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The carcinogenic profiles, trends, and cancer risks of regional smokeless tobacco products

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Thesis

THE CARCINOGENIC PROFILES, TRENDS, AND CANCER RISKS OF REGIONAL SMOKELESS TOBACCO PRODUCTS

by

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B.S., University of Florida, 2009

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ABSTRACT

Smokeless tobacco products have existed for centuries and vary significantly across geographical regions. The constituents found in each smokeless tobacco product depends on many factors, including social customs, manufacturing regulations, and the availability of local raw ingredients. Unfortunately, tobacco products have been linked to cancer over the past several decades, particularly of the oral cavity. In this study, the carcinogenic profiles, relative risks of oral cancer, and usage trends for three unique smokeless tobacco regions (United States, Sweden, and India) will be evaluated in order to determine the relative safety for each product.

In this paper, the chemical analysis of various products from United States, as well as Swedish snus and Indian gutkha were reviewed, to establish constituent profiles. The main carcinogens evaluated were the tobacco-specific n-nitrosamines; gutkha displayed the highest values of these ingredients, with
snus displaying the lowest. Studies examining the relative risks for oral cancers associated with each of the three region’s smokeless tobacco products were assessed. Indian gutkha expressed the highest relative risk for developing oral cancers, and Swedish snus expressed the lowest (a very slight increased risk at that). To establish usage habits for each region-specific smokeless tobacco product, various epidemiological studies were analyzed and showed that gutkha was the most prevalently used product in its respective region, with Swedish snus only slightly trailing in use. Smokeless tobacco products were used the least in the United States.

These studies concluded that the gutkha habits in India were the most damaging to the public health of the nation with regards to smokeless tobacco use, particularly due to the high prevalence of use and high relative risk of oral cancers. In a purely chemical sense, Swedish snus was less harmful than the counterparts often sold in the United States. Yet, with snus use significantly higher than smokeless tobacco use in the United States, it is thought that Sweden snus habits are more detrimental than those seen in the United States. However, smokeless tobacco is ultimately an unsafe practice in all three regions evaluated, and more should be done to remove carcinogens from the products and promote self-restraint for current users.
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WHO    World Health Organization
YRBS    Youth Risk Behavior Survey
Introduction

Historical evidence shows that smokeless tobacco of some form have been used for thousands of years, most likely originating in South American and Southeast Asia (National Cancer Institute, Centers for Disease Control and Prevention, & Stockholm Centre of Public Health, 2002). Through either chewing or sucking, an individual would release and absorb the nicotine found naturally within the tobacco, and desire for the associated psychotropic effect of nicotine has kept the habit alive for centuries.

It has only been within the last century or so that the detrimental effects of smokeless tobacco use have garnered attention, particularly with regards to cancers of the oral cavity and oropharynx. However, with the significant differences in tobacco products and habits seen around the world, each individual product deserves individual attention with respect to cancer association and risk. In this paper, smokeless tobacco products from three different regions (the United States, Sweden, and India) will be compared based on carcinogenic profile, trends of use, and associated cancer risk. By the end of the literature review, the general safety of each regions smokeless tobacco culture will be discussed.

Before looking over the literature, the characteristics of the different smokeless tobacco products and proposed carcinogenic mechanisms will be briefly covered.
Loose Leaf Tobacco

Until the early 20th century, particularly in the Americas, loose leaf tobacco (often referred to as chewing tobacco) was the prominent form of tobacco consumed. While manufacturing processes have certainly become more efficient and consistent with modern technology, loose leaf tobacco production varies little than historic production. Loose leaf tobacco is manufactured using an air-curing process on cigar leaf tobacco (Stanfill et al., 2011). The final product is then packaged in small, shredded strips into a bag. Brands of loose leaf tobacco vary based on the size of the shredded strips and are often flavored and sweetened with molasses or simple syrup, a mixture of processed sugar and water; the sugar content tends to be near 35%. Manufacturers often use licorice as one of these flavoring agents. To use the product, the consumer will pinch the desired amount of chewing tobacco (doses are around 0.75 to 1 inch diameter) out of the bag and place in their mouth (National Cancer Institute (NCI) et al., 2002). They will then either chew the tobacco or practice “dipping”. Dipping refers to when the consumer places the smokeless tobacco product between the gums and jaw, particularly in front of the lower incisors/canine teeth or in the rear of the mouth (Borgerding et al., 2012).

Loose leaf tobacco can also be manufactured in a “plug” form, characterized by the tobacco being pressed into bricks. Plug tobacco also tends to use heavier grades of the tobacco leaf for structure (Stanfill et al., 2011). Moisture levels of the plug brick will determine whether the product is marketed
as “firm” or “moist” plug tobacco (Borgerding et al., 2012). Additionally, “twist” tobacco is even another packaging option for chewing tobacco consumers. Twist tobacco is recognized as a twisted rope of between one and three high grade tobacco leaves (Stanfill et al., 2011). Twist tobacco products tend to have significantly less sweeteners added than traditional chewing tobacco (NCI et al., 2002).

The tobacco variety used in smokeless tobacco products found in the United States is *Nicotiana tabacum* L. This type of tobacco varies from the common Asian alternative *Nicotiana rustica* which expresses both a higher nicotine and tobacco-specific N-nitrosamines (TSNA) concentration than the alternative found in the United States market (Stanfill et al., 2011).

**Moist/Dry Snuff**

While chewing tobacco remained the most popular smokeless tobacco option after the boom of the cigarette industry, the introduction of moist snuff in the early 1970s changed this trend. In the United States population, moist snuff continues to be the most popular option among smokeless tobacco products. Dry snuff is a similar product with a few distinct differences (Stanfill et al., 2011).

Production of moist snuff starts with air or fire cured tobacco. Following either process, the cured tobacco goes through the process of fermentation for a set time period determined by the manufacturer. This fermentation process, involving the moistening and heating of the cured tobacco, leads to the high
levels of TSNAs and nitrite in the moist snuff. These components are created by naturally found bacteria that are stimulated by the fermentation (Rodu & Jansson, 2004). Unlike loose leaf tobacco, neither the stems nor the seeds are removed from the tobacco leaves during manufacturing (NCI et al., 2002).

If this cured and fermented tobacco becomes moist snuff, the tobacco will then be ground into fine particles or cut into millimeter long strands. Fine grinding or cutting snuff into these short strands increases the product’s surface area dramatically, which in turn increases nicotine absorption noticeably. Packing will either be loose tobacco in a tin or placed in small ready-to-use pouches, and either option is used in the same fashion as loose leaf tobacco. Moisture levels must maintain a minimal level of 25%, but a value closer to 50% is common practice (Borgerding et al., 2012). If dry snuff is the final product desired, the cured and fermented tobacco will be finely ground into small particles and dried to a moisture level of less that 25%, but moisture usually hovers around 10%. At this moisture and consistency, consumers may choose to inhale the dry snuff through the nasal passage in addition to using the product orally (NCI et al., 2002).

**Swedish Snus**

Although technically considered to be of the moist snuff variety of smokeless tobacco, unique manufacturing procedures and production restrictions make Swedish snus a distinct product. With daily snus use in Sweden hovering
around 20% with males and on the rise (yet still lower) with females, individual attention is importantly given to Swedish snus (Luo et al., 2007).

The major manufacturing difference between Swedish snus and traditional moist snuff, and the process which is responsible for the unique carcinogenic profile found in snus, is the replacement of fermentation with steam pasteurization (Coggins et al., 2012). By pasteurizing the air-cured tobacco instead of applying fermentation, the resulting sterilization destroys the bacteria responsible for much of the TSNA and nitrite production (Rodu & Jansson, 2004). As expected, the values of these constituents found in Swedish snus are significantly lower than other moist snuff options and will be discussed in further detail. In addition to the incorporation of steam pasteurization, Swedish snus manufacturers add sodium carbonate, sodium chloride, and humidifying agents to the list of ingredients. The final, packaged product is kept refrigerated while being stored, another attempt to avoid the fermentation process in the tobacco and further reducing TSNA and nitrite amounts (NCI et al., 2002).

