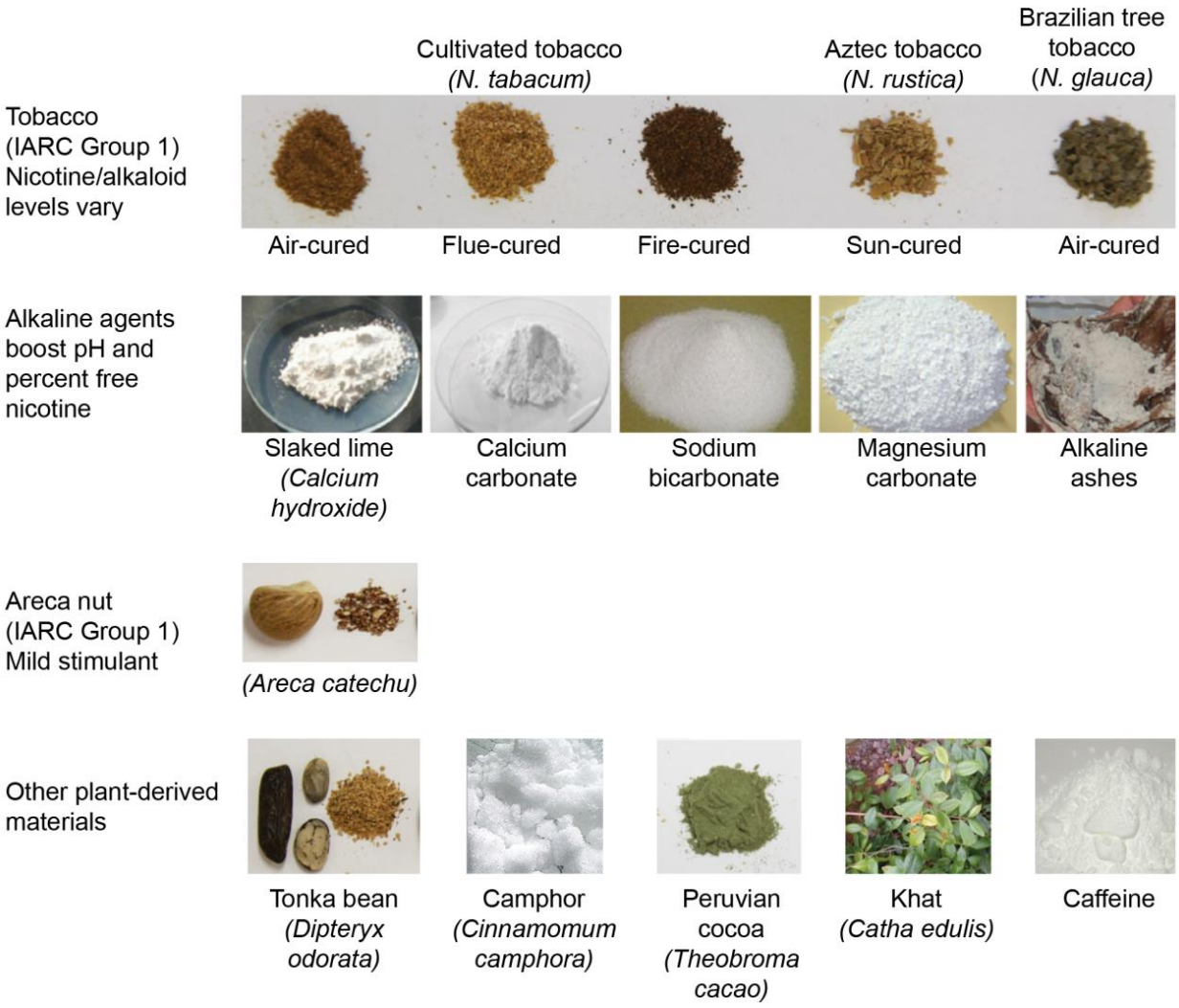
Increasingly, new varieties of manufactured smokeless products appear in a discrete, spit-less form that can be used where smoking is prohibited or socially inappropriate.[9] Since 2001, several tobacco companies, including those that have traditionally marketed cigarettes, have been introducing dissolvable ST products, which are made from finely milled tobacco pressed into tablets, rods and sticks, or flat strips that fully dissolve in the mouth[10–12] (Figure 3-2). Novel products introduced after about 2010 include tobacco-coated toothpicks, which are sucked on to release nicotine,[13] and an "energy-enhanced" ST product called Revved Up, made by Southern Smokeless, which is essentially moist snuff augmented with energy drink constituents.[14] A nicotine disk product called Verve, introduced by Altria in Virginia in 2012, is a chewable disc made of cellulose fibers and a polymer and impregnated with flavor and nicotine. The disk does not dissolve, but is chewed for about 15 minutes and then discarded.[15]

Premade cottage products can be in the form of pressed cakes (mawa), pellets (nasway), or pulverized tobacco (toombak, shammah), among others. Some premade products are used as the tobacco ingredient in custom-made products; for example, manufactured products (zarda and kiwam) or cottage products (mainpuri and toombak) can be used as the tobacco ingredient in betel quid and tombol. The "tobacco ingredient" used to make a custom-made product (tombol, betel quid) may itself be a mixture of tobacco with other ingredients such as areca nut, alkaline agents, spices, and silver flakes.[2,5]

Figure 3-2. Dissolvable products and their packaging

Source: Images courtesy of Clifford Watson, Centers for Disease Control and Prevention.

Figure 3-3. Ingredients added to some smokeless products that may influence their addictiveness, carcinogenicity, or toxicity



Abbreviation: IARC = International Agency for Research in Cancer.
Notes: Samples taken from the Federal University of Paraiba (Brazil). Products may also contain chemical additives (sweeteners, moisteners, flavor chemicals, binders, whiteners, preservatives), plant extracts, essential oils, spices, and other plant materials.
Source: Images for tobacco, areca nut, tonka bean, and Peruvian cocoa courtesy of Clifford Watson, Centers for Disease Control and Prevention.

Custom-made products, handmade by the user, a relative, or a vendor according to user preferences, are characteristic of countries in South Asia, Africa, the Middle East, North America (Alaska), and South America (Brazil) (see chapters 9–14). Custom-made products such as tombol and betel quid (also known as paan) are made by combining cured tobacco or a premade tobacco product (e.g., zarda) with one or more ingredients, such as ashes, alkaline agents, areca nut, spices, catechu, or other plant materials[16] (Figure 3-3).

Product Packaging

Approaches to the packaging of ST products are nearly as diverse as their formulations. Many manufactured ST products are packaged in tins, cylinders, or containers made of cardboard, plastic, or metal (e.g., snus, moist snuff, dry snuff); sealable pouches (zarda, chewing tobacco); tear packs (snuff, gutka, khaini); and toothpaste-like tubes (creamy snuff).[2] Some novel dissolvable tobacco products are packaged in paper packs (tobacco rods and sticks) or foil press-out packs (tablets). Manufactured packaging serves not only to protect product integrity but also to display recognizable logos or images that can promote brand image and use. Alternatively, the hand-prepared cottage products (mawa, mainpuri, toombak) are often portioned into non-conventional packaging (unlabeled paper or plastic bags or cellophane paper wrapping). Custom-made products may not be stored in any packaging as they are frequently prepared at the time of use by the user, a family member (as in the case of iqmik),[16] or a vendor (e.g., betel quid seller). Thus, cottage and custom-made products are likely to show substantial variation in product size, packaging, and composition as compared to manufactured products, which tend to be more consistent because of standardized production methods and quality control measures.[2,17]

Smokeless Tobacco Product Ingredients

Tobacco

Tobacco Types

Worldwide, approximately 70 species of tobacco (*Nicotiana*) occur in nature, although few are regularly used for smoked or smokeless tobacco products.[2,18] The identity of different tobacco species in products can be determined by a chemical analysis of the levels of nicotine and other tobacco alkaloids[19] and confirmed using infrared analysis.[20] Most commercial tobacco products worldwide contain the species *Nicotiana tabacum* (cultivated tobacco), but *N. rustica* is also frequently grown and used in regions of South America, Africa, and Asia.[2,18] In India, smoking tobacco tends to be made with *N. tabacum*, but most ST contains *N. rustica*, which has higher concentrations of nicotine and other alkaloids than *N. tabacum*.[17,21,22] Some products, such as khaini and kiwam from South Asia, may contain both *N. rustica* and *N. tabacum*.[2] *N. rustica* is also contained in some forms of naswar, Bangladeshi tobacco leaf, Indian chewing tobacco, maras, zarda, and toombak.[2,17,20,23] Smokeless tobacco products such as toombak may contain *N. glauca* (tree tobacco),[2,24] which has high levels of the alkaloid anabasine; ingestion of this form of tobacco has been linked to accidental poisoning and fatality in a few cases.[24,25] (Figure 3-3 includes images of different *Nicotiana* species.)

Changes in Chemical Composition of Tobacco During Growth

As tobacco grows, it absorbs metals, metalloids,[26] and nitrate from the soil[27,28] and synthesizes alkaloids, including nicotine and minor alkaloids (e.g., nornicotine, anabasine, and anatabine) in various concentrations, depending on species and variety.[19] Alkaloids are key chemical precursors in the formation of tobacco-specific nitrosamines (TSNAs),[29–31] some of which are potent carcinogens.[2,32]

Tobacco nitrate content and the presence of certain microorganisms on tobacco leaves contribute to the formation of TSNAs from alkaloids.[33] During cultivation, microorganisms (yeast, mold, fungi, and bacteria) and agricultural chemicals can be deposited on tobacco plants. On growing tobacco, bacteria are present at approximately 10^5 to 10^6 organisms per gram of leaf material. At harvest, tobacco is not generally washed, thus leaves with deposited microorganisms and agricultural chemicals will be processed, and the contaminants will be present in the final product. During the subsequent curing step, the tobacco leaves dry, and bacteria, which proliferate to levels 10 to 20 times higher than on the growing leaf,[34] begin converting the nitrate (NO_3^-) present in the plant tissue to nitrite (NO_2^-), a process called nitrate reduction. Once nitrite is produced, a chemical process of nitrosation occurs in which nitrite reacts with tobacco alkaloids to generate TSNAs.[35] (Figure 3-7 illustrates this process.) Amine compounds other than tobacco alkaloids can also react with nitrite to form nonvolatile *N*-nitrosamines, volatile nitrosamines, and *N*-nitrosamino acids.[5,36] The International Agency for Research in Cancer (IARC) has classified various nitroso compounds as IARC Group 1 (carcinogenic to humans), 2A (probably carcinogenic to humans), or 2B (possible carcinogenic) agents.[37] The IARC has also classified nitrate and nitrite as Group 2A agents[38] because of their potential to form nitroso compounds in the human body after ingestion. There are indications that additional amounts of nitrosamines can be formed in the mouth during ST use.[39]

Curing

Prior to use in products, tobacco is dried using sun, air, flue, or fire curing (Figure 3-3). Any given ST product can be produced using various tobacco-curing methods, depending on the manufacturer. The simplest method of tobacco processing is sun curing, the process of drying tobacco leaves in the sun, which is often used in making toombak, gutka, maras, khaini, and nass/naswar. Some tobaccos used in betel quid are also sun-cured.[2] Air curing, which involves placing tobacco stalks on wooden staves that are hung in a well-ventilated barn, is usually used in loose leaf and twist chewing tobaccos and moist snuff.[2,40] Iqmik can contain air- or fire-cured tobacco.[41] Flue curing involves hanging tobacco in an enclosed structure connected to an external heat source without exposing the tobacco directly to smoke[33,40]; this method is often used in making chewing tobacco. During fire curing, tobacco is hung in a large enclosed barn and exposed to smoke from hardwood fires that are continuously burning or smoldering, in a process directly analogous to producing smoked meat.[42] Fire-cured tobacco is used in the production of plug chewing tobacco, moist and dry snuff, and iqmik.[2,40,41] Fire curing not only causes chemical changes in the tobacco leaf, it also contaminates the tobacco with smoke-related chemicals. As a result, the levels of polycyclic aromatic hydrocarbons (PAHs), phenols, and volatile aldehydes tend to be higher in fire-cured tobacco than air-cured tobacco.[21,22,41,43]

Fermentation and Aging

Fermentation and aging of tobacco are common in the production of tobacco used in cigars[44] and smokeless tobacco (e.g., moist and dry snuff, toombak, taaba).[2,33,45] During fermentation or aging, the tobacco takes on a more agreeable flavor.[45] For manufactured products, fermentation can occur in a partially insulated tank,[33] which, because of increased microbial activity, can reach high temperatures (up to 65°C).[44] Fermentation of toombak, a cottage industry product, occurs in a closed container at 30 to 45ºC for a few weeks, then the tobacco is aged for a year.[2]

Tobacco fermentation involves chemical and biochemical changes (bacteria-mediated reactions).[2,33,44] During fermentation, a portion of nitrate in fire-cured tobacco is converted to nitrite, which then reacts with alkaloids to produce TSNAs.[33,44]

Chemical markers indicative of bacterial and fungal growth have been identified in tobacco of various types and at various stages of production.[46,47] In tobacco or tobacco products, a number of bacteria including *Bacillus*, *Enterobacter*, *Staphylococcus*, *Corynebacterium*, *Clostridium*, *Serratia*, and *Escherichia* species have been identified that are capable of converting nitrate to nitrite (nitrate reduction).[33,44,48–52] Additionally, several genera of fungi, such as *Cladosporium*, *Alternaria*, *Candida*, *Fusarium*, *Aspergillus*, and *Acremonium* are capable of nitrate reduction.[44,47,52,53] Throughout production, the combined capacity of product microorganisms to generate nitrite is a key determinant of the levels of TSNAs and other nitrosamines in the final product.[37,54] During one fermentation study, nitrite levels generated by bacteria resulted in an almost threefold increase in TSNA levels.[44]

Pasteurization, or heat-treating of tobacco, is a very effective means of eliminating microorganisms during ST production, and thus preventing the reduction of nitrate to nitrite.[55] Indeed, Swedish snus, a pasteurized product, generally has lower nitrite and TSNA levels than nonpasteurized products, such as moist snuff and khaini.[56,57] It has been shown that a further increase in nitrite and TSNA levels can be prevented by cleaning fermentation equipment before use and "seeding" the fermentation process with non-nitrate-reducing bacteria.[33] Together, these observations provide additional support for the idea that the levels of some carcinogenic and toxic agents in tobacco products can be substantially reduced by changing tobacco processing methods.

Following fermentation, tobacco may still contain substantial amounts of nitrate, nitrite, and bacteria (including endospore-forming bacteria such as *Bacillus* spp.) that are active across a wide temperature and pH range.[33,44,58] Moreover, moist snuff products, including South African smokeless tobacco, contain nitrate, nitrite, and viable nitrite-producing bacteria (e.g., *Bacillus* spp.).[49,56,58] Bacteria capable of initiating various infections (*Pseudomonas* spp. and *Staphylococcus* spp.) and periodontal abscesses (*Atopobium* spp. and *Klebsiella oxytoca*) have also been isolated from tobacco used to make cigarettes.[48] Research on black South African nasal snuff users has found an association between the use of nasal snuff and chronic bronchitis, which can be caused by *Staphylococcus* spp.[49] Although conditions in ST products are favorable for the presence of bacteria, it is not known which strains of bacteria are most common in ST products.

Products from India, such as zarda, mishri, gutka, creamy snuff, and toothpowder, have elevated nitrate levels but lower levels of nitrite. In contrast, Indian khaini contains higher levels of nitrite and TSNAs.[57] Accumulated nitrite may contribute not only to the formation of TSNAs but also to other nitroso compounds, such as *N*–nitrosamino acids and volatile *N*–nitrosamines, in some ST products.[5] The high levels of nicotine and other alkaloids in *N. rustica*[19,21] may contribute to extreme levels of TSNAs such as are found in the Sudanese product toombak.[20,23]

Other Tobacco Processing Methods

Tobacco is processed differently during the manufacture of some forms of ST products. For example, Swedish snus, a snuff-like product, is made from pasteurized and air-cured tobacco that is not fermented. Pasteurization reduces or eliminates bacteria, including those that convert nitrate to nitrite, a key precursor for TSNAs.[55] Similar processing is used in most novel "spitless" U.S. products that are also called "snus" but are slightly different from the traditional Swedish snus. Because bacterial activity is very low in snus products, it is not surprising that snus contains much lower levels of nitrite and TSNAs than moist snuff made with fermented fire-cured tobacco that is not pasteurized,[56] and levels of nitrite and TSNAs do not increase during long-term storage of snus as they do with moist snuff.[33,59,60] Also, because snus does not contain fire-cured tobacco, the levels of total PAHs and volatile aldehydes are lower than those found in moist snuff.[56,61]

The Swedish snus industry voluntarily complies with the GothiaTek industry standard, which sets maximum levels for nitrite, TSNAs, NDMA (a volatile nitrosamine), benzo[*a*]pyrene (a representative carcinogenic PAH compound), five metals (cadmium, lead, arsenic, nickel, and chromium), and various agrichemicals.[55] The StarCured process, which may lower the levels of some carcinogens, was used to produce the dissolvables Stonewall and Ariva, which were discontinued by Star Scientific at the beginning of 2013. Although snus contains nicotine and toxicants at some level, maintenance of toxicants below certain thresholds demonstrates that the tobacco industry can use manufacturing controls to reduce the levels of certain toxicants in ST products.

Additives

After curing, aging, and fermentation, further steps for manufacturing smokeless products include cutting the tobacco to the proper width, adding other substances, and adjusting moisture and pH levels.[62] Manufactured ST products, particularly Western-style forms (e.g., moist snuff, snus) are known to contain flavoring agents, spices, fruit juices, sweeteners, salt, humectants, and alkaline agents.[5,63–67] Flavorings used include cocoa, licorice, rum, spice powders, extracts, oleoresins, individual flavor compounds (menthol, vanillin, etc.), and more than 60 different essential oils (such as wintergreen, cinnamon, ginger).[5,63] The most common flavor chemicals detected in 85 brands of ST, primarily moist snuff, were methyl salicylate, ethyl salicylate, benzaldehyde, citronellol, menthol, nerol, menthone, and caryopyllene.[68] Among many mint and wintergreen moist snuff brands, Chen and colleagues found high levels of methyl salicylate (18.5–29.7 milligrams per gram [mg/g]), ethyl salicylate (0.17–5.78 mg/g), and menthol (undetectable–5.25 mg/g).[69] Sweeteners added to ST include honey, molasses, saccharin, brown sugar, sugar, and xylitol. Humectants, which are added to maintain product moisture, include agents such as glycerol, glycerin, and propylene glycol.[5,63,64] Dissolvable tobacco products (Figure 3-2)

include ingredients such as flavorings, sweeteners, humectants, and alkaline agents, as well as fillers, coatings, binders, colorings, and preservatives.[65–67]

Cottage ST products made in the Middle East, Africa, and South-East Asia may contain ingredients such as edible oils, metallic silver, potassium nitrate, and soil (chapters 11–13).

Alkaline modifiers used in manufactured ST products are predominantly chemicals including sodium bicarbonate, ammonium bicarbonate, various metallic carbonates (calcium, sodium, and ammonium), and slaked lime (calcium hydroxide) (Figure 3-3).[5,63] Chemical alkaline agents (mostly slaked lime or sodium bicarbonate) are also used in the preparation of cottage products (e.g., toombak, nass, shammah) or custom-made ST (iqmik). In some rural or tribal areas, custom-made or cottage industry ST products are prepared with ashes from the burning of certain woods, plants, or fungi (for example, wood: willow, mamón, paricá; plants: *Aloe vera, Amaranthus,* grapevine; fungi: punk fungi *[Phellinus igniarius]*), which significantly increases product pH.[2,70,71] Unlike rapé products that are mildly acidic (lower pH), the type of rapé used by the Kaxinawás Indians, who live in eastern Peru and in the States of Amazonas and Acre in Brazil, includes ashes from the paricá tree (*Schizolobium amazonicum*).[72] Products that contain alkaline ashes, such as iqmik[41] and nass,[73] have extremely high pH levels (≥pH 11). The effects of pH on nicotine levels are discussed later in this chapter.

Non-Tobacco Plant Material

In several regions of the world, especially South Asia, the Middle East, and South America, tobacco is commonly combined with substantial amounts of non-tobacco plant material. In those regions, several premade ST products (gutka, mawa, mainpuri, and some zarda products) and custom-made products (betel quid, dohra, tombol) contain areca nut, the seeds of the Areca palm (*Areca catechu*) (Figure 3-3)[2,6,17,20] (see chapters 11–13). Products in South Asia often contain appreciable amounts of spices (cardamom, clove, camphor, mint, saffron, pepper) or other plant materials such as betel leaf (*Piper betle*) and catechu (*Acacia catechu*).[2,6,17] Alternatively, packets containing non-tobacco condiments, such as supari or pan masala (a mixture of spices, flavorings, and other ingredients) can be purchased separately and combined with tobacco prior to use. In South Asian and Mediterranean countries, custom-made ST products, such as betel quid, dohra, or tombol, are often handmade from tobacco or premade ST (kiwam, zarda, toombak) combined with other ingredients, such as alkaline agents, areca nut, spices, condiments, or other plant material (such as coconut), and rolled in a betel leaf.[2,5,6,17] Some forms of tombol, such as those used in Yemen, contain khat (*Catha edulis*) (Ghazi Zaatari, personal communication, 2012), a plant that has psychoactive properties.[74] In South America, rapé and other indigenous forms of nasal ST used in Brazil and Peru contain tobacco mixed with ingredients such as tonka bean (*Dipteryx odorata*), cinnamon powder, clove buds, camphor, sunflower, Peruvian cocoa, and possibly cassava (Figure 3-3)[75,76] (André Oliveira da Silva, personal communication, 2012).

Product Categorization Based on Constituents

Although ST products range from simple to highly complex mixtures of tobacco and other ingredients, all known products can be grouped by key product constituents (see Figure 3-4) into four broad categories:

- Category 1 products contain tobacco with little or no alkaline modifiers.
- Category 2 products contain tobacco and substantial amounts of alkaline agents.
- Category 3 products contain tobacco, one or more alkaline agents, and areca nut.
- Category 4 products contain tobacco mixed with other chemical or plant ingredients that exhibit additional bioactivity (such as stimulants).

(A similar scheme of categorizing ST products was first presented in *Smokeless Tobacco and Some Tobacco-specific* N-*Nitrosamines,* International Agency for Research on Cancer monograph 89.[2,p.34])

To extend this categorization, ST products can be grouped in categories based on ingredients listed on packaging, but further analysis using gas chromatography–mass spectrometry, infrared spectroscopy, and pH measurements can be used for confirmation or when product ingredient information is unavailable.[20]

Category 1 products can have a wide range of total nicotine, depending on the tobacco used, but because they have a pH of 7 or less, they generally have lower free nicotine. Category 2 products can have a wide range of total nicotine, depending on the tobacco used, but have an alkalinity greater than pH 7 and thus higher free nicotine values. Category 3 products generally contain an appreciable amount of areca nut, which decreases the tobacco content, thus the amounts of total nicotine are generally lower. These products also contain areca-related compounds, such as arecoline, and other compounds that can contribute to the formation of areca-specific nitrosamines; the pH of this category varies based on this composition.[6,20] Category 4 products contain nicotine as well as other compounds like stimulants, flavoring agents, or spices. Some of these additives are toxicants, or carcinogens—for example, coumarin (a liver toxicant), which is found in tonka bean, cinnamon, and other substances. Products in category 4 have also been found to contain camphor, a cardiac toxicant. Figure 3-4 shows key ingredients and chemical markers for the four categories as well as products in each category. This categorization can help illuminate the relationship between ST product ingredients and the resulting levels of addiction, toxicity, and carcinogenicity associated with their use.

Figure 3-4. Smokeless tobacco product categorization by key constituents

Notes: Tombol (Category 4) shown on betel leaf prior to addition of noura (alkaline agent), fofal (areca nut), and tobacco. This figure groups products with similar constituents for further investigation and research and highlights constituents of concern. This categorization, which is based on product knowledge at the time of publishing, does not reflect the safety or the addictive properties of a product or product type. The composition of products of a given type can vary such that seemingly similar products may fit into different categories. Detailed product information is given in Appendix A.
Sources: All images except tombol with khat courtesy of Clifford Watson, Centers for Disease Control and Prevention. Image of tombol with khat courtesy of Dr. Mazen Abood Bin Thabit, University of Aden.

Toxic and Carcinogenic Agents in Smokeless Tobacco Products

In general, tobacco, and thus ST products, contains roughly 4,000 chemical constituents,[77] including nicotine and other toxicants and carcinogens, which are believed to play a crucial role in causing the negative health effects associated with ST use.[78–80] The U.S. Food and Drug Administration has also established a list of 93 harmful and potentially harmful constituents for regulatory purposes in the United States.[81] Based on epidemiologic evidence and animal carcinogenicity data, the IARC has classified ST as a Group I carcinogen: carcinogenic to humans.[2] Moreover, the IARC has classified two TSNAs present in ST, namely *N'*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), as Group 1 carcinogens. A list of carcinogens present in ST products based on the 2012 IARC list[37] is shown in Table 3-2.

Among the carcinogens in ST, TSNAs are considered the most potent because of their concentration and carcinogenicity.[32,82,83] The two main carcinogenic compounds in this group, NNK and NNN, are believed to be involved in the induction of oral cancer in ST users.[32] Other carcinogens in ST include *N*–nitrosamino acids, volatile *N*–nitrosamines, PAHs, volatile aldehydes, inorganic compounds, metals, and metalloids.[5,36,84,85] In addition, areca nut, a constituent of products such as mawa, betel quid, tamol (fermented areca nut), and mainpuri, is also classified as an IARC Group 1 carcinogen.[6] Some ST products contain plant materials (tonka bean, cinnamon) that have high levels of coumarin, which is moderately toxic to the liver and kidneys.[86,87]

The following sections of this chapter discuss some of the most important groups of ST constituents in greater detail: their origin, factors affecting their formation, and their reported levels in ST products used globally.

Nicotine and Free Nicotine

Nicotine in tobacco products leads to addiction and persistent use of tobacco products, and thus continuous exposure to numerous toxic and carcinogenic agents, which results in devastating health consequences and premature deaths worldwide.[88] Additionally, nicotine is a major precursor of carcinogenic NNK and NNN.[2] Nicotine has also been associated with fetal toxicity and an increase in cardiovascular risk factors.[88]

In an ST product, the entire amount of nicotine present is referred to as total nicotine, which includes both free (also called un-ionized or un-protonated) and bound (also called ionized and mono-proponated or di-proponated) forms of nicotine (Table 3-3). Free nicotine is of importance because it is the uncharged form that crosses cell membranes most readily. The amount of free nicotine in a product can be calculated using the Henderson–Hasselbalch equation.[89] The fraction of nicotine present as free nicotine depends on the pH of the ST product: A higher pH results in a greater proportion of nicotine being present as free nicotine, which is the most biologically available form.[90–93]

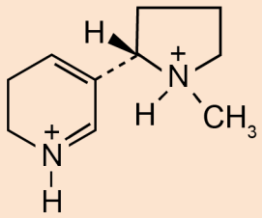
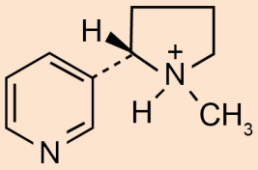
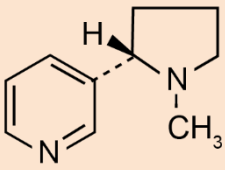
Table 3-2. Substances identified in smokeless tobacco products and their categorization as carcinogens