As with traditional moist snuff, users place either a pinch or pouch of snus between their gum and cheek/lip; placing snus behind the upper lip is common among snus users. However, snus users will continue to leave the dose in their mouths for extended periods of time, often averaging around 11 to 14 hours a day. Varying doses of pouched snus are also available for a snus user to choose from; either regular or “mini-portions”, at 1.0 g or 0.5 g of tobacco per dose, respectively (NCI et al., 2002).
**Gutkha**

Unlike the previous types of smokeless tobacco listed, Gutkha (also gutka) is unique in the sense that tobacco is not necessarily always the most prominent ingredient in this Southeast Asian product. Any gutkha sample will likely have much less tobacco present than a smokeless tobacco product from either the American or European market; the total percent tobacco is lowered due to the addition of key ingredients such as slaked lime and, more importantly, the areca nut (Stanfill et al., 2011). Common flavoring additives in gutkha include saffron, catechu, mustard, turmeric, and cloves, but the regional variety in percent composition and added ingredients is extreme (Centers for Disease Control and Prevention (CDC), 2011).

As mentioned, besides tobacco, the areca nut and slaked lime are the staple constituents found in the typical gutkha products. When a betel leaf is included with the areca nut and slaked lime, the subsequent combination is known as betel-quid (also, ’paan’), a common tobacco free chewing product. The Asian people use the areca nut in betel-quid and gutkha often enough to have it declared the “fourth most common psychoactive substance in the world”, following only caffeine, alcohol, and nicotine (Gupta & Ray, 2004). Betel-quid is formed when processed areca nut is wrapped in a betel leaf that has been spread with slaked lime, often with other varying additives present. Paan users will then chew or suck on the leaf-wrapped mixture until attaining the desired effect, either spitting or swallowing the saliva. Unlike betel-quid, gutkha is not
wrapped in the betel leaf; gutkha more resembles chewing tobacco and moist snuff in both appearance and method of consumption.

The areca nut is an additive native to the Southeast Asian market and derived from the areca palm plant, *Areca catechu*. Though this species of the areca palm is most common, different species exist throughout Asia. No matter the species, as noted by Gupta and Warnakulasuriya, areca nut use in Asian regions is “strongly interwoven into local art and craft, folklore, social customs, religious practices and cultural rituals.” In processing, the manufacturer or user (when homemade) has many options in which to treat the raw areca nut before use. Popular options include sun-drying or boiling the nuts before de-shelling, sometimes even burying them in moist pits allowing for fermentation to occur (Sharan et al., 2012). People and literature often refer to the areca nut as the betel nut, but this is a misnomer and a result of its popular use with the betel leaf of the *Piper betle* plant (Sharan et al., 2012).

Slaked lime (calcium hydroxide) isn’t, in and of itself, a potent carcinogen. However, the role it and other inorganic salts fill in smokeless tobacco products and betel-quid indirectly leads to higher incidents of harmful health effects of users. Slaked lime is obtained in two fashions and depends on the geographical location of production. In coastal regions, a process involving the heating of seashells is used; more inland regions incorporate quarried limestone into manufacturing (World Health Organization (WHO), 2004). As an alkaline modifier, slaked lime will raise the pH level of a substance it is added to. When
that substance includes nicotine as a constituent, a higher percentage of the total nicotine will be converted in to unionized ("free") nicotine. In the unionized form, nicotine is absorbed by the oral mucosa at a significantly faster rate and this, in turn, quickly increases the total blood nicotine concentration (Richter et al., 2008). Nicotine uptake has been widely established as the cause of tobacco product addiction, and the faster the faster the uptake, the higher the addictive potential.

**Tobacco-Specific N-Nitrosamines**

Although the blame for the addictive potential of tobacco falls on the naturally occurring alkaloid and drug nicotine, it is the TSNAs that are responsible for a majority of the detrimental health effects experienced by smokeless and smoking tobacco users alike. While there are several TSNAs present in smokeless tobacco products, the most detrimental are N'-nitrosonornicotine (NNN), N'-nitrosoanatabine (NAT), N'-nitrosoanabasin (NAB), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Figure 1). Of these four, NNN and NNK are the main TSNAs believed to play a significant role in the development of oral cancer of tobacco users (Stepanov, Jensen, & Hecht, 2008).

The listed N-nitrosamines are formed in tobacco products by the nitrosation and sometimes reduction of the nicotine found in tobacco, leading to the "tobacco-specific" title given to them (Hoffmann et al., 1995). While TSNAs are found in all tobacco products, the concentration and specific TSNAs found
depend entirely on type of tobacco plant, process used (or not used) during manufacturing, and constituent interactions. Curing the tobacco, and the optional step of fermenting the cured-tobacco, both significantly alter the TSNA concentration. The moisture level of the product and storage methods can further elevate the level after curing and fermentation (Borgerding et al., 2012). The mechanism of formation and chemical structure of the major TSNAs can be seen in Figure 1. The carcinogenicity and carcinogenic mechanisms of TSNA and other smokeless tobacco carcinogens are discussed in further detail.

**Figure 1**: TSNA formation and chemical structures. Relevant abbreviations: N’-nitrosonornicotine (NNN), N’-nitrosoanatabine (NAT), N’-nitrosoanabasin (NAB), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Hoffmann et al., 1995).
Carcinogenic Mechanisms and Toxicity

Tobacco-Specific N-Nitrosamines

As the most critical carcinogenic constituents found in most smokeless tobacco products, understanding the metabolism and role that TSNAs play in carcinogenesis is vital. As most of the other detected TSNAs in tobacco products contribute negligibly to cancer risk, research maintains focus on both NNN and NNK (Nilsson, 2011). While research on potential pathways are continuously being conducted and improved upon, and numerous possible mechanisms have been proposed, only several will be discussed within the scope of this paper.

Initially, biomarkers were established to provide evidence of TSNA uptake and to prove that TSNAs were actually metabolized in the body. The TSNA NNK is metabolized into the body into several different metabolites, specifically 4- (methyl-nitrosamino)-1-(3-pyridyl)-1-butilanol (NNAL) and the glucuronides of NNK (Hecht, 1998). Interestingly, while merely a metabolite of NNK, the carcinogenic properties of NNAL vary little to NNK. Conversely, the glucuronides of NNK retain none of the carcinogenic potential expressed by NNK. Analysts consider NNAL an ideal biomarker for tobacco usage, mainly for its “high reliability and specificity to tobacco products” of both the smokeless and smoking variety (Boffetta et al., 2008). In order to measure total absorption and uptake of NNK, the amount of both NNAL and NNK glucuronide concentration can be detected in
the urine and combined. Similar metabolites and glucuronides have been discovered for NNN and the other TSNAs.

Another byproduct of NNN and NNK’s metabolic pathways is available not only for detection, but also as evidence of carcinogenic trends. The cytochrome P450 enzymes, a superfamily of enzymes that metabolize a majority of drugs among having other roles, are known to interact with absorbed NNN and NNK to form pyridyloxobutyl (POB) diazonium ions (Jalas, Hecht, & Murphy, 2005). Once formed, these POB diazonium ions may then react with hemoglobin and form DNA adducts (a DNA molecule attached to a carcinogen) and subsequently release the compound 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) (Hecht, 1998). DNA adducts of all types are notorious for their tendency to cause DNA miscoding and mutation errors, key components of carcinogenesis (Nilsson, 2011). HPB, the final compound released from this pathway, is the biomarker often used to detect NNN and NNK.

These biomarkers are important when comparing TSNA absorption between tobacco products, either smoking or smokeless. For example, one study compared smokeless tobacco users’ NNK absorption to that of smokers using total urinary NNAL as the metric; smokeless tobacco users showed significantly higher concentrations. A similar study was performed with NNN absorption and the detectable urinary HPB, and smokeless tobacco users (particularly snuff) again expressed higher concentrations of total urinary HPB than smokers (Hecht et al., 2007).
Highlighting on the danger of the DNA adducts formed by NNK and NNN helps to explain the damage potential of TSNAs found in tobacco. These DNA adducts form in tissues that are directly exposed to the TSNAs such the oral cavity when smokeless tobacco products are used (Hecht, 1998). DNA adducts are not immediately detrimental, however, as the natural DNA repair cycle has the opportunity to reverse the damage. If not repaired, mutations and miscoding may then occur. Vulnerable DNA regions for these errors include the RAS oncogene and the P53 tumor suppressor gene, responsible for promoting and suppressing carcinogenesis respectively (Warnakulasuriya & Ralhan, 2007). Severe enough DNA damage may arrest the cycle of affected cells and therefore induce neoplastic growth. Figure 2 illustrates this process with a simple flow chart. Furthermore, other carcinogenic promoting processes brought on by smokeless tobacco include oxidative stress, chronic local inflammation, and oral lesion formation. No matter the underlying cause, only repetitive exposure to tobacco carcinogens will lead to carcinogenesis over an extended period of time (Boffetta et al., 2008).
**Figure 2**: Proposed carcinogenic process of tobacco carcinogens (Boffetta et al., 2008).
With a unique delivery of tobacco and accompanying toxins to the body, a study by Wang and colleagues focused on NNK’s role in laryngeal carcinoma, slightly associated with dry snuff use. Only nasal inhalation of dry snuff exposes laryngeal cells to TSNAs when disregarding smoking tobacco. In the laboratory, the TSNA NNK has shown to increase protein levels of the enzyme DNA methyltransferase 1 (DNMT 1). The increased concentration levels of this enzyme then will hypermethylate tumor suppressor genes in laryngeal tissue cells, specifically laryngeal squamous cells. The particular laryngeal squamous cell concentrated on this study was of the Hep-2 variety (Wang et al., 2012). Normal DNA methylation, which plays a key role in cell growth management, is then compromised and can lead to carcinogenesis. Similar mechanisms of tumorigenesis by hypermethylation are seen in other cancers of the body that are not associated with tobacco use. So while these results were only observed under laboratory conditions, further studies may show similar mechanisms caused by NNK in vivo (Wang et al., 2012).

**Areca Nut**

An essential component of gutkha, the Areca nut and its impact on the carcinogenicity of products must be explored. Chemically the most detrimental compounds observed in the Areca nut are the alkaloids and tannins, particularly the alkaloid arecoline, but Figure 3 shows other potential oncogenic mechanisms. Arecoline, along with having the greatest carcinogenic
significance, is also the most abundant alkaloid in the Areca nut (Sharan et al., 2012).

One experiment focused on the effects of Areca nut extract and the alkaloid arecoline on SAS oral epithelial cells as both are known for demonstrating genotoxicity (Ko et al., 1992). These analytes were used in assays to test this claim. The first assay checked for CHK1 and CHK2 activation by the extract or arecoline. Both CHK1 and CHK2 are checkpoint kinases and are integral in DNA repair during the cell cycle; activation of these kinases indicate damage to the DNA. Their activations can eventually lead to alteration of the cell cycle and apoptosis. Twenty-four hour exposure to either analyte marked an increase of CHK1 and CHK2 phosphorylation in the SAS cells, a display of kinase activation (Chang et al., 2012).

The MMP-9 assay of these analytes provided further genotoxicity evidence. Metalloproteinase-9, an inflammatory mediator, plays a role in the inflammatory response and can lead to cellular proliferation, angiogenesis, and ultimately metastatic cancer (Chang et al., 2012). High levels of this mediator are associated with oral squamous cell carcinoma. Following treatment with the Areca nut extract, SAS cell MMP-9 levels increased by a factor of two or more, depending on concentration levels. Oddly, arecoline exposure instead caused a decrease in MMP-9 levels, more so with higher arecoline concentration levels. The reduction may be attributed to the cytotoxicity of arecoline that has been determined in other earlier studies (Chang et al. 2012).
Nut of Areca catechu or betel nut (BN)

- Betel nut extract (BNE)
  - ROS generated by BNE at pH > 9.5 induces SCE, MN and CA types of mutagenic cells. Causes DNA SSBs and DSBs, reduces glutathione synthetase level, lowers poly-ADP ribosylation of BRCA1 proteins in mice, and lowers p53, BRCA1 and DRCA2 proteins in mice, induces mutation in mouse BRCA1 gene.

- Trace elements
  - Cu increases lysyl oxidase activity leading to OSF

Polyphenols, tannins

- Auto-oxidation of polyphenols generates ROS, tannins induce gene conversion.

Alkaloids

- Arecaidine
- Arecolidine
- Gyracine
- Curacine
- Arecoline

- Inhibits protein synthesis, stimulates collagen production, increases fibroblast proliferation, cytotoxicity, decreases neoplastic changes in mice and hamsters, decreases hepatic GSH, induces MN, CA, SCE, decreases BRCA1 expression in HGF.

BSNA upon nitrosation

- DNA adducts and DNA strand breaks

Figure 3: A flow-chart displaying the Areca nut’s numerous cancer pathways (Sharan et al., 2012).
Arecoline is also linked to the inhibition of the tumor suppressor gene \textit{p53}. One study involved an assay exposing KB and HEp-2 cells, both well-established cancer cell lines, to arecoline. Results determined that arecoline repressed expression of the \textit{p53} gene; further testing exhibited \textit{p53} mRNA repression and inhibition of \textit{p53} target genes (Tsai et al., 2008). The exact mechanism of this inhibition has yet to be determined but may involve inhibited promoter gene activity. Even so, Tsai et al. believe this inhibitive property of arecoline “should play a critical role in the tumorigenesis” of \textit{Areca} nut related cancers.

According to Wang and colleagues,

“dysregulation of the caretaker genes lead to an increase in the overall rates of DNA damage, mutation, and chromosomal missegregation.”

The spindle assembly checkpoint gene falls into this “caretaker” category. The checkpoint prevents mitotic progress into metaphase if the chromosomes are misaligned by the microtubules and will become inactive once the chromosomes align properly. The effect of arecoline on the spindle assembly checkpoint was analyzed by treating KB and HEp-2 cells and microscopically determining if the cells get held in the prometaphase stage (Wang et al., 2010). Prometaphase is marked by the nuclear envelope dissolving and microtubules attaching to the kinetochores at the chromosome centers (Malmanche et al., 2006). The arecoline treatment matched the concentration found in the saliva of \textit{gutkha} users to mimic those conditions. Treatment caused over 50% of the KB and HEp-2 cells to remain in prometaphase compared to the 10% found in the
control. Additionally, spindle assembly checkpoint genes appeared upregulated, possibly a result of the checkpoint trying to correct the cellular arrest (Wang et al. 2010). Malmache and colleagues tell how “a weakened [spindle assembly checkpoint] could facilitate tumour development in cells that are undergoing tumorigenesis.”

Similar to the TSNAs found in tobacco, areca nut-specific N-nitrosamines have been discovered and detected in users of areca nut products. The notable N-nitrosamines are 3-methylnitrosamino-propionaldehyde (MNP), 3-methylnitrosamino-propionitrile (MNPN), N-nitrosoguvacine (NGC), and N-nitrosoguvacoline (NGL). These compounds are all derived from the alkaloid arecoline and seem to be created during the chewing process and blending with saliva. (WHO, 2004). Although no studies have been completed to determine the carcinogenic potential of these areca nut nitrosamines in humans, this potential has been seen in rats.

**Combination of Areca Nut and Tobacco**

In 2011, Joshi et al. explored the combined toxic effect of both areca nut and tobacco carcinogens in buccal mucosal (cheek) cells of gutkha chewers using a micronucleus assay, as micronuclei are the “biomarker of genetic damage in buccal mucosal cells” (Stich and Rosin, 1983). Cells that were either micronucleated or showed evidence of other nuclear anomalies were noted. The
study involved both chewers and non-chewers of gutkha with each participant having 1000 buccal cells assayed.

Assay results showed an increase in frequency of micronuclei in the buccal mucosal cells of gutkha chewers (0.57 ± 0.08) when compared to non-chewers. Similarly, with all other nuclear anomalies included, the total frequency of normal differentiated cells in chewers (85.81 ± 0.98) was lower than non-chewers (88.42 ± 0.53) (Joshi et al., 2011). These findings support the belief that the constituents in gutkha, as seen in other tobacco products, are genotoxic.