Compound/substance	IARC group*	Source†
Tobacco-specific nitrosamines		
N'-nitrosonornicotine + 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone {NNN+NNK}	1	IARC 2007 (2)
Volatile *N*-nitrosamines		
N-Nitrosodimethylamine (NDMA)	2A	IARC 2007 (2)
N-Nitrosopyrrolidine (NPYR)	2B	IARC 2007 (2)
N-Nitrosopiperidine (NPIP)	2B	IARC 2007 (2)
N-Nitrosomorpholine (NMOR)	2B	IARC 2007 (2)
N-Nitrosodiethanolamine (NDELA)	2B	IARC 2007 (2)
Nitrosamino acids		
N-Nitrososarcosine (NSAR)	2B	IARC 2007 (2)
Inorganic compounds		
Nitrate (under conditions resulting in endogenous nitrosation)	2A	Stepanov et al. 2008 (56)
Nitrite (under conditions resulting in endogenous nitrosation)	2A	Stepanov et al. 2008 (56)
Volatile aldehydes		
Formaldehyde	1	Stepanov et al. 2008 (56)
Acetaldehyde	2B	Stepanov et al. 2008 (56)
Fermentation-related compound		
Ethyl carbamate (urethane)	2A	Faizi et al. 2010 (157)
Mycotoxins		
Aflatoxins (mixtures of)	1	Varma et al. 1991 (152)
Aflatoxin M1	2B	Varma et al. 1991 (152)
Ochratoxin A	2B	Varma et al. 1991 (152)
Polycyclic aromatic hydrocarbons		
Benzo[*a*]pyrene (BaP)	1	Hearn et al. 2013 (41), Stepanov et al. 2010 (61)
Dibenz[*a,h*]anthracene (DBahA)	2A	Hearn et al. 2013 (41), Stepanov et al. 2010 (61)
Benz[*a*]anthracene (BaA)	2B	Hearn et al. 2013 (41), Stepanov et al. 2010 (61)
Benzo[*b*]fluoranthene (BbF)	2B	Hearn et al. 2013 (41), Stepanov et al. 2010 (61)
Benzo[*j*]fluoranthene (BjF)	2B	Hearn et al. 2013 (41), Stepanov et al. 2010 (61)
Benzo[*k*]fluoranthene (BkF)	2B	Hearn et al. 2013 (41), Stepanov et al. 2010 (61)
Dibenzo[*a,i*]pyrene (DBaiP)	2B	Hearn et al. 2013 (41)
Indeno[*1,2,3-cd*]pyrene (IcdP)	2B	Hearn et al. 2013 (41), Stepanov et al. 2010 (61)
5-Methylchrysene (5MC)	2B	Hearn et al. 2013 (41)
Naphthalene (NAP)	2B	Hearn et al. 2013 (41), Stepanov et al. 2010 (61)
Plant material		
Areca nut	1	IARC 2004 (6)
Betel quid (with or without tobacco)	1	IARC 2004 (6)

Compound/substance	IARC group*	Source†
Metals/metalloids		
Arsenic	1	Pappas et al. 2011 (26)
Beryllium	1	Pappas et al. 2011 (26)
Cadmium	1	Pappas et al. 2011 (26)
Cobalt	2B	Pappas et al. 2011 (26)
Chromium VI	1	Pappas et al. 2011 (26)
Lead/Inorganic lead compounds	2B/2A	Pappas et al. 2011 (26)
Nickel compounds	1	Pappas et al. 2011 (26)
Polonium-210	1	Syed et al. 2009 (158)

*IARC = International Agency for Research in Cancer, 2012 (37).
†Numbers in parentheses correspond to full citations in the References at the end of this chapter.
Notes: All carcinogen designations are current as of October 2012 (IARC [37]). Includes agents that are added or that can be absorbed from the soil, result from microbial contamination, or form chemically. Some smokeless products contain nutmeg, which contains methyleugenol (IARC Group 2B) and safrole (IARC Group 2B). The concentration of these compounds would depend on the chemical composition of the nutmeg and the amount of nutmeg used in the product. The compound 3-(*N*-nitrosomethylamino) propionitrile (MNPN), which is an IARC 2B carcinogen, can be formed during the chewing of areca nut or products/preparations containing areca nut.

Table 3-3. Forms of nicotine: Chemical structures, ionic charge, alternative names, and health implications

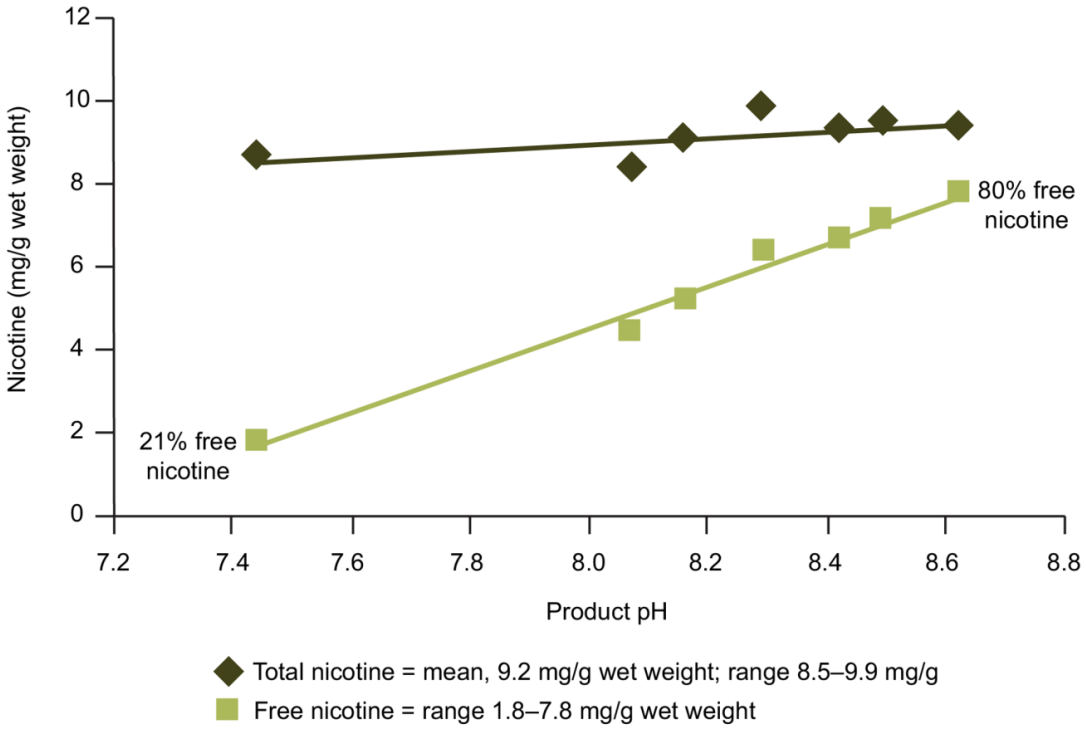
Forms of nicotine (Ionic charge)	Di-protonated (++)	Mono-protonated (+)	Unprotonated (Neutral)
Chemical structure			
	Ionized	Ionized	Un-ionized
	Total nicotine is the combination of the ionic forms existing at a given pH.		
Alternative names	Not applicable*	Salt form Tobacco nicotine Protonated nicotine	Free nicotine Free-base nicotine Non-protonated nicotine
Health implications	Doubly charged nicotine is not prevalent at pH levels typically found in smokeless tobacco products (generally greater than pH 5.5).	For singly charged nicotine, absorption across cellular membranes is not efficient. This is the predominant form in unprocessed tobacco.	Uncharged nicotine rapidly crosses cellular membranes and diffuses into the bloodstream. Smokeless tobacco products with higher levels of this form of nicotine may be more addictive.

*A few smokeless tobacco products (plug, twist, zarda, and some forms of rapé) have a pH below pH 5.5 and would contain some di-protonated nicotine.
Note: The fraction of nicotine present in a particular ionic form is pH-dependent. Di-protonated and mono-protonated nicotine is present at acidic conditions below pH 5.5. In the majority of smokeless tobacco products, a combination of mono-protonated and unprotonated nicotine is present. The fraction of nicotine in the unprotonated (free) form increases as pH increases above pH 5.5.

The pH of unprocessed tobacco is generally slightly acidic (pH approximately 5–6.5)[43]; thus, generally less than 5% of the nicotine is present as free nicotine. During ST production, various alkaline agents are added that boost pH and increase the amount of free nicotine that can be delivered to the user. Some products, such as iqmik or nass,[41,73] are highly alkaline (pH 11–12; Figure 3-6); hence, greater than 99% of nicotine is present as free nicotine in these products.

The common practice of adding alkaline agents to ST products increases pH and thus free nicotine levels, which increases nicotine emissions and exposure, subsequently resulting in adverse health effects. Products with similar total nicotine concentrations can contain a wide range of free nicotine concentrations, depending on pH[2,94] (Figure 3-5).

Figure 3-5. The effect of pH on free nicotine amounts (in products produced by the same manufacturer, with equivalent amounts of total nicotine and product moisture)



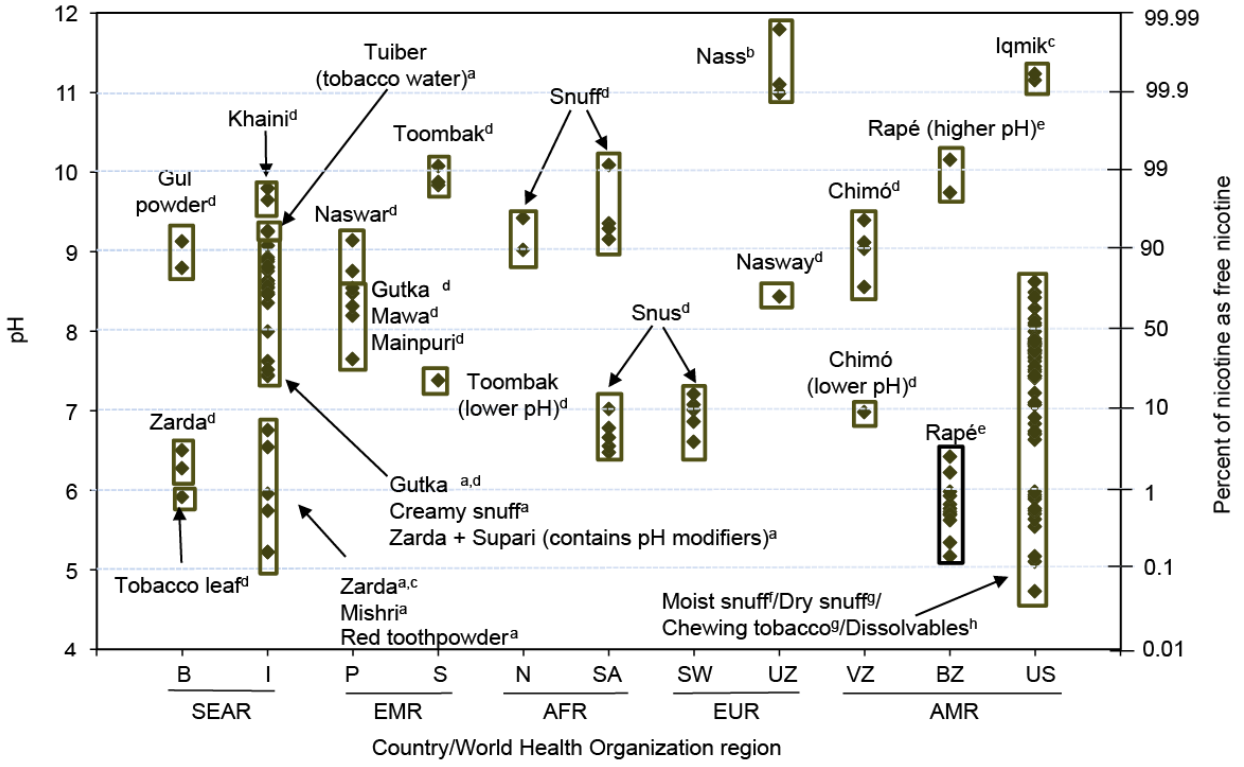
Abbreviation: mg/g = milligram per gram.
Notes: An increase of 1.2 pH units increases free nicotine by 433%. Each square or diamond symbol represents a different product. Percent moisture = 51.9–53.9%; trends remain the same when expressed on a dry weight basis.
Source: Richter et al. 2008 (94).

Clinical studies indicate that absorption of nicotine through cell membranes is more rapid for products with higher pH than for products with lower pH.[90,92,95] Products with higher free nicotine concentrations generate faster spikes in blood nicotine concentrations and could cause such products to be more addictive.[96,97] Addition of alkaline agents and the resulting pH increase in some products may play a decisive role in the targeted delivery of nicotine (Figure 3-5). The availability of products spanning a

wide pH range may make it easier for ST users to move on to products with increasingly higher nicotine levels (i.e., the graduation strategy).[96,98]

The wide ranges of pH, total nicotine, and free nicotine levels in various products have been clearly demonstrated in numerous studies.[2,12,20,41,73,91,93,94,99–102] Combined, these studies include more than 20 product types (such as zarda, chimó, gutka) from 12 countries. Products with the lowest pH include chewing tobacco[2,73,101] and some forms of dry snuff, zarda, and snus[20,101] (Figure 3-6). Toombak, khaini, chimó, naswar, tuiber (tobacco water), and some varieties of African snuff and gutka have pH values generally between pH 8 to pH 10[2,20,41,73,99,100,102]; products such as iqmik and nass have the highest known values (pH 11.0 to pH 11.8).[41,73]

Figure 3-6. pH values and % free nicotine in selected smokeless tobacco products from 11 countries in 5 World Health Organization regions



Country abbreviations: B = Bangladesh; I = India; P = Pakistan; S = Sudan; N = Nigeria; SA = South Africa; SW = Sweden; UZ = Uzbekistan; VZ = Venezuela; BZ = Brazil; US = United States.
World Health Organization regions: SEAR = South-East Asia Region; EMR = Eastern Mediterranean Region; AFR = African Region; EUR = European Region; AMR = Region of the Americas.
Note: Each diamond represents the pH value for a single product; the rectangles are added to aid in visualization.
Sources: [a]Gupta and Sreevidya 2004 (100); [b]Brunnemann et al. 1985 (73); [c]Hearn et al. 2013 (41); [d]Stanfill et al. 2011 (20); [e]Stanfill, Oliveira da Silva, unpublished results, 2013: [f]Richter et al. 2008 (94); [g]Stepanov et al. 2005 (57); [h]Rainey et al. 2011 (11).

In a 2010 study of 30 naswar products, reported pH values ranged from pH 8.10 to pH 8.96.[102] Extensive surveys in the United States found pH values between pH 5.54 and pH 8.62 for moist snuff.[2,91,93,94] Among 74 brands of chewing tobacco sold in Massachusetts, the pH values ranged from pH 5.07 to

pH 6.91; for 33 brands of dry snuff the values ranged from pH 5.50 to pH 7.61. The pH for 106 brands of moist snuff ranged from pH 5.41 to pH 8.38,[2,103] and a study of 40 moist snuff brands reported a similar range (pH 5.54 to pH 8.62).[94] Several zarda products combined with supari mixes had pH values ranging from pH 8.56 to pH 8.90.[100]

The content of nicotine and other alkaloids in growing tobacco plants is affected by numerous factors, including genetics, geographic location, climate, fertilization rates, stalk and leaf position, and maturity of the leaf. The wide variation of nicotine levels in various ST products used worldwide depends on the method of tobacco curing (air-cured, fire-cured, or flue-cured), variety within the type of tobacco, curing processes, manufacturing techniques, and tobacco blending approaches used.[104,105] Because ST products differ in moisture content, which affects the amount of tobacco present in one gram of product, constituent levels are often reported per gram dry weight. This chapter presents nicotine values found in the product "as is" or per wet weight. While this approach could have limitations when applied to some products such as dry snuff, it makes it possible to compare the greatest number of values among published reports. All values are expressed on a wet weight basis unless noted otherwise.

Most ST products have a total nicotine content of 20 mg/g or less, but products such as nass, gul powder, chewing tobacco (India), iqmik, zarda, toombak, chimó, and twist tend to have the highest total nicotine concentrations, as high as 95 mg/g.[2,20,23,41,61,73,94,99,101] Products that contain a considerable amount of areca nut, such as gutka, mawa, and mainpuri, had the lowest total nicotine values due to tobacco dilution with other material based on weight (0.16–4.20 mg/g).[2,20,73,101] Moist snuff, the most popular form of ST in the United States, had values that ranged from 7.06 to 24.3 mg/g in one study as reported by IARC[2,103] and from 4.42 to 25.0 mg/g in another study.[94] A 2010 study of 30 brands of naswar from a Pakistani market found total nicotine values ranging from 7.35 to 26.7 mg/g.[102] The nicotine values for toombak varied widely (7.0–95 mg/g).[23] The high nicotine concentrations found in many samples of toombak may be due to the use of *N. rustica* tobacco, which has higher concentrations of nicotine than *N. tabacum*.[20,23] Other high total nicotine values were observed for dry snuff (U.S.) (4.70–24.8 mg/g), iqmik (38.3–38.9 mg/g), nass (11.8–28.7 mg/g), chimó (5.29–30.2 mg/g), gul powder (33.4–34.1 mg/g), twist tobacco (21.6–40.1 mg/g), and zarda (14.6–65.0 mg/g).[20,41,73,101,102]

One global survey investigated *N. rustica* tobacco and its higher nicotine content.[20] The presence of *N. rustica* was indicated by elevated nicotine concentrations and comparisons of infrared spectra of the product with known *N. rustica* samples. In one toombak sample containing *N. rustica,* nicotine concentrations were almost three times higher than in the toombak samples that contained *N. tabacum* (28.2 mg/g vs. 10.2 mg/g). Nicotine in several other *N. rustica* products, including gul, zarda, and tobacco leaf (Bangladesh), ranged between 19.7 and 33.4 mg/g. Some chimó samples had high nicotine values (27.5–30.1 mg/g), but the tobacco type could not be determined conclusively.[20] Products that have high pH values (due to alkaline agents) and contain the nicotine-enriched *N. rustica* can deliver extremely high levels of free nicotine.[20,23,41,73]

An analysis of ST products across several countries found that free nicotine amounts were generally less than 10 mg/g, with the exception of chimó (1.32–30.1 mg/g), gul powder (29.1–31.0 mg/g), and naswar (8.84–13.2 mg/g).[20] Free nicotine concentrations in moist snuff products sold in the United States ranged from 0.01 to 7.81 mg/g[91] (Table 3-4). Products that tended to have the lowest levels of free nicotine

included gutka (handmade, cottage-made, and manufactured: 0.12–3.33 mg/g), tobacco leaf (0.15 mg/g), zarda (0.05–0.63 mg/g), mawa (0.11 mg/g), mainpuri (0.38 mg/g), and South African and Swedish snus (0.29–2.03 mg/g).[20]

Table 3-4. Ranges of moisture content, pH, free nicotine, total nicotine, and 5 TSNAs in 39 top-selling brands of U.S. moist snuff

Constituent	Minimum value	Brand	Maximum value	Brand
Moisture, %	27.4	Hawken Rough Wintergreen	54.5	Rooster Long Cut Bold Wintergreen
pH	5.54	Hawken Rough Wintergreen	8.62	Kodiak Ice Long Cut Regular
Free nicotine, %	0.3	Hawken Rough Wintergreen	79.9	Kodiak Ice Long Cut Regular
Total nicotine (mg/g wet)	4.42	Hawken Rough Wintergreen	25.0	W.B. Cut Regular
Free nicotine (mg/g wet)	0.01	Hawken Rough Wintergreen	7.81	Kodiak Ice Long Cut Regular
NNK (μg/g wet)	0.382	Red Seal Long Cut Wintergreen	9.95	Skoal Key
NNN (μg/g wet)	2.20	Copenhagen LC Regular	42.6	Skoal Key
NAT (μg/g wet)	0.938	Hawken Rough Wintergreen	31.9	Skoal Key
NAB (μg/g wet)	0.123	Red Seal Fine Cut Wintergreen	4.24	Skoal Key
NNAL (μg/g wet)	0.021	Copenhagen LC Regular	1.41	Skoal Key
Total TSNAs (μg/g wet)	4.87	Red Seal Long Cut Wintergreen	90.0	Skoal Key

Abbreviations: For nicotine values, mg/g = milligram per gram, and μg/g = microgram per gram. For TSNAs (tobacco-specific nitrosamines): NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN = *N'*-nitrosonornicotine; NAT = *N'*-nitrosoanatabine; NAB = *N'*-nitrosoanabasine; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.
Note: In the original report, one herbal brand (Oregon Mint Snuff) did not contain detectable levels of nicotine and was excluded from the data presented in this table.
Source: Richter et al. 2008 (94).

Several other reports published since 2000 have provided information on pH and nicotine content in ST products used in India, South Africa, and Pakistan. A report to the World Health Organization (WHO) South-East Asia Regional Office showed that 20 ST products used in India had pH values between pH 5.2 and pH 10.1, and the total nicotine content ranged from 1.24 to 10.2 mg/g product, with free nicotine values ranging from 0.03 to 4.06 mg/g.[100] A report on moist snuff products used in South Africa showed the pH of these products to range between pH 7.1 and pH 10.1, and total nicotine content to vary between 11.6 and 29.3 mg/g dry weight.[106] In the 30 brands of naswar from the Pakistani market, total nicotine ranged from 7.35 to 26.7 mg/g, and free nicotine levels ranged from 5.52 to 21.4 mg/g. The pH averaged 8.56,[102] resulting in an average 77% of total nicotine in these products being present in free form.

Of the ST products available on the U.S. market, moist snuff contains the highest level of free nicotine. According to 2003 data from the Massachusetts Department of Public Health (MDPH) as reported by IARC, the average pH of moist snuff was pH 7.43, compared to pH 6.36 for dry snuff and pH 5.82 for chewing tobacco.[2,103] The mean value for free nicotine in moist snuff was 3.52 mg/g, which is five times

higher than the proportion of free nicotine in dry snuff (0.71 mg/g) and 32 times higher than the free nicotine level in chewing tobacco (0.11 mg/g). In addition to the differences by the type of smokeless product, the MDPH report showed that pH and free nicotine in U.S. products vary by brand and over time. Thus, of the most popular brands of moist snuff, Kodiak has had the highest pH since 1999, and the free nicotine level in this brand has increased greatly, from 35.2% to 60.3% of total nicotine over a six-year period (1997–2003). In contrast, average nicotine levels in Copenhagen and Skoal decreased during this time.[2,103]

A 2008 survey of 39 top-selling brands of U.S. moist snuff showed a more than fivefold variation in total nicotine levels and a more than 500-fold range in free nicotine.[94] The ranges for moisture content, pH, total/free nicotine, and TSNA levels in this sample of U.S. moist snuff are summarized in Table 3-4. A 2003 study described nicotine levels for some of the brands that were later included in the 2008 study.[89] Comparing the data for the two time points shows the following ranking of differences in free nicotine content for the U.S. moist snuff brands: Hawken Wintergreen had the lowest free nicotine content in both studies (0.01 mg/g wet weight in 2003 and 2008), followed by Skoal Bandits Mint (0.97 mg/g in 2003 and 0.37 mg/g in 2008), Copenhagen Long Cut (2.04 mg/g in 2003 and 5.67 mg/g in 2008), and Kodiak Wintergreen (5.81 mg/g in 2003 and 7.14 mg/g in 2008). This observation supports the idea that moist snuff manufacturers target particular brands to specific consumers by controlling free nicotine levels in their products, most likely as a part of the continued use of the graduation strategy.[98]

In 2012, nicotine levels were reported for a large sample of novel oral spit-less and dissolvable ST products being marketed to U.S. smokers as an alternative to smoking.[12,107] A total of 117 samples were analyzed, including various flavors of Marlboro Snus (rich, mild, spearmint, peppermint) and Camel Snus (mellow, frost, robust, winterchill), as well as dissolvable products Camel Orbs (mellow, fresh), Camel Sticks (mellow), Camel Strips (fresh), Ariva (java, citrus, cinnamon, wintergreen), and Stonewall (java, wintergreen) (Table 3-5). Overall, the results of these analyses supported previous observations that, with the exception of Camel Snus, these products generally contain relatively low amounts of free nicotine compared with most traditional U.S. moist snuff brands.[12] Although the dissolvable Camel products have very low total nicotine levels, they have a higher pH and thus a larger portion of free nicotine, exceeding the amount of free nicotine in Marlboro Snus and the dissolvable brands Ariva and Stonewall.[12]

The varying levels of free nicotine across these novel products may affect how acceptable they are to current or new tobacco users. On one hand, smokeless products with higher nicotine content may be more effective at satisfying smokers' cravings than products with less nicotine,[108,109] and this may in part explain the greater popularity of Camel Snus compared to Marlboro Snus.[110] On the other hand, products that are low in free nicotine could be more easily accepted by young people initiating tobacco use.

Table 3-5. Moisture content, pH, total/free nicotine, and TSNA concentrations in novel U.S. smokeless tobacco products

Brands (number of samples)	Moisture content, %	pH	Total Nicotine mg/g wet	Free nicotine		NNN µg/g wet	NNK µg/g wet
				% of total	mg/g wet		
Marlboro Snus (71)	16.6	6.75	20.5	5.2	0.88	0.36	0.13
Camel Snus (36)	29.6	7.42	11.6	21.4	2.47	0.62	0.31
Camel Orbs (4)	13.0	8.10	3.0	54.5	1.65	0.21	0.28
Camel Sticks (3)	13.1	7.76	3.9	35.8	1.44	0.26	0.31
Camel Strips (3)	17.7	7.88	2.7	41.9	1.11	0.15	0.22
Ariva (4)	2.5	6.92	5.0	7.3	0.37	0.09	0.07
Stonewall (2)	3.8	7.10	6.9	10.6	0.73	0.12	0.06

Abbreviations: TSNA = tobacco-specific nitrosamines; NNN = *N'*-nitrosonornicotine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; mg/g = milligram per gram; µg/g = microgram per gram.
Note: The dissolvables Ariva and Stonewall were discontinued at the beginning of 2013 by Star Scientific, Inc.
Source: Stepanov et al. 2012 (12).

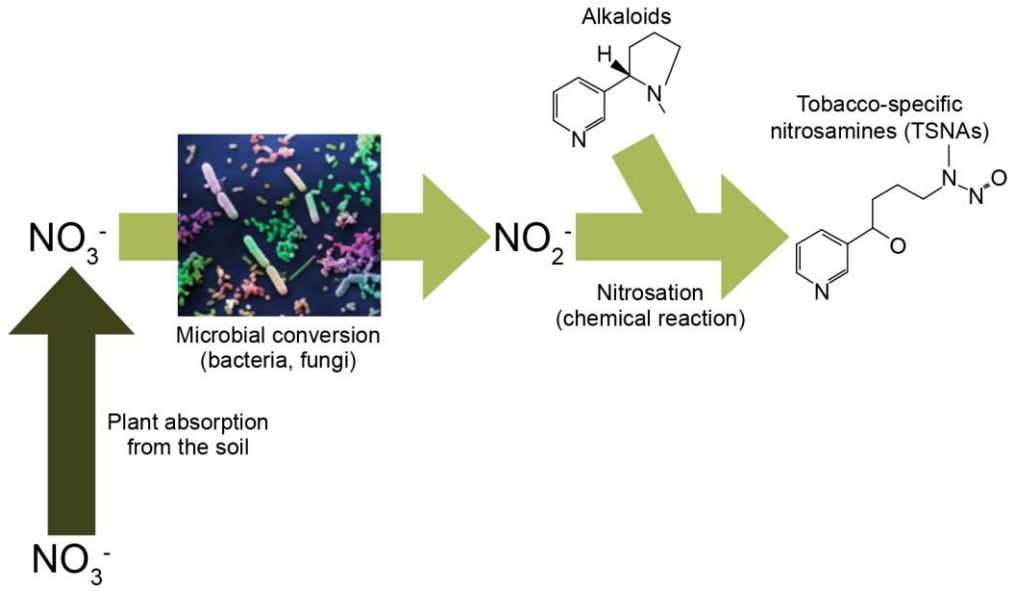
Tobacco-Specific Nitrosamines

TSNAs are commonly considered among the most potent carcinogens in ST products.[2,32] A total of five TSNAs have been identified in tobacco products: *N'*-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), *N'*-nitrosoanatabine (NAT), and *N'*-nitrosoanabasine (NAB). NNN, NNK, and NNAL are among the more common TSNAs and are the most carcinogenic.[2,32,82] The carcinogenicity of NNN and NNK has been reviewed and established by the IARC,[2] and the pulmonary and pancreatic carcinogenicity of NNAL has been demonstrated in a few animal studies (reviewed in Hecht 1998[32]). NNN, NNK, and NAT generally occur in greater quantities than the other TSNAs.[20,32,56,57,59,85,94,111,112]

Because of NNAL's potential for carcinogenicity, the levels of NNAL present are also important, but these have been reported in smokeless products only occasionally.[20,94,113,114] However, regardless of the sparse reporting, NNAL carcinogenicity should always be taken into consideration because it is metabolically formed from NNK in ST users. Moreover, NNAL is commonly utilized as a biomarker of exposure to carcinogenic NNK.[115]

In the growing plant, TSNAs are not generally present at elevated levels,[21,22,35] but they can accumulate to extremely high levels in certain products (e.g., toombak).[23] The levels of TSNAs present in ST products are attributable to numerous factors, including plant genetics (tobacco species/varieties), growth factors (nitrate levels, climate), cultivation practices (fertilization rates, harvesting methods), processing (curing, fermentation), and storage conditions[2,27,114,116–121] (Figure 3-7). Many studies have investigated techniques for reducing TSNA levels in tobacco.[121–123] One study by Wiernik and colleagues proposed a method of quick-drying tobacco at 70°C for 24 hours to remove excess water and reduce growth of microorganisms, which resulted in decreased nitrite and TSNA levels.[34] Drying tobacco quickly at this stage of curing reduces the microbial activity but lowers tobacco leaf quality.