The assay results reinforce another claim, that frequency of use is directly related to cellular damage done. A dose-dependent relation was exhibited in the micronuclei frequency among gutkha chewers. For those that used gutkha five times or less throughout a single day, the frequency was determined at 0.70. When gutkha use reached or surpassed ten times a day, micronuclei frequency reached 0.90 (Joshi et al., 2011).

**Carcinogenic Profiles**

**United States**

While the carcinogenic mechanisms of most smokeless tobacco component are established, the abundance of a component in smokeless tobacco products is imperative to understanding its effect on cancer risk.

A recent large investigation evaluated the chemical composition of 43 different US brands of various types of smokeless tobacco sold between 2006
and 2007. Past chemical studies of smokeless tobacco tended to only review a few brands and types, so the significance of the review can be found in its inclusion of many brands for each major tobacco type across each price range. The major tobacco types included in this survey are moist and dry snuff, loose leaf, dissolvable, and plug. The methods used for composition analysis follow protocol presented by Health Canada, AOAC INTERNATIONAL, and LabStat, and were repeated three times to establish accuracy (Borgerding et al., 2012). The ingredients analyzed were, NNN, NAT, NNK, NAB, nitrite, metals (cadmium, lead, arsenic, nickel, chromium, chloride), and nicotine.

The mean total TSNA (NNN, NAT, NAB, NNK) concentrations for each US tobacco type were determined and were ranked from least to highest: dissolvable (399 ng of total TSNA / g of product), loose leaf (3350 ng/g), plug (8388 ng/g), moist snuff (9786 ng/g), and dry snuff (14,768 ng/g). Some individual brand’s total TSNA concentrations reached upwards of 40,000 ng/g. Even though dry snuff in the tested brands had the highest mean concentration, the range was broad and some brands were significantly lower than others coming in as low as 1750 ng/g. In all of the US tobacco types, with the exception of dissolvable tobacco, NNN had the highest recorded mean concentration for individual TSNAs. Conversely, NAB had the lowest mean concentration across all tobacco types (Borgerding et al., 2012).

Similar to the TSNA results, dry snuff and moist snuff had the highest and second highest mean concentration of toxic metals among US smokeless
tobacco products at 768 ng/g and 5239 ng/g, respectively. Loose leaf tobacco had the lowest value in this category. When breaking down the total toxic metal concentrations into individual metal concentration, mean concentrations did vary wildly among product types and brands. For instance, while moist snuff had the highest mean concentration of arsenic (214 ng/g), chromium recorded higher in dry snuff (2838 ng/g). The US snus brands had the lowest mean total metal concentrations in their respective dry and moist snuff varieties (Borgerding et al., 2012).

Although not directly carcinogenic, nitrite and nicotine values are important statistics to consider. The concentration pattern continues with nitrite, exhibiting overall higher concentrations in moist and dry snuff when compared to other varieties. As mentioned earlier, nitrite acts as a nitrating agent and can form TSNAs during the processing and storage of tobacco, thereby increasing carcinogenicity. The high nitrite concentrations observed in the snuff products indicate future rises in total TSNA concentrations throughout the product life. Mean nicotine concentrations, representing the element responsible for the addictive property of tobacco, ranged from 6.0 to 15.8 mg/g. The more bioavailable “free nicotine” made up between 0.5% and 36.0% of the total nicotine concentration in smokeless tobacco products. Moist snuff made up the largest portion of smokeless tobacco products at the top end of “free nicotine” concentrations, ranging all the way up to 68.0% (Borgerding et al., 2012). Table 1 summarizes the findings for several of the categories discussed.
**Table 1**: Smokeless tobacco component levels determined in US brands (Borgerding et al., 2012).

<table>
<thead>
<tr>
<th>Tobacco Type</th>
<th>Products Tested</th>
<th>Total TSNA (ng/g)</th>
<th>NNN (ng/g)</th>
<th>NNK (ng/g)</th>
<th>Nitrite (µg/g)</th>
<th>&quot;FreeNicotine&quot; (% of Total Nicotine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolvable</td>
<td>2</td>
<td>399</td>
<td>107</td>
<td>&lt;114</td>
<td>4.9</td>
<td>16.1</td>
</tr>
<tr>
<td>Loose Leaf</td>
<td>7</td>
<td>3350</td>
<td>1798</td>
<td>523</td>
<td>5.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Plug</td>
<td>1</td>
<td>8388</td>
<td>5053</td>
<td>1230</td>
<td>6.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Moist Snuff</td>
<td>23</td>
<td>9786</td>
<td>4058</td>
<td>1394</td>
<td>113.5</td>
<td>36</td>
</tr>
<tr>
<td>Dry Snuff</td>
<td>10</td>
<td>14,768</td>
<td>5535</td>
<td>2522</td>
<td>9.3</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Unlike the previous study, the aim of the analysis on smokeless tobacco carcinogen levels by Stepanov and colleagues was to compare levels in newer products to traditional brands. Two relatively new US snus brands (Camel Snus and Marlboro Snus), a dry snuff (Skoal Dry), and a snus-like product named Taboka. All of these newer products are sold as pouches placed around gums, and do not require spitting, mirroring the delivery method of Swedish products. Different flavors of these brands were analyzed and compared against popular, traditional moist snuff as well as a Swedish snus product. TSNA, nitrate/nitrite, and PAH concentrations were determined by gas (TSNA) and ion (nitrate/nitrite, PAH) chromatography (Stepanov et al., 2008). Chemical extractions were performed before analysis and followed a protocol of tobacco homogenization, high speed centrifugation, and chemical washes (Stepanov et al., 2005).

Results show that all of the newer tobacco products analyzed had lower NNN values than traditional moist snuff products with exclusion of Marlboro Snus.
Mint and Skoal Dry. This particular mint snus had a NNN concentration similar to traditional moist snuff at 3.28 µg of NNN per gram of tobacco. NNK concentrations were lower in all of the new tobacco products when compared to the traditional moist snuff products; Taboka exhibited the lowest level among all the new products. Overall, the total TSNA concentration found in the new products was lower than the traditional counterparts in comparison with Skoal dry having the highest. The nitrite levels in the newer products were ten times lower than traditional products, lowered from 0.030 mg of nitrite per gram of tobacco to 0.0030 mg/g. Similarly, nitrate was also determined to be lower but by only a factor of three. Observed polycyclic aromatic hydrocarbon (PAH) concentration followed the trend and was significantly lower in all newer products (Stepanov et al., 2005).

Sweden

Unique in chemical and physical composition for a moist snuff, Swedish snus displays a significantly different carcinogenic profile than the traditional smokeless tobacco types found in the American market. Manufacturing procedures and practices account for the distinct ingredient values and proportions. Due in part because of strict production protocol found in the Swedish smokeless tobacco industry to be discussed later, data revealing snus’ profile is readily available.
A particular study reviewing the composition of smokeless tobacco products around the global market was performed at the Centers for Disease Control’s (CDC) Tobacco Analysis Laboratory in 2011. While the laboratory reviewed a total of 53 smokeless tobacco products from several different World Health Organization (WHO) regions, five popular Swedish snus products were extensively evaluated. Chemical profiles which included the composition, nicotine quantification, and TSNA level of each product were created through the use of spectroscopy, gas chromatography, and mass spectrometry (Stanfill et al., 2011).

**Table 2:** Swedish Snus component levels found in 5 popular brands (Stanfill et al., 2011).

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Mean Total TSNA (ng/g)</th>
<th>Mean NNK (ng/g)</th>
<th>Mean NNN (ng/g)</th>
<th>Mean “Free” Nicotine (% of Total Nicotine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Original Snus</td>
<td>723</td>
<td>96.4</td>
<td>345</td>
<td>8.98</td>
</tr>
<tr>
<td>General Loose Snus</td>
<td>652</td>
<td>105</td>
<td>293</td>
<td>3.77</td>
</tr>
<tr>
<td>General White Wintergreen Snus</td>
<td>601</td>
<td>89.8</td>
<td>267</td>
<td>10.0</td>
</tr>
<tr>
<td>General White Portion Snus</td>
<td>648</td>
<td>96.8</td>
<td>296</td>
<td>6.48</td>
</tr>
<tr>
<td>Catch Peppermint Snus</td>
<td>630</td>
<td>84.5</td>
<td>295</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Study results display a pH of the Swedish snus products ranging from 6.61 to 6.86. Paired with nicotine levels between 7.76 and 15.2 mg/g, the
determined percent of bioavailable free nicotine in the Swedish snus products stretched from 3.77% up to 13.3%. These percentages vary significantly from and are much lower than the 36% average found for the 23 American moist snuff products discussed previously. Differences in this value could explain any possible differences in the addictive potential of Swedish snus when compared to their American counterparts (Stanfill et al., 2011).