Figure 3-7. Plant-related absorption, microbiological, and chemical steps involved in the formation of tobacco-specific nitrosamines



Abbreviations: NO_3^- = nitrate; NO_2^- = nitrite; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.
Note: Nitrosation is the chemical reaction of nitrite with various secondary and tertiary amines. This reaction produces nitrosamines, including tobacco-specific nitrosamines, nitrosoamino acids, volatile nitrosamines, and areca-specific nitrosamines.

Nitrate, nitrite, and alkaloids are present in ST products at the time of purchase, and prolonged storage can lead to further accumulation of TSNAs, with larger amounts accumulating if storage occurs at elevated temperatures and humidity.[121,124] Adding nitrate-containing agents could contribute to increased levels of TSNAs in ST products. One product, Ghana traditional snuff, contains tobacco mixed with potassium nitrate (saltpeter)[125] (chapter 12).

Worldwide, the use of different tobacco types, processing techniques, and tobacco blending approaches leads to wide variation of TSNA levels in various ST products. Several comparative international reports[2,20,73,99] and individual studies on ST products used in different countries[12,23,57,94,112] provide an informative view of the variations in TSNA levels among countries and product types. Concentration data in this section are expressed as microgram per gram (µg/g) wet weight, which allows for comparison of a larger global dataset of ST products.

The highest levels of TSNAs ever observed in tobacco products have been found in Sudanese toombak. Calculations based on dry weight values and moisture content reported by Idris and colleagues[23] reveal that NNN content in some samples of this product were as high as 2,860 µg/g, and NNK content as high as 7,300 µg/g. Lower levels of TSNAs were reported in 1985 by Brunnemann and colleagues for moist snuff samples purchased in Canada; total TSNA was up to 115 µg per gram of product.[73] Some tobacco products sold in India also have very high TSNA levels, but Stepanov and colleagues[57] reported a large variation in TSNA levels among Indian products. The largest quantities of TSNAs were found in khaini; amounts were also relatively high in zarda products, but the levels of these carcinogens in gutka were

relatively low. Stepanov and colleagues found that, overall, total TSNA content varied from 0.04 to 127.93 µg/g product.[57] Such a wide range is not surprising given the variety of ST products and approaches to tobacco processing and product manufacturing used in India.

TSNA levels also vary widely in moist snuff products sold in the United States, although they do not reach the levels seen in Indian products. A comprehensive survey of moist snuff conducted by the Centers for Disease Control and Prevention (CDC)[94] showed an 18-fold variation in TSNA content among 39 top-selling U.S. brands of moist snuff (see Table 3-4 for a summary). The levels of NNN ranged from 2.2 to 42.6 µg/g; levels of NNK ranged from 0.38 to 9.9 µg/g. The survey also recorded information about NNAL levels in the studied brands, and an almost 70-fold difference was found in NNAL content among brands. Thus, even though TSNA levels had declined overall in some U.S. smokeless tobacco products since the 1980s,[116] some U.S. moist snuff brands still contained high levels of these carcinogens as of 2008.

TSNA levels in snus sold in Sweden are reported to have declined by about 85% over a 20-year period. In 2002, amounts of NNN in 27 samples of Swedish snus averaged 0.49 µg/g product, whereas the NNK amounts averaged 0.19 µg/g product.[112,126] These levels are among the lowest seen in commercial ST products. The oral spit-less and dissolvable ST products marketed in the United States after about 2008 also contain relatively low levels of TSNAs.[12,56] According to an analysis of 117 samples of these products reported in 2012, total TSNAs (the sum of NNN, NNK, NAT, and NAB) ranged from 0.53 µg/g in dissolvable Camel Strips to 1.19 µg/g in Camel Snus.[12] Thus, considerable variation of TSNA levels has been observed even in this low-TSNA category.

The most current and comprehensive analysis of international samples showed wide variation in TSNA levels in more than 53 products from 9 countries reported in 2011[20] (Table 3-6). The concentration of total TSNAs (that is, the sum of NNK, NNN, NAT, NAB, and NNAL) in the products ranged from 0.084 to 992 µg/g. As mentioned earlier, the highest NNK concentrations were found in Sudanese toombak and dry zarda (Bangladesh) (3.84 µg/g). The highest NNN concentrations were observed also in toombak (Sudan), dry zarda (Bangladesh), khaini (India), and handmade gutka (India). Handmade gutka and mawa from Pakistan had the lowest NNK concentrations. The study found that NNAL levels ranged from 0.004 to 6.77 µg/g, with the highest NNAL concentrations in toombak, dry zarda, and khaini.[20] Extremely high concentrations of TSNAs were found in saliva from toombak users.[23,127,128] Given the high carcinogenic potency of NNN and NNK, it is not surprising that over 50% of oral cancers in Sudanese men are attributed to the use of toombak or other oral products.[5,128–130]

Table 3-6. Ranges of pH, free nicotine, total nicotine, and 5 TSNAs in 53 international smokeless tobacco products

Constituent	Minimum value	Brand or type	Maximum value	Brand or type
pH	5.22	Baba Zarda120	10.1	Toombak, sample 2; Super Taxi Snuff
Free nicotine, %	0.16	Baba Zarda120	99.1	Toombak, sample 2
Total nicotine (mg/g wet)	0.16	Mawa	34.1	Mawa
Free nicotine (mg/g wet)	0.11	Mawa	31.0	Eagle Gul Powder
NNK (μg/g wet)	0.004	Mawa	516	Toombak, sample 5
NNN (μg/g wet)	0.045	Gutka (handmade, Karachi)	368	Toombak, sample 5
NAT (μg/g wet)	0.014	Gutka (handmade, Karachi)	59.6	Toombak, sample 5
NAB (μg/g wet)	0.005	Gutka (handmade, Karachi)	41.5	Toombak, sample 5
NNAL (μg/g wet)	0.004	Mawa	6.77	Toombak, sample 5
Total TSNAs (μg/g wet)	0.084	Gutka (handmade, Karachi)	992	Toombak, sample 5

Abbreviations: NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN = *N'*-nitrosonornicotine; NAT = *N'*-nitrosoanatabine; NAB = *N'*-nitrosoanabasine; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.
Notes: Values for nicotine (total and free), mg/g wet = milligrams per gram as received (wet weight). Values for TSNAs (tobacco-specific nitrosamines, NNN, NNK, NAT, NAB and NNAL), μg/g wet = micrograms per gram as received (wet weight).
Source: Stanfill et al. 2011 (20).

Metals and Metalloids

Metals and metalloids are naturally present in tobacco, and amounts of these substances in tobacco are influenced by soil pH, soil composition, and industrial contamination.[131,132] Smokeless tobacco products have been reported to contain detectable levels of several metals that are classified as IARC Group 1 carcinogens (arsenic, beryllium, chromium VI, cadmium, nickel compounds, polonium-210) or Group 2B carcinogens (e.g., cobalt, lead).[133] A review of studies of ST products from Ghana, Canada, India, and the United States found detectable concentrations of arsenic (0.1–3.5 μg/g), beryllium (0.01–0.038 μg/g), chromium (0.71–21.9 μg/g), cadmium (0.3–1.88 μg/g), nickel (0.84–13.1 μg/g), lead (0.23–13 μg/g), and cobalt (0.056–1.22 μg/g).[26] A report of metals values in Pakistani naswar showed detectable levels of arsenic (0.15–14.04 μg/g), chromium (0.8–54.05 μg/g), cadmium (0.25–9.2 μg/g), nickel (2.2–64.85 μg/g), lead (12.4–111.15 μg/g), and even higher levels of several other metals.[102]

Some ST products also contain mercury, a systemic toxicant, and barium, a dermal irritant,[26,125,134,135] and metals such as aluminum and chromium, which may cause biologic sensitization.[26,125,136] The potential for exposure to several of the toxic metals listed above (barium, beryllium, cadmium, cobalt, nickel, and lead) was demonstrated by determining how much of these metals transferred from tobacco to artificial saliva.[136]

The amount of copper in ST products is also of interest. The copper content of areca nuts is higher than that found in other nuts.[137] A study of seven ST product types from India (zarda, creamy snuff, khaini, etc.) revealed very high levels of copper in four gutka products (237–656 μg/g) compared with other gutka products or other types of ST products (0.012–36.1 μg/g).[135] Areca nut use has been linked to oral submucous fibrosis (OSF), a condition that affects the mouth, pharynx, and esophagus. It has been suggested that copper upregulates lysyl oxidase, resulting in the excessive cross-linking and accumulation of collagen that occurs in OSF.[137]

Among the previously mentioned GothiaTek standards set for the Swedish tobacco industry are guidelines for the allowable levels of metals in Swedish snus: cadmium (1.0 μg/g), lead (2.0 μg/g), arsenic (0.5 μg/g), nickel (4.5 μg/g), and chromium (3.0 μg/g). The average levels of metals in Swedish snus in 2009 were low: cadmium (0.6 μg/g), lead (0.3 μg/g), arsenic (0.1 μg/g), nickel (1.3 μg/g), and chromium (0.8 μg/g),[55] which demonstrates that the levels of metals in ST can be monitored and held below certain limits.

Polycyclic Aromatic Hydrocarbons

Compounds such as polycyclic aromatic hydrocarbons (PAHs), phenols, and volatile aldehydes are formed from burning wood and sawdust.[42,61] During fire curing, tobacco is exposed to this wood smoke, and these substances can be deposited on the curing leaf. Indeed, levels of PAHs and phenols tend to be higher in tobacco that is fire-cured rather than air-cured.[21,22,41,43] Products made with fire-cured tobacco (e.g., moist snuff) have higher levels of PAHs, including PAHs that are IARC Group 1 or 2 carcinogens, than products such as snus, which do not contain fire-cured tobacco.[56,61]

Ten PAH compounds have been designated as IARC carcinogens or potential carcinogens (see Table 3-2): in Group 1, benzo[*a*]pyrene (BaP); in Group 2A, dibenz[*a,h*]anthracene; and in Group 2B, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, dibenzo[*a,i*]pyrene, indeno[*1,2,3-cd*]pyrene, 5-methylchrysene, naphthalene, and benz[*a*]anthracene.[37] All of these compounds have been found in smokeless tobacco.[61]

Among U.S. products, total PAH levels (that is, the sum concentration of 23 PAH compounds) in moist snuff that contained fire-cured tobacco ranged between 921 and 9,070 nanograms per gram of product (ng/g), which was generally higher than levels found in snus that did not contain fire-cured tobacco (660–1,100 ng/g).[61] Overall, among products with detectable levels of BaP, moist snuff had higher BaP levels (9.7–44.6 ng/g) than snus (3.0–12.3 ng/g); however, 41% of the snus brands had BaP levels below the detectable limit of 1.6 ng/g. The levels of naphthalene in moist snuff that contained fire-cured tobacco (409–1,110 ng/g) were comparable to naphthalene amounts in snus that was made with air-cured tobacco (636–1,065 ng/g). When naphthalene was excluded from the total PAH concentration, the remaining PAHs in moist snuff (145–8,120 ng/g) were higher than those found in snus (21–213 ng/g). One brand of moist snuff, Hawken Long Cut Wintergreen, which could be viewed as a starter brand, contained only 145 ng/g of PAHs other than naphthalene (776 ng/g).[61] It is clear that amounts of PAHs can be reduced by eliminating or reducing the use of fire-cured tobaccos.

Areca Nut

Areca nut, an ingredient in some ST products, is an IARC Group 1 carcinogen.[133] Areca nuts are seeds from the Areca palm (*Areca catechu*), which is native to South-East Asia and Eastern Africa (Figure 3-3). The seed can be used in the ripe or unripe form; can be dried, baked, or roasted; and then cut into slices, crushed, or consumed whole. Betel quid often contains areca nut, among other ingredients, such as tobacco, catechu (an extract of the *Acacia* plant), alkaline agents, and spices, wrapped in a piper betel leaf.[6]

Areca nut contains compounds such as arecoline and guvacoline that can react with nitrite to form areca-specific nitrosamine compounds (ASNAs).[6] These ASNAs are also formed in the mouth during use of products containing areca nut.[39] The areca-derived *N*-nitrosoguvacoline (NGL) has been shown to induce pancreatic tumors in lab animals, and a mixture of NG and NNK has been shown to induce lung tumors.[138] Another ASNA compound, 3-(*N*-nitrosomethylamino)propionaldehyde, is both highly cytotoxic and genotoxic to human buccal epithelial cells, a finding that is important to understanding tumor induction among users of areca nut–containing products.[139] Areca nut is a carcinogen and a very harmful substance that should not be included in tobacco products.[6]

Tonka Bean

Tonka (*Dipteryx odorata),* a flowering tree in the pea family (*Fabaceae*), is native to Brazil and is cultivated in Central and South America. The tree produces seed pods containing black wrinkled seeds with a fragrance reminiscent of vanilla, which are known as tonka beans.[140]

Coumarin, a benzopyrone compound, is present at high concentrations in tonka bean (35,000 µg/g), as well as in cassia (*Cinnamomum aromaticum*) (17,000–87,300 µg/g), cinnamon (900–40,600 µg/g), and Peru balsam (*Myroxylon balsamum*) (4000 µg/g).[141] In the mid-1950s, Hazelton and others identified coumarin as a liver toxin in dogs and rats following oral administration of coumarin.[142] Coumarin and tonka beans were banned as flavoring agents in the United States,[143] and because of this ban, daily human exposure is thought to occur at very low levels (60 ng/g), primarily resulting from use of fragrances and foodstuffs made with flavor substances (cinnamon) containing low levels of naturally occurring coumarin.[144] Detectable levels of coumarin have been found in the filler from several brands of Indonesian clove cigarettes[145] due to the use of flavor materials containing tonka bean.[146]

Tonka bean is widely used in a tobacco product called rapé (chapter 9).

Other Harmful Agents

Flavoring agents are added to ST products worldwide.[63,64,147]

Diphenyl ether, a flavor compound with a harsh metallic aroma,[148] and camphor have been identified as highly concentrated constituents of some tobacco products and certain spice condiment packs used to make betel quid.[149] Diphenyl ether irritates mucus membranes and can damage the liver, kidney, spleen, or thyroid after prolonged exposure.[149,150] Camphor can adversely affect the neurological, respiratory, cardiovascular, and gastrointestinal systems. Even small amounts of camphor have caused convulsions

followed by depression.[151] Ingestion of these substances is of note since betel quid can be swallowed during use.

Brazilian rapé, a nasal product, contains tobacco mixed with tonka bean, cinnamon powder, or clove buds, but usually lacks alkaline agents. Varieties of rapé produced in the Minas Gerais State of Brazil are known to contain extremely high levels of coumarin, a liver toxicant, which is derived from tonka bean and cinnamon (André Oliveira da Silva, personal communication, 2013).

Energy-enhanced smokeless products such as Revved Up contain stimulants (caffeine, ginseng), taurine, and vitamins B and C.

Some forms of tombol contain khat (*Catha edulis*), a plant that contains cathinone, an alkaloid with amphetamine-like stimulant properties, which purportedly causes euphoria, excitement, and loss of appetite.[74]

Gaps and Limitations of the Current Evidence Base

Further research is required to better characterize the chemical contents of a wider range of products. Research is also needed into the role of microorganisms (bacteria and mold) in altering product chemistry (i.e., generating nitrite and nitrosamines, producing mycotoxins). The effects of bacteria and mold on TSNA levels in products and the conditions that increase TSNA levels are also subjects in need of further study.

Because of the complexity of ST products—which can include a variety of tobacco types, chemical additives, non-tobacco plant ingredients, and microorganisms—ST products should not be viewed as a single homogenous product category for assessing composition or health effects. This wide variety of ST products worldwide differs in terms of addictiveness, toxicity, carcinogenicity, health effects, and impact on public health. Categorizing the products into groups with similar properties may provide a means of determining the health effects associated with particular product chemistries. However, drawing conclusions about the health consequences of different types of ST products (snuff, chimó, gutka) based on limited data from a small sample set from specific localities could be very misleading.

Summary and Conclusions

The widely diverse group of tobacco-containing products known as ST are distributed and used around the world. Smokeless tobacco products vary greatly in chemical composition and, in some cases, contain extremely high levels of total nicotine, free nicotine, and carcinogens. Most ST products contain tobacco and chemical/plant-derived additives, and may also contain microorganisms.

From the growing process to the final product, ST undergoes numerous chemical changes. Many constituents in ST products are present at very low levels in the growing tobacco plant. Chemicals are formed or introduced at one or more stages in the process of transforming the harvested tobacco into the final ST product. During curing, nitrite, TSNAs, *N*–nitrosamino acids, and volatile *N*–nitrosamines can be formed. Fire curing can also lead to the formation of PAHs, aldehydes, and phenols.[21,22,33,43,56,61] The fermentation stage may result in the formation of chemicals such as ethyl carbamate, nitrite, and

TSNAs.[2,33,44] During production or preparation, compounds from areca nut may be added, and storage conditions may increase TSNA levels in ST products.[114,121,124] Also, during use of products containing areca nut, areca-specific nitrosamines are formed endogenously in the mouth.[39]

A number of studies have begun to address the presence of bacteria, fungi, and mold in tobacco.[46,47] Especially important are (1) bacteria and mold that convert nitrate to nitrite, which contribute to the formation of TSNAs and other nitrosamines,[33,44,53] (2) bacteria and mold that are potentially pathogenic,[48] and (3) fungi that produce mycotoxins, including aflatoxins (i.e., *Aspergillus*).[152]

High nitrite concentrations in ST are a clear indication of past or ongoing conversion of nitrates to nitrites by microorganisms.[33,44] Nitrite concentrations should be monitored and controlled in all ST products as they are a key precursor in the formation of carcinogenic nitrosamines, including TSNAs.[2,38,54,133] Eliminating or decreasing the population size of nitrite-forming microorganisms (by pasteurization, cleaning fermentation equipment, seeding with non-nitrite-forming bacteria)[33,55] or lowering nitrite levels by other means (use of nitrite scavengers, modifying production processes, etc.) generally results in lower TSNA levels in smokeless tobacco.[33,44,53,55–57]

Among the means of controlling microorganism during ST production, pasteurization or heat-treating of tobacco is one of the most effective methods of preventing nitrate-to-nitrite conversion.[55] Indeed, snus, a pasteurized product, generally has lower nitrite and TSNA levels than non-pasteurized products such as moist snuff and khaini.[56,57] These observations suggest that changing tobacco processing methods shows promise as a means of reducing the levels of some carcinogenic and toxic agents in tobacco products.

The user's actual absorption of nicotine, toxicants, and carcinogens from a given ST product is affected by the product's characteristics (product design, pH, moisture, cut width, additives content, pouched/non-pouched, buffering capacity),[8,90,153] use parameters (dosage and frequency, duration, and intensity of use),[154,155] mode of use (oral: chewing, sucking, dental application, etc.; nasal),[1] and physiologic factors (salivary volume and pH).[85,153]

Areas of concern regarding manufacturing smokeless products include, but are not limited to, fire curing (introduction of PAHs and other smoke-related chemicals), bacterial contamination (potential pathogenicity), fermentation (formation of nitrite and carcinogenic nitrosamines), the addition of areca nut (an IARC Group 1 carcinogen), nicotine-enriched tobacco species (high total nicotine levels), alkaline agents (which boost free nicotine levels), and storage methods that allow continued formation of nitrosamines. Another matter of concern is the addition of stimulants to tobacco products, such as the addition of caffeine to moist snuff products (e.g., Revved Up energy dip[14]), and the mixing of khat, a plant with amphetamine-like properties, with tobacco to form tombol in Middle Eastern countries such as Yemen[74] (Ghazi Zaatari, personal communication, 2013). Maintenance of toxicants below certain feasible, but not necessarily safe, thresholds demonstrates that the tobacco industry has the ability to use manufacturing controls to reduce toxicants as recommended by the World Health Organization[156]; however, only one company has set its own voluntary toxicant reduction standards.

References

1. Boffetta P, Hecht S, Gray N, Gupta P, Straif K. Smokeless tobacco and cancer. Lancet Oncol. 2008 Jul;9(7):667–75.
2. International Agency for Research on Cancer. Smokeless tobacco and some tobacco-specific *N*-nitrosamines. IARC monographs on the evaluation of carcinogenic risks to humans. Vol. 89. Lyon, France: World Health Organization, International Agency for Research on Cancer; 2007 [cited 2012 April 24]. Available from: http://monographs.iarc.fr/ENG/Monographs/vol89/index.php
3. World Health Organization. WHO report on the global tobacco epidemic, 2011. Appendix VIII—Table 8.2: Crude smokeless tobacco prevalence in WHO member states. Geneva: World Health Organization; 2011. Available from: http://www.who.int/tobacco/global_report/2011/en_tfi_global_report_2011_appendix_VIII_table_2.pdf
4. Eriksen M, Mackay J, Ross H. The tobacco atlas. 4th ed. Atlanta: American Cancer Society; New York: World Lung Foundation; 2012. Available from: http://www.tobaccoatlas.org
5. Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). Health effects of smokeless tobacco products. Brussels: European Commission; 2008 [cited 2010 July 8]. Available from: http://ec.europa.eu/health/archive/ph_risk/committees/04_scenihr/docs/scenihr_o_013.pdf
6. International Agency for Research on Cancer. Betel-quid and areca-nut chewing and some areca-nut-derived nitrosamines. IARC monographs on the evaluation of carcinogenic risks to humans. Vol. 85. Lyon, France: World Health Organization, International Agency for Research on Cancer; 2004 [cited 2012 April 24]. Available from: http://monographs.iarc.fr/ENG/Monographs/vol85/index.php
7. Swedish Match. Firebreak – the smoke-free tobacco product of the future [Internet]. 2006 Mar 23 [cited on 2012 May 2]. Available from: http://www.swedishmatch.com/en/Media/Pressreleases/Press-releases/Other/Firebreak--the-smoke-free-tobacco-product-of-the-future/
8. Nasr MM, Reepmeyer JC, Tang Y. In vitro study of nicotine release from smokeless tobacco. J AOAC Int. 1998;81(3):540–3.
9. Carpenter CM, Connolly GN, Ayo-Yusuf OA, Wayne GF. Developing smokeless tobacco products for smokers: an examination of tobacco industry documents. Tob Control. 2009;18:54–9. doi: 10.1136/t.c.2008.026583
10. Connolly GN, Richter P, Aleguas A, Pechacek TF, Stanfill SB, Alpert HR. Unintentional child poisonings by ingestion of conventional and novel tobacco products. Pediatrics. 2010;125(5):896–9.
11. Rainey CL, Conder PA, Goodpaster JV. Chemical characterization of dissolvable tobacco products promoted to reduce harm. J Agric Food Chem. 2011;59:2745–51.
12. Stepanov I, Biener L, Knezevich A, Nyman AL, Bliss R, Jensen J, et al. Monitoring tobacco-specific *N*-nitrosamines and nicotine in novel Marlboro and Camel smokeless tobacco products: findings from Round 1 of the New Product Watch. Nicotine Tob Res. 2012 Mar;14(3):274–81. Epub 2011 Oct 29. doi: 10.1093/ntr/ntr209
13. Seidenberg AB, Rees VW, Connolly GN. R.J. Reynolds goes international with new dissolvable tobacco products. Tob Control. 2012 May;21(3):368–9. Epub 2011 Oct 7. doi: 10.1136/tobaccocontrol-2011-050116
14. Southern Smokeless Tobacco Company. Revved up [Internet]. [no date] [cited 2012 Apr 18]. Available from: http://www.southernsmokeless.com/Revved-up.html
15. Lawson K. Altria will launch verve – a non-dissolving nicotine disc [Internet]. Tobacco Pub. 2012 Jun 18 [cited 2013 Apr 23]. Available from: http://www.tobaccopub.net/tobacco-info/altria-will-launch-verve-a-non-dissolving-nicotine-disc
16. Bhonsle RB, Murti PR, Gupta PC. Tobacco habits in India. In: Gupta PC, Hamner JE 3rd, Murti PR, editors. Control of tobacco-related cancers and other diseases. Proceedings of an international symposium, Tata Institute of Fundamental Research. Bombay, January 15–19, 1990. Bombay: Oxford University Press; 1992. p. 25–46.
17. World Health Organization. Report on oral tobacco use and its implications in South-East Asia [Internet]. World Health Organization, Regional Office for South-East Asia; 2004. Available from: http://www.searo.who.int/LinkFiles/NMH_OralTobaccoUse.pdf
18. Lewis R, Nicholson J. Aspects of the evolution of *Nicotiana tabacum* L. and the status of the United States *Nicotiana* germplasm collection. Genet Resour Crop Evol. 2007;54(4):727–40.
19. Sisson VA, Severson RF. Alkaloid composition of *Nicotiana* species. Beitr Tabakforsch. 1990;14:327–39.
20. Stanfill SB, Connolly GN, Zhang L, Jia LT, Henningfield JE, Richter P, et al. Global surveillance of oral tobacco products: total nicotine, unionised nicotine and tobacco-specific *N*-nitrosamines. Tob Control. 2011 May;20(3):e2. Epub 2010 Nov 25. doi:10.1136/tc.2010.037465
21. Bhide SV, Nair J, Maru GB, Nair UJ, Kameshwar Rao BV, Chakraborty MK, et al. Tobacco-specific *N*-nitrosamines [TSNA] in green mature and processed tobacco leaves from India. Beit Tabakforsch. 1987;14:29–32.

22. Bhide SV, Kulkarni JR, Padma PR, Amonkar AJ, Maru GB, Nair UJ, et al. Studies on tobacco specific nitrosamines and other carcinogenic agents in smokeless tobacco products. In: Sanghvi LD and Notani PP, editors. Tobacco and health: the Indian scene. Proceedings of the UICC workshop "Tobacco or Health." Bombay: UICC and Tata Memorial Centre, Bombay; 1989:121–31.
23. Idris AM, Nair J, Ohshima H, Friesen M, Brouet I, Faustman EM, et al. Unusually high levels of carcinogenic tobacco-specific nitrosamines in Sudan snuff (toombak). Carcinogenesis. 1991;12(6):1115–8.
24. Steenkamp PA, van Heerden FR, van Wyk BE. Accidental fatal poisoning by *Nicotiana glauca*: identification of anabasine by high performance liquid chromatography/photodiode array/mass spectrometry. Forensic Sci Int. 2002 Jul 17;127(3):208–17.
25. Furer V, Hersch M, Silvetzki N, Breuer GS, Zevin S. *Nicotiana glauca* (tree tobacco) intoxication—two cases in one family. J Med Toxicol. 2011;7(1):47–51.
26. Pappas RS. Toxic elements in tobacco and in cigarette smoke: inflammation and sensitization. Metallomics. 2011 Nov;3(11):1181–98. Epub 2011 Jul 28. doi: 10.1039/c1mt00066g
27. Burton HR, Bush LP, Djordjevic MV. Influence of temperature and humidity on the accumulation of tobacco-specific nitrosamines in stored burley tobacco. J Agric Food Chem. 1989;37:1372–7.
28. Burton HR, Childs GH, Anderson RA, Fleming PD. Changes in chemical composition of burley tobacco during senescence and curing. 3. Tobacco-specific nitrosamines. J Agric Food Chem. 1989;37:426–30.
29. Hecht SS, Ornaf RM, Hoffman D. Chemical studies on tobacco smoke. XXXIII. *N'*-nitrosonornicotine in tobacco: analysis of possible contributing factors and biologic implications. J Natl Cancer Inst. 1974;54(5):1237–44.
30. Hecht SS, Chen CB, Hirota N, Ornaf RM, Tso TC, Hoffman D. Tobacco-specific nitrosamines: formation from nicotine in vitro and during tobacco curing and carcinogenicity in strain A mice. J Natl Cancer Inst. 1978;60(4):819–24.
31. Spiegelhalder B, Fischer S. Formation of tobacco-specific nitrosamines. Crit Rev Toxicol. 1991;21:241.
32. Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specific *N*-nitrosamines. Chem Res Toxicol. 1998;11(6):559–603.
33. Fisher MT, Bennett CB, Hayes A, Kargalioglu Y, Knox BL, Xu D, et al. Sources of and technical approaches for the abatement of tobacco specific nitrosamine formation in moist smokeless tobacco products. Food Chem Toxicol. 2012;50:942–8.
34. Wiernik A, Christakopoulos A, Johansson L, Wahlberg I. Effect of air-curing on the chemical composition of tobacco. Recent Adv Tob Sci. 1995;21:39–80.
35. Djordjevic M, Gay SL, Bush LP, Chaplin JF. Tobacco-specific nitrosamine accumulation and distribution in flue-cured tobacco alkaloid isolines. J Agric Food Chem.1989;37(3):752–6.
36. Hoffmann D, Djordjevic MV, Fan J, Zang E, Glynn T, Connolly GN. Five leading U.S. commercial brands of moist snuff in 1994: assessment of carcinogenic *N*-nitrosamines. J Natl Cancer Inst. 1995;87(24):1862–9.
37. International Agency for Research on Cancer. Agents classified by the IARC monographs, Volumes 1–106. Updated June 28, 2012. [cited 2012 July 16]. Available from: http://monographs.iarc.fr/ENG/Classification/ClassificationsAlphaOrder.pdf
38. International Agency for Research on Cancer. Ingested nitrate and nitrite and cyanobacterial peptide toxins. IARC monographs on the evaluation of carcinogenic risks to humans. Vol. 94. Lyon, France: World Health Organization, International Agency for Research on Cancer; 2010.
39. Nair J, Nair UJ, Ohshima H, Bhide SV, Bartsch H. Endogenous nitrosation in the oral cavity of chewers while chewing betel quid with or without tobacco. In: Bartsch H, O'Neill IK, Schulte-Hermann R, editors. Relevance of N-nitroso compounds to human cancer: exposures and mechanisms. IARC Scientific Publications no. 84. Lyon, France: International Agency for Research on Cancer; 1987. p. 465–9.
40. Peedin GF. Production practices: flue-cured tobacco. In: Davis DL, Nielsen MT, editors. Tobacco: production, chemistry, and technology. London: Blackwell Publishing; 1999. p. 104–42.
41. Hearn BA, Ding YS, England L, Kim S, Vaughan C, Stanfill SB, et al. Chemical analysis of Alaskan iq'mik smokeless tobacco. Nicotine Tob Res. 2013;15(7):1283–8. Epub 2013 Jan 3. doi: 10.1093.ntr/nts270
42. Miller RD, Fowlkes DJ. Dark fire-cured tobacco. In: Davis DL, Nielson MT, editors. Tobacco: production, chemistry, and technology. London: Blackwell Publishing; 1999. p. 164–82.
43. Leffingwell JC. Leaf chemistry: basic chemical constituents of tobacco leaf and differences among tobacco types. In: Davis DL, Nielson MT, editors. Tobacco: production, chemistry, and technology. London: Blackwell Publishing; 1999. p. 265–84.