Stanfill’s study of Swedish snus TSNA values focused on the specific N-nitrosamines NNK, NAT, NNN, and NNAL. The ranges of each TSNA in the five tested snus products were quite small, as can be seen in Table 2. The highest total TSNA value recorded was from the General Original Snus sample at 723 ng/g of tobacco. As was seen with the comparison between Swedish snus and American moist snuff free nicotine percentages, even the snus sample with the highest total TSNA was significantly lower than the American total TSNA average of 9786 ng/g. The values for NNK and NNN found in Swedish snus follows this trend and also display significantly lower values. Nitrite values were not studied in this experiment and thus not available for comparison (Stanfill et al., 2011). It is important to note that this study only reviewed 5 snus products (numerous samples of each), 4 of which came from the same manufacturer, so a larger sample size could provide even stronger evidence.
India

The carcinogenic profile of gutkha is extremely difficult to assess due to the wide variety of types seen and lack of manufacturing regulation. The Stanfill review provides a great example of how much carcinogen presence can vary in the gutkha market. The total TSNA range (in ng TSNA per gram of product) stretched remarkably from 83.9 all the way up to an astounding 23,900 ng TSNA/g (Stanfill et al., 2011). This incredibly high TSNA value was seen in one of the handmade varieties of gutkha; other handmade gutkha products had only slightly lower total TSNA values than this extreme value. Disregarding high-TSNA count handmade gutkha, most Indian gutkha TSNA levels closely resembled Swedish snus values. This low TSNA value for the highly carcinogenic gutkha products can be attributed to the low percent of tobacco used in them. Betel-quid presence decreases the “ng TSNA per g product” value via dilution.

An additional problem with establishing gutkha’s carcinogenic profile is quantifying levels of the betel-quid carcinogens. When gutkha is analyzed with other smokeless tobacco products, only common compounds are included in the metrics (nicotine, TSNA, etc.) This leaves betel quid-specific carcinogens such as arecoline and areca nut-specific N-nitrosamines, which are not found in most other smokeless tobacco products, too often ignored. Relative risk determinations for gutkha and related cancers may be the only effective current method to quantify gutkha’s carcinogenic potential.
Epidemiology of Smokeless Tobacco Use

United States

As smokeless tobacco use was first recognized as a public health problem in the United States in the mid-1980’s, this epidemiological analysis starts by looking at a review of data from that period and beyond (Nelson et al., 2006). Adult trends were gathered from the National Health Interview Surveys from 1987, 1991, 1992, 1994, 1998, and 2000. Each year had anywhere between 98,000 and 128,000 adult participants. As defined by this survey, users were “those who had used either chewing tobacco or snuff 20 or more times in their life and who reported that they were now current users of either product” (Nelson et al., 2006).

Adult smokeless tobacco use had a steady decline between 1987 and 2000 with an average annual percentage point change of -0.08. Assessing men specifically shows a -0.14 percentage point change. The only demographic subgroup that displayed increased smokeless tobacco use over this period and had a positive annual percentage point change (+ 0.02) included users between the ages of 25 and 44 years. Overall, the decrease in adult use approached a total of 26% over the study period (Nelson et al., 2006).

Nelson et al. also analyzed the adolescent user trend during this period by using The National Institute for Drug Abuse’s Monitoring the Future (MTF) survey. The MTF survey looked at 8th, 10th, and 12th grade students’ smokeless tobacco use. A range of 130 to 150 schools were evaluated annually; both
private and public institutions were considered. Questionnaires for 12\textsuperscript{th} graders were available from the years 1986 to 1989, and 1992 to 2003. Similar questionnaires were available for 8\textsuperscript{th} and 10\textsuperscript{th} graders from 1991 to 2003. Also investigated was the CDC’s Youth Risk Behavior Survey (YRBS), given across the nation, from the years 1991 to 1993, 1995 to 1999, and 2001 to 2003. In both the MTF and YRBS, smokeless tobacco use was defined as any adolescent who had used either chewing tobacco or snuff once or more in the past 30 days (Nelson et al., 2006).

By exploring the MTF surveys, the overall average annual percentage point changes of 8\textsuperscript{th}, 10\textsuperscript{th}, and 12\textsuperscript{th} graders from the early 1990s was calculated at -0.36, -0.38, and -0.24, respectively. Since adolescent girl smokeless tobacco use is very low and unchanged over this period, focusing at changes in adolescent boy use proved to be much more significant. Boys in the 8\textsuperscript{th}, 10\textsuperscript{th}, and 12\textsuperscript{th} grade had an average annual percentage point change of -0.62, -0.74, and -0.50, in that order. The MTF surveys show a relative decrease of use of 48% for 8\textsuperscript{th} and 10\textsuperscript{th} graders, and 43% for 12\textsuperscript{th} grade boys. In comparison, the YRBS displayed an average annual percentage point change of -0.38 among adolescents. Again, when focusing on adolescent boys only, this value increased to -0.76; overall, this represents a decrease in adolescent use of 43% (Nelson et al. 2006).

One study of smokeless tobacco use determined the trends from 2000 until 2010 by also using the National Health Interview Surveys, however the
researchers used more current versions of the surveys (2000, 2005, and 2010). The survey includes questions asked regarding both chewing tobacco and snuff products, specifically on whether those surveyed had tried either product, and if so, how frequently they used either. While the survey participants included adults of all ages, in this study specific focus was given to the young adult demographic with the age range from 18 to 44 years as this group is seen to be particularly vulnerable to smokeless tobacco exposure. A total of 86,270 adults participated across all three surveys (Bhattacharyya, 2012).

Results indicate that the number of adults who had ever tried chewing tobacco had increased from 7.1% to 9.2% over the study period. Similarly, those who tried snuff also rose from 4.4% to 8.4%. While the percent of adults who were frequent users of chewing tobacco was relatively unchanged over this ten year period, the percent of frequent snuff users significantly increased from 1.4% to 2.0%. This resembles around 2.52 million and 4.34 million adult chewing tobacco and snuff users in 2010, respectively (Bhattacharyya, 2012).

When focus is shifted to the 18 to 44 year young adult subgroup, the number of participants who had tried chewing tobacco escalated from 8.4% to 9.9%, while with snuff products the rise was from 5.7 to 11.8%. Not unlike the adult demographic, frequent chewing tobacco use among young adults showed no change; however, snuff use changed significantly from 1.8% to 2.8%. Therefore, in 2010, approximately 1.52 million young adults used chewing tobacco and 2.89 million used snuff (Bhattacharyya, 2012).
This study also evaluated risk factors for smokeless tobacco use, finding that being male, non-Hispanic, Caucasian, and a non-graduate of high school increased the risk of using smokeless chewing tobacco products. Conversely, being older and having a higher family income were associated with decreased risk of use. The same risk factors apply to snuff (Bhattacharyya, 2012).

While the previously reviewed studies provide a broad perspective on smokeless tobacco use in the United States, trends vary significantly when comparing one state to another. Using data from the 2009 Behavioral Risk Factor Surveillance System, the CDC evaluated the state-specific prevalence of smokeless tobacco use among adults. According to the CDC, the Behavioral Risk Factor Surveillance System is a

“state-based, landline telephone survey of non-institutionalized adults conducted annually in all 50 states, the District of Columbia (DC), Guam, Puerto Rico, and USVI [the U.S. Virgin Islands].”

The survey included 432,607 adults with a 52% response rate when including those eligible who did not finish the survey or were not contacted (CDC, 2010).