44. Di Giacomo M, Paolino M, Silvestro D, Vigliotta G, Imperi F, Visca P, et al. Microbial community structure and dynamics of dark fire-cured tobacco fermentation. Appl Environ Microbiol. 2007;73:825–37. doi: 10.1128/AEM.02378-06
45. Tso TC. Seed to smoke. In: Davis DL, Nielson MT, editors. Tobacco: production, chemistry, and technology. London: Blackwell Publishing; 1999. p. 1–31.
46. Larsson L, Szponar B, Ridha B, Pehrson C, Dutkiewicz J, Krysinska-Traczyk E, et al. Identification of bacterial and fungal components in tobacco and tobacco smoke. Tob Induc Dis. 2008;4:4. doi: 10.1186/1617-9625-4-4
47. Pauly JL, Paszkiewicz G. Cigarette smoke, bacteria, mold, microbial toxins, and chronic lung inflammation. J Oncol. 2011;2011:819129.
48. Sapkota AR, Berger S, Vogel TM. Human pathogens abundant in the bacterial metagenome of cigarettes. Environ Health Perspect. 2010 March;118(3):351–6. Epub 2009 Oct 22. doi: 10.1289/ehp.0901201
49. Ayo-Yusuf OA, Reddy PS, van den Borne BW. Association of snuff use with chronic bronchitis among South African women: implications for tobacco harm reduction. Tob Control. 2008;17:99–104. doi:10.1136/tc.2007.022608
50. Bao P, Huang H, Hu ZY, Haggblom MM, Zhu YG. Impact of temperature, CO2 fixation and nitrate reduction on selenium reduction, by a paddy soil clostridium strain. J Appl Microbiol. 2013;114(3):703–12.
51. Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, et al. Koneman's color atlas and textbook of diagnostic microbiology. 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2006. Available from: http://books.google.com/books/about/Koneman_s_color_atlas_and_textbook_of_di.html?id=xzlsZo44GkoC
52. Cockrell WT, Roberts JS, Kane BE, Fulghum RS. Microbiology of oral smokeless tobacco products. Tobacco Int. 1989;55–7. Available from: http://legacy.library.ucsf.edu/documentStore/m/t/d/mtd27d00/Smtd27d00.pdf
53. Wahlberg I, Wiernik A, Christakopoulos A, Johansson L. Tobacco-specific nitrosamines: a multidisciplinary research area. Agro Food Industry Hi Tech. 1999 Jul/Aug;23–8.
54. Brunnemann KD, Prokopczyk B, Djordjevic MV, Hoffmann D. Formation and analysis of tobacco-specific *N*-nitrosamines. Crit Rev Toxicol. 1996;26(2):121–37.
55. Rutqvist LE, Curvall M, Hassler T, Ringberger T, Wahlberg I. Swedish snus and the GothiaTek standard. Harm Reduct J. 2011;8:11. Epub 2011 May 16. doi: 10.1186/1477-7517-8-11
56. Stepanov I, Jensen J, Hatsukami D, Hecht SS. New and traditional smokeless tobacco: comparison of toxicant and carcinogen levels. Nicotine Tob Res. 2008 Dec;10(12):1773–82. doi: 10.1080/14622200802443544
57. Stepanov I, Hecht SS, Ramakrishnan S, Gupta PC. Tobacco-specific nitrosamines in smokeless tobacco products marketed in India. Int J Cancer. 2005 Aug 10;116(1):16–9.
58. Rubinstein I, Pederson GW. Bacillus species are present in chewing tobacco sold in the United States and evoke plasma exudation from the oral mucosa. Clin Diagn Lab Immunol. 2002 Sept;9(5):1057–60. doi: 10.1128/CDLI.9.5.1057-1060.2002
59. Brunnemann KD, Qi J, Hoffmann D. Chemical profile of two types of oral snuff tobacco. Food Chem Toxicol. 2002;40(11):1699–703.
60. Hatsukami DK, Ebbert JO, Feuer RM, Stepanov I, Hecht S. Changing smokeless tobacco products: new tobacco-delivery systems. Am J Prev Med. 2007;33(6S):S368–78.
61. Stepanov I, Villalta PW, Knezevich A, Jensen J, Hatsukami DK, Hecht SS. Analysis of 23 polycyclic aromatic hydrocarbons in smokeless tobacco by gas chromatography–mass spectrometry. Chem Res Toxicol. 2010 Jan 18;23(1):66–73. doi: 10.1021/tx900281u. Erratum in: Chem Res Toxicol. 2010 Apr 19;23(4):845.
62. Dube MF, Cantrell DV, Mua JP, Holton DE, Stokes CS, Figlar JN, inventors. R.J. Reynolds Tobacco Company, assignee. United States patent application 20080029110. 2008 Feb 7. Available from: http://appft1.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PG01&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.html&r=1&f=G&l=50&s1=%2220080029110%22.PGNR.&OS=DN/20080029110&RS=DN/20080029110
63. U.S. House of Representatives. Smokeless tobacco ingredient list as of April 4, 1994. U.S. House of Representatives, Report to the Subcommittee on Health and the Environment, Committee on Energy and Commerce, Washington, DC: Patton, Boggs, and Blow; May 3, 1994. Brown and Williamson. Bates No. 566415479/5524. Available from: http://legacy.library.ucsf.edu/tid/pac33f00/pdf
64. Swedish Match. Ingredients in snus [Internet]. 2012 [cited on 2012 Jul 16]. Available from: http://www.swedishmatch.com/en/Our-business/Snus-and-snuff/Ingredients-in-snus/
65. R.J. Reynolds Tobacco Company. Brand compounds [Internet database].Winston-Salem, NC: R.J. Reynolds; 2012 [cited 2012 July 16]. Available from: http://www.rjrt.com/smokelessingredientsbybrand.aspx

66. U.S. Smokeless Tobacco Company. Ingredients [Internet]. C2009–2013 [cited 2012 July 16]. Available from: http://www.ussmokeless.com/en/cms/Products/Ingredients_Nav/Ingredients/default.aspx
67. Phillip Morris USA. Smokeless tobacco: Ingredients [Internet]. Richmond, VA: Phillip Morris USA; c1999–2012 [cited 2012 May 30]. Available from: http://www.philipmorrisusa.com/en/cms/Products/Smokeless_Tobacco/Ingredients/default.aspx
68. Stanfill SB, Jia L, Tewfik R, Lawler T, Watson C, Ashley D. Utilizing GC/MS to eliminate flavor-related interferences in nicotine analysis [poster presentation]. 5th National Summit on Smokeless and Spit Tobacco. Madison, WI. September 2009.
69. Chen C, Isabelle LM, Pickworth WB, Pankow JF. Levels of mint and wintergreen flavorants: smokeless tobacco products vs. confectionery products. Food Chem Toxicol. 2010 Feb;48(2):755–63.
70. Renner CC, Enoch C, Patten CA, Ebbert JO, Hurt RD, Moyer TP, et al. Iqmik: a form of smokeless tobacco used among Alaska Natives. Am J of Health Behav. 2005;29(6):588–94.
71. Blanchette RA, Renner CC, Held BW, Enoch C, Angstman S. The current use of *Phellinus igniarius* by the Eskimos of western Alaska. Mycologist. 2002;16(4):142–5.
72. Lagrou E. Xamanismo e representagao entre os kaxinawá [Shamanism and representation among the Kaxinawa]. In: Langdon EJ, editor. Xamanismo no Brasil: novas perspectivas [Shamanism in Brazil: new perspectives]. Florianópolis, Brazil: Federal University of Santa Catarina Publishing House; 1996. p. 197–231.
73. Brunnemann KD, Genoble L, Hoffmann D. *N*-nitrosamines in chewing tobacco: an international comparison. J Agric Food Chem. 1985;33:1178–81.
74. Lee MM. The identification of cathinone in khat (*Catha edulis*): a time study. J Forensic Sci. 1995;40(1):116–21.
75. Wilbert J. Tobacco and shamanism in South America. New Haven, CT: Yale University Press; 1987.
76. McKenna T. Food of the gods: the search for the original tree of knowledge: a radical history of plants, drugs, and human evolution. New York: Bantam; 1993.
77. Rodgman A, Perfetti T. The chemical components of tobacco and tobacco smoke. Boca Raton, FL: CRC Press; 2009. doi: 10.1201/9781420078848
78. Khariwala SS, Carmella SF, Stepanov I, Fernandes P, Lassig AA, Yueh B, et al. Elevated levels of 1-hydroxypyrene and *N'*-nitrosonornicotine in smokers with head and neck cancer: a matched control study. Head Neck. Epub 2012 Jul 17. doi: 10.1002/hed.23085
79. Yuan JM, Gao YT, Wang R, Chen M, Carmella SG, Hecht SS. Urinary levels of volatile organic carcinogen and toxicant biomarkers in relation to lung cancer development in smokers. Carcinogenesis. 2012 Apr;33(4):804–9. Epub 2012 Jan 31. doi: 10.1093/carcin/bgs026
80. Yuan JM, Gao YT, Murphy SE, Carmella SG, Wang R, Zhong Y, et al. Urinary levels of cigarette smoke constituent metabolites are prospectively associated with lung cancer development in smokers. Cancer Res. 2011;71(21):6749–57. Epub 2011 Oct 25. doi:10.1158/0008-5472.CAN-11-0209
81. U.S. Food and Drug Administration. Tobacco products: harmful and potentially harmful constituents (HPHCs) [Internet]. [cited 2012 Sep 11]. Available from: http://www.fda.gov/TobaccoProducts/GuidanceComplianceRegulatoryInformation/ucm297741.htm#
82. Hecht SS, Hoffmann D. Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. Carcinogenesis. 1988;9(6):875–84.
83. Rivenson A, Djordjevic MV, Amin S, Hoffman R. A study of tobacco carcinogenesis XLIV. Bioassay in A/J mice of some *N*-nitrosamines. Cancer Lett. 1989 Sep 15;47(1–2):111–4.
84. Brunnemann KD, Hoffmann D. Chemical composition of smokeless tobacco products. In: National Cancer Institute. Smokeless tobacco or health: an international perspective. Smoking and tobacco control monographs. Vol. 2. Bethesda, MD: National Cancer Institute; 1992. p. 96–108.
85. Hoffmann D, Djordjevic MV. Chemical composition and carcinogenicity of smokeless tobacco. Adv Dental Res. 1997;11(3):322–9. doi: 10.1177/08959374970110030301
86. Material Safety Data Sheet. Coumarin, ACC# 52685 [Internet]. St. Louis: Fisher Scientific Sigma Chemical; 2007 [cited 2012 May 4]. Available from: https://fscimage.fishersci.com/msds/52685.htm
87. Lungarini S, Aureli F, Coni E. Coumarin and cinnamaldehyde in cinnamon marketed in Italy: a natural chemical hazard? Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2008 Nov;25(11):1297–305.

88. U.S. Department of Health and Human Services. The health consequences of smoking: a report of the Surgeon General. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2004. Available from: http://www.cdc.gov/tobacco/data_statistics/sgr/2004/complete_report/index.htm
89. Richter P, Spierto FW. Surveillance of smokeless tobacco nicotine, pH, moisture, and unprotonated nicotine content. Nicotine Tob Res. 2003 Dec;5(6):885–9.
90. Tomar SL, Henningfield JE. Review of the evidence that pH is a determinant of nicotine dosage from oral use of smokeless tobacco. Tob Control. 1997;6(3):219–25.
91. Henningfield JE, Radzius A, Cone EJ. Estimation of available nicotine content of six smokeless tobacco products. Tob Control. 1995;4:57–61.
92. Fant RV, Henningfield JE, Nelson RA, Pickworth WB. Pharmacokinetics and pharmacodynamics of moist snuff in humans. Tob Control. 1999;8:387–92.
93. Djordjevic MV, Hoffman D, Glynn T, Connolly GN. U.S. commercial brands of moist snuff, 1994. I. Assessment of nicotine, moisture, and pH. Tob Control. 1995;4:62–6.
94. Richter P, Hodge K, Stanfill S, Zhang L, Watson C. Surveillance of moist snuff: total nicotine, moisture, pH, un-ionized nicotine, and tobacco-specific nitrosamines. Nicotine Tob Res. 2008 Nov;10(11):1645–52.
95. Benowitz NL. Nicotine addiction. Prim Care. 1999;26:611–31.
96. Alpert HR, Koh H, Connolly GN. Free nicotine content and strategic marketing of moist snuff tobacco products in the United States: 2000–2006. Tob Control. 2008;17:332–8.
97. Henningfield JE, Fant RV, Tomar SL. Smokeless tobacco: an addicting drug. Adv Dental Res. 1997;11:330–5. doi: 10.1177/08959374970110030401
98. Connolly GN. The marketing of nicotine addiction by one oral snuff manufacturer. Tob Control. 1995;4:73–9.
99. McNeill A, Bedi R, Islam S, Alkhatib MN, West R. Levels of toxins in oral tobacco products in the UK. Tob Control. 2006;15(1):64–7. doi: 10.1136/tc2005.013011
100. Gupta P, Sreevidya S. Laboratory testing of smokeless tobacco products. Final report to the India Office of the WHO (Allotment No.: SE IND TOB 001.RB.02). New Delhi; 2004.
101. Lawler TS, Stanfill SB, Zhang L, Ashley DL, Watson CH. Chemical characterization of domestic oral tobacco products: total nicotine, pH, unprotonated nicotine and tobacco-specific *N*-nitrosamines. Food Chem Toxicol. 2013;57:380–6. Epub 2013 Mar 19. doi 10.1016/j.fct.2013.03.011
102. Zakiullah, Saeed M, Muhammad N, Khan SA, Gul F, Khuda F, et al. Assessment of potential toxicity of a smokeless tobacco product (naswar) available on the Pakistani market. Tob Control. 2012 Jul;21(4):396–401. Epub 2011 Jun 3. doi:10.1136/tc.2010.042630
103. Massachusetts Department of Public Health (MDPH). Smokeless tobacco data base. Boston; 2004 [cited 2012 April 24].
104. Borgerding MF, Perfetti TA, Ralapati S. Determination of nicotine in tobacco, tobacco processing environments and tobacco products. In: Gorrod JW, Jacob P 3rd, editors. Analytical determination of nicotine and related compounds and their metabolites. Amsterdam: Elsevier; 1999. p. 285–391.
105. Burton HR, Dye NK, Bush LP. Distribution of tobacco constituents in tobacco leaf tissue. 1. Tobacco-specific nitrosamines, nitrate, nitrite, and alkaloids. J Agric Food Chem.1992;40(6):1050–5.
106. Ayo-Yusuf OA, Swart TJP, Pickworth WB. Nicotine delivery capabilities of smokeless tobacco products and implications for control of tobacco dependence in South Africa. Tob Control. 2004;13:186–9.
107. Tobacco Products Scientific Advisory Committee. The nature and impact of the use of dissolvable products on the public health. A compilation of the materials from the July 21–22, 2011, January 18–20, 2012, and March 1, 2012, meetings of the Tobacco Products Scientific Advisory Committee. Available from: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/TobaccoProductsScientificAdvisoryCommittee/UCM295994.pdf
108. Kotlyar M, Hertsgaard LA, Lindgren BR, Jensen JA, Carmella SG, Stepanov I, et al. Effect of oral snus and medicinal nicotine in smokers on toxicant exposure and withdrawal symptoms: a feasibility study. Cancer Epidemiol Biomarkers Prev. 2011;20(1):91–100. doi: 10.1158/1055-9965. Erratum in: Cancer Epidemiol Prev. 2011 May; 20(5):1048.
109. Hatsukami DK, Jensen J, Anderson A, Broadbent B, Allen S, Zhang Y, et al. Oral tobacco products: preference and effects among smokers. Drug Alcohol Depen. 2011;118:230–6. doi:10.1016/j.drugalcdep.2011.03.026
110. Rogers JD, Biener L, Clark PI. Test marketing of new smokeless tobacco products in four U.S. cities. Nicotine Tob Res. 2010;12(1):69–72. doi:10.1093/ntr/ntp166

111. Chamberlain WJ, Schlotzhauer WS, Chortyk OT. Chemical composition of nonsmoking tobacco products. J Agric Food Chem. 1988;36:48–50.
112. Österdahl BG, Jansson C, Paccou A. Decreased levels of tobacco-specific *N*-nitrosamines in moist snuff on the Swedish market. J Agric Food Chem. 2004 Aug;11;52(16):5085–8.
113. Prokopczyk B, Wu M, Cox JE, Amin S, Desai D, Idris AM, et al. Improved methodology for the quantitative assessment of tobacco-specific *N*-nitrosamines in tobacco by supercritical fluid extraction. J Agric Food Chem. 1995;43:916–22.
114. Djordjevic MV, Fan J, Bush LP, Brunnemann KD, Hoffmann D. Effects of storage conditions on levels of tobacco-specific *N*-nitrosamines and *N*-nitrosamino acids in U.S. moist snuff. J Agric Food Chem. 1993;41:1790–4.
115. Hecht SS, Carmella SG, Murphy SE, Riley WT, Le, C, Luo X, et al. Similar exposure to a tobacco-specific carcinogen in smokeless tobacco users and cigarette smokers. Cancer Epidemiol Biomarkers Prev. 2007;16:1567–72. doi: 10.1158/1055-9965.EPI-07-0227
116. Djordjevic MV, Brunnemann KD, Hoffmann D. The need for regulation of carcinogenic *N*-nitrosamines in oral snuff. Food Chem Toxicol. 1993 Jul;31(7):497–501.
117. Fischer S, Spiegelhalder B, Preussmann R. Preformed tobacco-specific nitrosamines in tobacco—role of nitrate and influence of tobacco type. Carcinogenesis. 1989;10(8):1511–7.
118. Chamberlain WJ, Chortyk OT. Effects of curing and fertilization on nitrosamine formation in bright and burley tobacco. Beitr Tabakforsch. 1992;15:87–92.
119. Burton HR, Dye NK, Bush LP. Relationship between tobacco-specific nitrosamines and nitrite from different air-cured tobacco varieties. J Agric Food Chem. 1994;42:2007–11.
120. Peele DM, Riddick MG, Edwards ME. Formation of tobacco-specific nitrosamines in flue-cured tobacco. Recent Adv Tob Sci. 2001;27:3–12.
121. Andersen RA, Burton HR, Fleming PD, Hamilton-Kemp TR. Effect of storage conditions on nitrosated, acylated, and oxidized pyridine alkaloid derivatives in smokeless tobacco products. Cancer Res. 1989;49:5895–900.
122. Lewis RS, Jack AM, Morris JW, Robert VJM, Gavilano LB, Siminszky B, et al. RNA interference (RNAi)–induced suppression of nicotine demethylase activity reduces levels of a key carcinogen in cured tobacco leaves. Plant Biotechnol J. 2008 May;6(4):346–54. Epub 2008 Feb 14. doi: 10.1111/j.1467-7652.2008.00324.x
123. Rickert WS, Joza PJ, Sharifi M, Wu J, Lauterbach JH. Reductions in the tobacco specific nitrosamine (TSNA) content of tobaccos taken from commercial Canadian cigarettes and corresponding reductions in TSNA deliveries in mainstream smoke from such cigarettes. Regul Toxicol Pharmacol. 2008 Aug;51(3):306–10. Epub 2008 Apr 24.
124. Andersen RA, Fleming PD, Burton HR, Hamilton-Kemp TR, Sutton TG. Nitrosated, acylated, and oxidized pyridine alkaloids during storage of smokeless tobaccos: effects of moisture, temperature, and their interactions. J Agric Food Chem. 1991;39(7):1280–7.
125. Addo MA, Gbadago JK, Affum HA, Adom T, Ahmed K, Okley GM. Mineral profile of Ghanaian dried tobacco leaves and local snuff: a comparative study. J Radioanal Nucl Chem. 2008;277:517–24.
126. Österdahl BG, Slorach SA. Volatile *N*-nitrosamines in snuff and chewing tobacco on the Swedish market. Food Chem Toxicol. 1983 Dec;21(6):759–62.
127. Idris AM, Nair J, Friesen M, Ohshima H, Brouet I, Faustman EM, et al. Carcinogenic tobacco-specific nitrosamines are present at unusually high levels in the saliva of oral snuff users in Sudan. Carcinogenesis. 1992;13(6):1001–5.
128. Idris AM, Prokopczyk B, Hoffmann D. Toombak: a major risk factor for cancer of the oral cavity in Sudan. Prev Med. 1994;23:832–9.
129. Idris AM, Ahmed HM, Malik MO. Toombak dipping and cancer of the oral cavity in the Sudan: a case-control study. Int J Cancer. 1995;63:477–80.
130. Ahmed HG, Mahgoob RM. Impact of toombak dipping in the etiology of oral cancer: gender-exclusive hazard in the Sudan. J Cancer Res Ther. 2007;3:127–30.
131. Adamu CA, Bell RE, Mulchi CL, Chaney RL. Residual metal levels in soils and leaf accumulations in tobacco a decade following farmland application of municipal sludge. Environ Pollut. 1989;56:113–26.
132. Mulchi CL, Adamu CA, Bell PF, Chaney RL. Residual heavy metal concentrations in sludge amended coastal plain soils – II. Predicting metal concentrations in tobacco from soil test information. Commun Soil Sci Plant Anal. 1992;23(9&10):1053–69.

133. International Agency for Research on Cancer. A review of human carcinogens: personal habits and indoor combustions. IARC monographs on the evaluation of carcinogenic risks to humans. Vol. 100E. Lyon, France: World Health Organization, International Agency for Research on Cancer; 2012 [cited 2012 July 16]. Available from: http://monographs.iarc.fr/ENG/Monographs/vol100E/mono100E.pdf
134. Shaikh AN, Khandekar RN, Anand SJS, Mishra UC. Determination of some toxic trace elements in Indian tobacco and its smoke. J Radioanal Nucl Chem. 1992;163:349–53.
135. Dhaware D, Deshpande A, Khandekar RN, Chowgule R. Determination of toxic metals in Indian smokeless tobacco products. Scientific World Journal. 2009;9:1140–7.
136. Pappas, RS, Stanfill SB, Watson C, Ashley DL. Analysis of toxic metals in commercial moist snuff and Alaskan iqmik. J Anal Toxicol. 2008 May;32(4):281–91.
137. Trivedy C, Baldwin D, Warnakulasuriya S, Johnson N, Peters T. Copper content in *Areca catechu* (betel nut) products and oral submucous fibrosis. Lancet. 1997 May 17;349(9063):1447.
138. Rivenson A, Hoffmann D, Prokopczyk B, Amin S, Hecht SS. Induction of lung and exocrine pancreas tumors in F344 rats by tobacco-specific and areca-derived *N*-nitrosamines. Cancer Res. 1988 Dec 1;48(23):6912–7.
139. Sundqvist K, Liu Y, Nair J, Bartsch H, Arvidson K, Grafström RC. Cytotoxic and genotoxic effects of areca nut–related compounds in cultured human buccal epithelial cells. Cancer Res. 1989 Oct 1;49(19):5294–8.
140. Jang DS, Park EJ, Hawthorne ME, Vigo JS, Graham JG, Cabieses F, et al. Potential cancer chemopreventive constituents of the seeds of *Dipteryx odorata* (tonka bean). J Nat Prod. 2003:66(5):583–7.
141. Duke, J.A. Handbook of phytochemical constituents of GRAS herbs and other economic plants. Boca Raton, FL: CRC Press; 1992.
142. Hazelton LW, Tusing TW, Zeitlin, BR, Thiessen R Jr, Murer HK. Toxicity of coumarin. J Pharmacol Exp Ther. 1956;118:348–58.
143. U.S. Food and Drug Administration. Substances prohibited from use in human food – coumarin. 21 C.F.R. Sect. 189.130 (2013). Available from: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=189.130
144. Lake, B.G. Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. Food Chem. Toxicol. 1999;37:423–53.
145. Stanfill SB, Brown CR, Yan J, Watson CH, Ashley DL. Quantification of flavor-related compounds in the unburned contents of bidi and clove cigarettes. J Agric Food Chem. 2006;54(22):8580–8.
146. Hanusz M. Kretek: the culture and heritage of Indonesia's clove cigarettes. Tortola, British Virgin Islands: Equinox Publishing; 2000.
147. Dabur India, Ltd. Dabur red toothpowder. [cited 2012 May 1]. Available from: http://www.dabur.com/Export-Dabur%20Red%20Toothpowder
148. Burdock GA, editor. Fenaroli's handbook of flavor ingredients. Vol. II. 3rd ed. Boca Raton, FL: CRC Press; 1995.
149. Lisko, JG, Stanfill, SB, Watson, CH. Quantitation of ten flavor compounds in unburned tobacco products. Anal Methods. 2014;6(13):4698–704. doi: 10.1039/C4AY00271G
150. Material Safety Data Sheet. Diphenyl ether, MSDS# BWXKJ [Internet]. St. Louis: Sigma 1212 Chemical Co; 1994 [cited 2012 Apr 24]. Available from: http://hazard.com/msds/f2/bwx/bwxkj.html
151. International Programme on Chemical Safety. Camphor. INCHEM Database. PIM095; 1988 [cited 2013 April 24]. Available from: http://www.inchem.org/documents/pims/pharm/camphor.htm
152. Varma SK, Verma RA, Jha AK. Ecotoxicological aspects of *Aspergilli* present in the phylloplane of stored leaves of chewing tobacco (*Nicotiana tobaccum*). Mycopathologia. 1991 Jan;113(1):19–23.
153. Ciolino LA, McCauley HA, Fraser DB, Wolnik KA. The relative buffering capacities of saliva and moist snuff: implications for nicotine absorption. J Anal Toxicol. 2001;25(1):15–25.
154. Lemmonds CA, Hecht SS, Jensen JA, Murphy SE, Carmella SG, Zhang Y, et al. Smokeless tobacco topography and toxin exposure. Nicotine Tob Res. 2005 Jun;7(3):469–74. doi:10.1080/14622200500135640
155. Hecht SS, Carmella SG, Edmonds A, Murphy SE, Stepanov I, Luo X, et al. Exposure to nicotine and a tobacco-specific carcinogen increase with duration of use of smokeless tobacco. Tob Control. 2008;17(2):128–31.
156. World Health Organization Study Group on Tobacco Product Regulation. Report on the scientific basis of tobacco product regulation. Third report of a WHO study group. WHO technical report series no. 955. Geneva: World Health Organization; 2009. Available from: http://www.who.int/tobacco/global_interaction/tobreg/publications/tsr_955/en/index.html

157. Faizi A, Kimpton H, Rodu B, McAdam K. Ethyl carbamate levels in U.S. and Swedish smokeless tobacco products [poster presentation]. CORESTA Conference. Edinburgh. September 2010.
158. Syed U-F, Bari A, Husain L. Leaching of ^{210}Po in human saliva from smokeless tobacco. J Radioanal Nucl Chem. 2009;281(3):541–6. doi: 10.1007/510967-009-0038-2