The results of the CDC evaluation exhibited a wide range of smokeless tobacco use among the states and territories included, from 0.8% in the USVI to 9.1% in Wyoming. Alongside Wyoming, West Virginia (8.5%) and Mississippi (7.5%) had the highest observed use. On the opposite end of the spectrum with the USVI were California (1.3%), DC (1.5%), and Massachusetts/Rhode Island (1.5% each). Figure 4 provides a graphic of smokeless tobacco prevalence
among the states and clearly shows significant use in the southeastern, midwestern, and northwestern regions (CDC, 2010).

**Figure 4**: Prevalence of smokeless tobacco use among adults, by state (CDC, 2010).

In all states and territories, males used smokeless tobacco products more than females. Smokeless tobacco use was most prevalent in those males between the ages of 18 and 24 years who had less than a high school education, a similar finding to the earlier study. Across all included areas, the range of use among those with less than a high school education was 0.6% to 14.2%. Those in the upper tier of education (college degree or further) displayed a prevalence of 0.9% to 6.1% (CDC, 2010).
Sweden

Use of snus in Sweden has increased dramatically since its surge in the late 1960s, overtaking cigarette sales around 1996, but it has been around since the beginning of the 1800s (Rutqvist et al., 2011). The replacement of smoking with moist snuff (snus) has led to this change in snus use. Overall use of tobacco (smoking and smokeless) was stable from 1986 to 1999 even with the use of cigarettes dropping 9%; this was countered by a simultaneous 8% increase in snus use (Rodu et al., 2002). In fact, smokeless tobacco is so popular in Sweden that it has the highest consumption of snuff as a country, primarily of the snus variety (Luo et al., 2007).

The 2012 National Public Health report offered the most useful data on snus use in the Swedish population. Figure 5 shows a breakdown of daily snus use, surveyed at the end of four different decades, for different age groups and gender. The highest prevalence of snus use for men is seen in the 25-44 years age range, with use at 31% (Danielsson, Gilljam, & Hemstrom, 2012). Adolescent males (16-24 years) have the next highest percentage of snus use with 26.5%. Unlike their male counterpart, adolescent females use snus the most for their gender at just under 5%. For all men between the ages of 16 and 84, 21% use snus daily. (Danielsson et al., 2012).
India

Unfortunately, prevalence studies for smokeless tobacco and gutkha usage are difficult to establish. For one, response rates and participation across India in smokeless tobacco surveys are significantly low. Additionally, determining the incidence of Areca nut and gutkha use separately is difficult as different studies vary in their classification of both (Gupta et al., 2002). Nonetheless, regional epidemiological studies on smokeless tobacco and gutkha use do provide a perspective of prevalence.

Worldwide, it is estimated that approximately 10 to 20% of the population uses Areca nut of some kind. A study of the inhabitants in Bombay, India, revealed a 32.1% prevalence rate for gutkha usage. In that same population, the use of Areca nut or betel quid without tobacco was considerably lower at a 0.5% rate. Among those included in the study, the prevalence of gutkha use was higher in men than women at a rate of 34.5% to 27.2% (Gupta et al., 2002).

Figure 5: Daily snus use of males and females from different decades (Danielsson et al., 2012)
Using some earlier epidemiological surveys on gutkha use, comparisons can be drawn between rural populations and those populations living in an urban environment. A survey given to the inhabitants of the rural Bahavnagar District in Gujarat, India, detailed tobacco and areca-nut use. A total of 3124 surveyed male villagers (27.7%) in this district had chewing habits of either areca-nut or tobacco, mixed or alone. The majority of males (20.4%), however, used the ingredients simultaneously in various forms of gutkha. The same could not be said about the surveyed female villagers; only 11.7% chewed areca-nut or tobacco and all except 0.1% were only dry snuff users (Gupta et al., 1998).

Surveyed inhabitants of Indian urban areas provided results that paralleled those of the rural regions with respect to male habits, but not with regards to females. In Trivandrum, a city in the Kerala state, men and women of low socioeconomic status were asked about areca nut and tobacco chewing habits. The survey shows 26.8% of the 25,453 male participants and 26.4% of the 34,441 female responders admitted to having gutkha habits (Sankaranarayanan et al., 2000). Urban participants of lower socioeconomic status have more similarities with rural inhabitants than wealthier urban groups, so perhaps this is why male gutkha habits for both regions emulate one another. There was no explanation discussed for the seemingly high gutkha habit prevalence in the female urban population.

Currently, gutkha is being heavily marketed towards the Indian youth population; the effectiveness of these campaigns is observed in the study of a
small Indian fishing village. In this location, 27.4% of 5 to 20 year old boys use gutkha. As seen in the epidemiologic studies in the United States, education level also seems to play a role in determining those at risk for smokeless tobacco usage in India, with less use associated with higher education levels. A survey of 1200 Indian college students in the Maharashtra state revealed 9.6% of the participants used gutkha, a significantly less than the rate found in the Bombay study (Hans, 1998).

Smokeless Associated Cancer Risk

Now that smokeless tobacco trends and carcinogenicity have been established, the next logical step is to review studies determining the cancer risk associated with using the different regional products. Proposed carcinogenic mechanisms may explain how cancers may be instigated, but do not necessarily explain how often. Moreover, comparing dose dependent cancer risk profiles for each smokeless tobacco type with their respective user habits may reveal how safely the products are being used.

United States

The largest cancer study examined was a meta-analysis of cancer risk estimates associated with smokeless tobacco. The authors reviewed decades of epidemiological studies for past and present North American and Swedish products. A total of 89 studies were included in the meta-analysis, with a wide
variety of cancer types covered, and only those studies performed within the regions analyzed (North America and Europe) were included (Lee and Hamling, 2009). When appropriate, relative risk (RR) estimates were presented for never-smokers if studies provided such information. For each cancer variety, RR estimates were combined from the associated studies and an overall RR estimate was calculated. Any value noted as “adjusted for smoking” means that the influence of smoked tobacco to the RR calculated was removed from the calculation. Methods for performing this adjustment vary so smoked tobacco influence may be over or under estimated.

Disregarding the Swedish studies involving snus for future review, the results of the meta-analysis can be seen on Table 3. Smokeless tobacco use shows a significant RR elevation for oropharyngeal cancer in the US, along with several other cancer groupings. The very high RR value of 3.33 for oropharyngeal cancer among participants who have never smoked was determined much higher than the overall RR calculated for this variety of cancer, but only 5 studies contributed to the never-smoked RR (Lee and Hamling, 2009). Once adjusted for smoking, the relative risks for oropharyngeal, esophageal, and larynx cancer associated with smokeless tobacco were 1.65, 1.89, and 2.01, respectively. Interestingly, when only including studies that focus on oropharyngeal cancer after the year 1990 (a total of 18 studies), the RR estimate drops from 1.65 to 1.28 (Lee and Hamling, 2009). A decrease of this magnitude
reflects the lower carcinogenic levels associated with modern era smokeless tobacco when compared to earlier products.

**Table 3**: Meta-analysis results of smokeless tobacco associated cancer risk in the United States. 'n' displays the number of study estimates used, 'RR' refers to relative risk, and ‘CI’ is the confidence interval (Lee and Hamling, 2009).

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Overall Data (n) RR (95% CI)</th>
<th>Smoking Adjusted (n) RR (95% CI)</th>
<th>Never Smokers (n) RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oropharyngeal</td>
<td>(31) 2.16</td>
<td>(12) 1.65</td>
<td>(5) 3.33</td>
</tr>
<tr>
<td>Esophageal</td>
<td>(6) 1.56</td>
<td>(3) 1.89</td>
<td>(3) 1.89</td>
</tr>
<tr>
<td>Laryngeal</td>
<td>(4) 1.56</td>
<td>(1) 2.01</td>
<td>-</td>
</tr>
</tbody>
</table>

While the large scope of this meta-analysis provides stronger RR estimates than an individual study, some conclusions are left to be desired. For example, relative few of the studies included in the meta-analysis reported on dose or frequency of smokeless tobacco use; frequency of exposure plays a critical role in carcinogenesis and would explain differing RRs. Additionally, each study varies in participant pool, exclusion and inclusion criteria, methods of assessment, and other parameters. Moreover, even though only chewing tobacco and snuff products were examined, many different brands were encountered. Lastly, some studies did not specify whether or not the RR estimates were determined from current or former users of smokeless tobacco (using old or current products). All weaknesses aside, Lee and Hamling concluded that “the consistency of these findings suggest that smokeless
tobacco may increase the risk of cancer” although this risk is “clearly very much less than that from smoking.”

Another meta-analysis considered, authored by Weitkunant, Sanders, and Lee combined the results of 32 separate studies. In comparison, the selection of studies was much more specified than those seen in Lee and Hamling’s meta-analysis with extensive inclusion and exclusion criteria. Also, the analysis only included cancer risk assessments for the most common of oral cancers, squamous-cell carcinoma.

The fifteen studies included in the meta-analysis focusing on the US region displayed a mean RR of 1.76 for oral cancer from smokeless tobacco use. Ten of these studies adjusted for smoking, and with those intrusive variables calculated out, the resulting mean RR was 1.39 (Weitkunat, Sanders, and Lee, 2007). As seen in the adjusted smoking RR calculated in the Lee meta-analysis, oral cancer (oropharyngeal cancer) RR decreased with the influence of smoking removed. The analysis concludes that

“at most a minor increased risk of oral cancer [is] associated with the use of a wide range of currently used Western chewing tobacco and snuff” (Weitkunat, Sanders, and Lee, 2007).

Though the meta-analysis provides a good estimate for oral cancer risk, changes could strengthen the results. For one, studies from as early as the 1920s met the inclusion criteria and were included even though they do not
reflect modern day risk. Also, the number of RR estimates adjusted for smoking was limited.

**Sweden**

Epidemiological data reviewed displayed a relatively high percentage of snus use in the Swedish population compared to smokeless tobacco use in the United States. Fortunately, for research purposes, the popularity of snus is matched by an increase in research on its effects on health, specifically regarding oral malignancies. Several of these studies were reviewed to determine the magnitude of risk involved with snus use.

The objective of a study by Roosar et al. was to calculate the cancer incidence in male users of snus. In this cohort study, 9,976 total participants were selected in 1973; the males were all from Uppsala, Sweden, and needed to be above the age of 15. Individuals with previous cancers were excluded, as were women, since little snus use was observed in their population (Roosar et al., 2008). Upon follow-up in 2002, participants either were placed in “never daily use” or “ever daily use” groups for snus use. Participant incidences of cancer and death (from cancer or other factors) were recorded. Factors such as alcohol and smoking tobacco were adjusted for.

Collected results showed that 9% of participants were ever daily users of snus, 53% were ever daily smokers, and 7% were ever daily users of both. There was no significant increase of any cancer incidence in ever daily users of
snus (RR of 0.99) when compared to never daily users, even when adjusted for smoking and alcohol (Roosar et al., 2008). When focusing only on smoking-related cancers, such as those in the oral cavity, lungs, and larynx, there was only a minor 1.1 RR for snus users (not adjusted for smoking). Only with focus particularly on oropharyngeal cancers was a real increase of risk noted; this had a RR value of 3.1 among users of snus (Roosar et al., 2008). For the overall mortality of snus users, the RR for death was merely 1.10, a slim elevation (adjusted for smoking). These RR measures argue against the popular claim that snus is a healthy alternative to smoking and that the results are “inconsistent with claims that the use of [Swedish] moist snus is without demonstrable risk” (Roosar et al., 2008). However, older participants may have been exposed to more of the earlier, high-concentration TSNA products than younger participants, possibly altering the calculated RR from the modern day RR.

A second cohort review studied Swedish construction workers, men who had a high prevalence of snus exposure, and established a RR for oral cancer. The study began in 1978 and continued until the final follow-up in 1992. For the 279,897 participants, information on snus use, grams used daily, smoking status, and grams of smoking used daily was gathered (Luo et al., 2007). RR associated for snus use was adjusted for smoking when necessary.

A total of 31% of the participants had or were currently using snus, a slightly higher percentage than that established for all Swedish men. Of the 125,576 participants who had no history of smoking tobacco use, there were 60
oral cancer incidences (Luo et al., 2007). Surprisingly, the RR estimated for oral cancer from snus use was 0.8, less than participants who had never used any tobacco product. Ten of the 60 oral cancer incidences were from snus users, the other 50 incidences represented users who had never used tobacco. An increase of RR, albeit a slight increase, would be expected. Of the 154,321 ever smokers, the RR associated with smoking and oral cancer was 2.0 (198 cases) (Luo et al., 2007). The lack of an increase of oral cancer RR for snus users conflicts with the previous studies results. Perhaps the lack of variation in the participant pool is partly responsible for this unusual end result.

**India**

The first study reviewed oropharyngeal cancer cases from three separate hospital centers in Nagpur, a central Indian city. A total of 123 cases of oropharyngeal cancer were included when determining RR estimates associated with tobacco. For the study participants, tobacco habits were recorded which revealed dose, frequency of use, composition of tobacco mix used, and other related metrics. Most of the participants were male (59%) and between the ages of 41 and 60 (67%) (Wasnik et al., 1998). A large percentage (42.3%) of the cancer patients used gutkha, and over 61% of the participants used tobacco products 6 or more times a day. The largest percentage of patients had been using tobacco chewing products for 20 and 40 years (Wasnik et al., 1998).
Overall, analysis of the data generated a 7.98 RR for oropharyngeal cancer associated with chewing tobacco use. For comparison, a 2.25 RR was found for smoking tobacco use (Wasnik et al., 1998). Probing into the individual RR for chewing tobacco use, the RR generated with only gutkha use was 8.25; for betel nut alone, an 1.85 RR was established. As expected, an increase in either the daily frequency of use or lifetime duration of use was matched with an increase in RR for oropharyngeal cancer. These RR estimates as a whole provide evidence that gutkha use significantly increases your risk for oral cancer (Wasnik et al., 1998).

A weakness in this study, and with most gutkha studies, is the wide variation in gutkha composition used by participants. The lack of standardization makes it difficult to make claims regarding the risks of gutkha as a whole if a standard product is nonexistent.

The other study used information gathered from the Bhopal Cancer Registry database out of Bhopal, India. A total of 247 oropharyngeal cancer cases and 148 oral cavity cancer cases were evaluated based on tobacco habits and history. In regards to oropharyngeal cancer, the RR associated with gutkha use was 1.1, and 1.2 when adjusted for smoking (Dikshit and Kanhere, 2000). A much more significant increase in RR was found when reviewing the oral cavity cancer cases. The RR for this cancer type when associated with gutkha was 5.5, and 5.8 when adjusted for smoking (Dikshit and Kanhere, 2000). Daily-dosage and lifetime exposure to gutkha both greatly influenced the RR of both cancers.
involved. For instance, if gutkha was only chewed between 1 to 5 times daily, a 0.5 RR for oropharyngeal cancer and a 2.0 RR for oral cancer was estimated. However, at the extreme level with gutkha chewed over 10 times a day, these values saw an increase to 3.6 and 13.9, respectively (Dikshit and Kanhere, 2000). Similar trends were seen with length of gutkha habit (in years), with much higher RRs estimated for longer gutkha habits.

Discussion and Conclusion

Clearly, the use of smokeless tobacco is embedded in the cultures of many differing global regions. While the presence of smokeless tobacco is common, the variety of the smokeless tobacco product and associated public health impact vary considerably. Together, the differing products, health consequences, and usage trends seen in India, Sweden, and North America demonstrate the relative safety of each region’s smokeless tobacco environment.

When comparing the carcinogenic profiles of each region’s associated smokeless tobacco product, there seems to be a direct association between production standards and carcinogen exposure. The carcinogenic levels shown for TSNA concentration in gutkha products vary wildly and reached extraordinarily high levels, reflecting its disordered manufacturing. While commercial gutkha products are available in many Indian districts, much gutkha is of the homemade variety through the combination of betel quid and chewing tobacco. Therefore, the amount of tobacco used (and consequently the amount
of TSNA) is dependent on individual desires. Smokeless tobacco products in the United States, on the other hand, show more consistency in the TSNA levels. While particular smokeless tobacco types vary in carcinogen levels (for instance, a mean of 9786 ng/g total TSNA in moist snuff compared to 3350 ng/g in loose leaf), the individual brands of each type are reasonably similar in constituent concentrations (Borgerding et al., 2012). However, Sweden displayed the most stable mean total TSNA values in their snus as well as the lowest, both a result of the strict manufacturing policies in place.

While snus has been around for decades, only since 1971 has snus manufacturing been regulated under the Swedish Food Act (Rutqvist et al., 2011). Known since its conception that year as the GothiaTek® standard, Swedish snus production follows stringent protocol and quality control. Included in the protocol, heating the snus was initially performed to eliminate an earlier microbial problem; lower TSNA values are responsible for the continued use of the heating process. To this day, the GothiaTek® standard (current standard set in 1990) also determines maximum acceptable TSNA levels, criteria for natural material selection, and the required information to be displayed to consumers (Rutqvist et al., 2011). Consequently, the Tobacco Regulatory Committee of the World Health Organization has determined that Swedish snus has one of the lowest TSNA concentrations on the global smokeless tobacco market (World Health Organization Study Group on Tobacco Production Regulation, 2009).
While gutkha and American smokeless tobacco products both fall under the authority of their respective government’s Food and Drug Administration, this authority is comparatively young when compared to Sweden’s (Swedish Food Act) by several decades. If both the United States and India adopt stricter manufacturing guidelines for domestic smokeless tobacco manufacturers to reflect the GothiaTek® standard, more products may exhibit the TSNA levels seen in snus. Perhaps this change would lower the RR for oral cancers associated with gutkha and American products similar to Swedish snus.

With reference to the RR for oral cancers, the reviewed cancer studies clearly demonstrated that the highest association between smokeless tobacco use and the development of oral cancer was observed with gutkha products. The RR associated with gutkha use was greater than that of US smokeless tobacco products by a factor between 3 and 4, and even more so when compared to the RR associated with Swedish snus. The difficulty encountered when interpreting the results for the RR associated with gutkha use is determining which constituents are most carcinogenically significant, the TSNAs or the betel quid. This difficulty is compounded when factoring in how unique the constituent blends can be among gutkha products. While the RR for oral cancer associated with pure betel quid use was mentioned in one of the studies (1.85), it is unknown whether or not areca nut and tobacco carcinogens interact with one another, possibly increasing (or decreasing) carcinogenicity (Wasnik et al., 1998).
The extremely low (and possibly nonexistent) increase in the RR for oral cancers associated with Swedish snus was particularly interesting. TSNA presence in snus would typically point toward an increase in carcinogenicity, and thus a rise in RR. In order to further explore the specific carcinogenicity of Swedish snus, a toxicology study was reviewed.

The early section discussing the TSNA’s carcinogenic mechanisms may have explained how the TSNA found in tobacco products cause damage that may lead to eventual tumorigenesis, but an investigative approach to the toxicology of Swedish snus provides conflicting results in the laboratory of Coggins and colleagues. The purpose of the study was to determine if Swedish snus was active in four popular in vitro toxicology assays that are used to predict and determine the carcinogenicity of a substance. The products tested included three popular Swedish snus brands and a moist snuff reference. The four assays used were: The Ames assay (S. typhimurium reverse-mutation assay), the mouse lymphoma assay (MLA), an in vitro micronucleus assay (MNAvit), and the neutral red uptake (NRU) assay. Each sample was formed and extracted (500 mg of product per mL of extract) and further diluted to be tested. The consistency of concentrations was determined by testing the nicotine content of each extract (Coggins et al., 2012).

The Ames assay tests for the mutagenic and carcinogenic potential of a sample by determining whether or not the sample can revert a mutated S. typhimurium strain back to its natural state. If a compound exhibits carcinogenic
characteristics, the strain will show signs of reverting (Mortelmans and Zeigar, 2000). Numerous concentrations of each extract were tested on petri dishes, with results measured in revertant ("reverted") colonies. The presence of revertant colonies shows mutagenic potential; more colonies present are associated with a substance having a higher mutagenic potential. Of all the extract concentrations analyzed, only the two highest concentrations provided results of significant levels, concentrations which were “well above those suggested by regulatory guidelines” for snus use and not found commercially (Coggins et al., 2012). The three other assays (the MLA, MNAvit, and NRU) displayed similar results concerning the toxicity of snus.

With the exception of snus extract concentration levels far exceeding commercial levels, no significant positive results were demonstrated, suggesting that snus is not significantly carcinogenic. On the other hand, the moist snuff reference demonstrated significant positive results in both the Ames assay and MLA, and a positive control confirmed the accuracy of the assays used. Although interpretation of positive assay results vary among those in the field, the Coggins’ study claims Swedish snus to be less carcinogenic than traditional moist snuff and significantly less carcinogenic than cigarettes (Coggins et al., 2012).

Even so, the carcinogenic potential of these regions’ products only affect those who use them. Keeping up with the previous trend, gutkha use is highest with its respective region’s population, followed by snus in Sweden and then
lastly the smokeless tobacco products in the United States. The research reviewed earlier revealed gutkha use by males in India ranged from around 20% to just over 30%, and slightly lower for females (Gupta et al., 2002). Male snus use in Sweden is comparable to the lower range of male gutkha use in India, but the female population in Sweden uses smokeless tobacco products far less than in India. Smokeless tobacco use in the United States is significantly lower than either the Indian or Swedish population, not even surpassing a 10% user base in any state (CDC, 2010).

The varying cultures and perspectives on smokeless tobacco account for the differences in the percentage of smokeless tobacco users. As stated earlier, gutkha use is deeply engrained in many Indian societies’ traditions which may explain the large user base. However, as information on the detrimental effects of gutkha spreads, the Indian government has begun banning its sale. For instance, the Indian state of Maharashtra had areca nut products banned from the market in 2002, including gutkha (Sharan et al., 2012). Unfortunately, the public circumvents these restrictions by purchasing the constituents separately, sometimes from surrounding states, and making their own gutkha products.

In the United States, cigarettes remain to this day the preferred method of tobacco consumption. The slight rise in smokeless tobacco use seen in the United States (and simultaneous slight decrease in smoking tobacco use) is partially a result of the tobacco industry encouraging the use of smokeless tobacco where cigarettes and cigars have been recently banned (Bhattacharyya,
The shift from smoking to smokeless tobacco may continue if restrictions on smoking tobacco tighten further and the tobacco industry increases the marketing of snuff (Richter et al., 2008).

In Sweden, the belief of snus as a “healthier” alternative to other tobacco options, especially cigarettes, holds much of the responsibility for the increase in snus users over the decades (Roosar et al., 2008). With the low TSNA concentrations found in snus products and comparative RR for oral cancers between snus and smoking tobacco, snus seems to be less harmful. To distinguish snus as a “healthy” alternative seems misleading, though. TSNAs are carcinogens no matter their concentration, and no matter how slight the increase in RR for cancer is found to be, the risk is higher than if tobacco was not consumed at all.

Although none of the smokeless tobacco products studied can be considered safe, each region differs in the severity and potential danger of their smokeless tobacco habits. With the highest associated RR for oral cancer, most carcinogens, and highest percentage of users, gutkha has a significant detrimental effect on the public health of India. Luckily, recent actions are being taken to address this issue. Swedish snus may appear much safer than gutkha, particularly at the chemical level, but the large male user population is still exposing itself to unnecessary toxins that are linked to oral cancer. The tendency to refer to snus as a healthy tobacco option has to change. Finally in the United States, where the imprint of smokeless tobacco products may
currently be small, recent trends provide a glimpse at a future problem if snuff use continues to increase. Measures must be taken to discourage the use of any tobacco products, including snuff, to avoid usage trends presently seen in Sweden and India.

**Future Research**

Most of the cancer risk studies reviewed were long-term cohort studies, spanning several decades of smokeless tobacco use. As TSNA levels in smokeless tobacco products have decreased drastically over this same time period, some of the cancer cases recorded will be a consequence of the earlier, more carcinogenic products. Future studies should try to include only those users who have used the more modern products which will reflect the current market better.

Additionally, it would be interesting to test the toxicology of Indian gutkha products with different constituents removed. By running Ames (and similar) tests on a gutkha product, once with the areca nut ingredients removed and once with the tobacco ingredients removed, it may be possible to compare the carcinogenicity of individual constituents in a single product.
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