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# Tobacco Carcinogen Research to Aid Understanding of Cancer Risk and Influence Policy

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Education regarding the health effects associated with tobacco use has made important progress worldwide over the last few decades. Still, tobacco remains a significant cause of cancer and other diseases. As a result, significant worldwide morbidity and mortality is still attributable to tobacco use in modern times. Research into tobacco products, the carcinogens they contain, and how users metabolize them is an important benefit to the advancement of research aimed at reducing harm associated with tobacco use. This review summarizes the use of this type of research to study tobacco users' risk of developing cancer, especially head and neck cancer. In addition, we discuss the use of tobacco research to provide support for increasing levels of federal regulation of tobacco products.

**Key Words:** Head and neck cancer, tobacco, carcinogen, nitrosamines, DNA adduct, Level of Evidence.
**Level of Evidence:** 4.

## INTRODUCTION

Tobacco has been used in its various forms around the world for many years. In the last 50 years, the true scope of health problems associated with tobacco use has come to light and prompted strong efforts in research, consumer education, and regulation. While much progress has been made, there continues to be widespread use of tobacco on a global scale, with some regions of the world demonstrating extremely high levels of associated morbidity and mortality. In this review, we will describe data derived from a specific line of tobacco-related research in which the study of tobacco-related carcinogens can be used to inform regulatory policies as well as the risk of cancer in users of tobacco products.

## BACKGROUND AND EPIDEMIOLOGY

### *History of Tobacco Use*

Tobacco plants are believed to have been growing in the Americas as far back as 6000 BC. The first mention of tobacco dates to the time of Christopher Columbus. Upon landing in North America, he was greeted with fruits, spears, and tobacco as gifts by the indigenous population. He noted that the tobacco leaves had a pungent smell and were thrown overboard. In that period, dried tobacco leaves were prized possession among the native population who often bartered with them and bestowed them as gifts. Tobacco was then introduced into Europe, and in the mid-1600s, it was introduced to Africa. Over the next 200 years, tobacco products experienced significant spread across the world.[1] By 1890, approximately 4 billion cigarettes were sold per year, increasing to over 70 billion in the year of 1924 in the US alone.[1]

Over the long history of tobacco production, many different forms of tobacco have been developed. Largely, they include combustible and smokeless tobacco products. According to the IARC monograph, the different forms of combustible products used all over the world include cigarettes, cigars, cigarillos, bidis, chutta, kretek, and many others.[2] Similarly, there are various tobacco products used as a noncombustible/smokeless form including betel quid with tobacco, chimo, chewing tobacco, creamy snuff, gudhaku, gul, gutka, areca nut, iqmik, khaini, khiwam, loose leaf, maras, mawa, moist snuff, naswar, red tooth powder, shammah, toombak, tuibur, and zarda.[2] There is significant worldwide geographic variation in tobacco product preferences. For example, smokeless tobacco is more prevalent in South Asian countries such as India. According to the GATS 2 survey, every fifth adult in India uses smokeless tobacco.[3] This number of users in India is equivalent to the entire US adult population. In contrast, in the US, cigarettes are the most popular form of tobacco used. Forty million American adults smoke cigarettes and 4.7 million middle and high school students use at least one tobacco product, including e-cigarettes. Tobacco use in various forms is implicated in the



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death of nearly 500,000 Americans and 7 million persons worldwide each year.[4]

## HISTORY OF TOBACCO FROM MEDICINAL HERB TO FULL-FLEDGED CARCINOGEN

In the 1500s, tobacco was used as a medicinal herb. It was believed to have properties that were antidiarrheal, narcotic, emollient, and pain relieving. It was used in powdered form and applied locally to heal burns and ulcers. As tobacco use became more widespread, reservations were expressed by some about its abuse. Franciscan monk Andre Thevet in Brazil warned that smoking of such leaves caused fainting and weakness which was well supported by Conrad, a botanist, physician, and scientist.[5] Scientific evidence regarding the dangers of tobacco began to accumulate in 1791 when a British doctor reported cases in which tobacco snuff caused nasal cancers. The foundation for modern understanding and regulation of tobacco products was released in 1964 via the Surgeon General's Report on Smoking and Health. Based on more than 7000 articles relating to smoking and disease already available at that time in the biomedical literature, the Advisory Committee assembled to create the report concluded that cigarette smoking is: 1) a cause of lung cancer and laryngeal cancer in men, 2) a probable cause of lung cancer in women, and 3) the most important cause of chronic bronchitis in addition to noting the linkage with emphysema and heart disease.[6] This report was a critical catalyst for action in tobacco research that has transpired over the ensuing 50 to 60 years.

## TOBACCO-RELATED CARCINOGENS

Over 5300 compounds have been identified in tobacco smoke. Of those, there are over 70 known carcinogens which have been described in cigarette smoke.[7] One approach to understand the risk of cancer among tobacco users is through the investigation of tobacco carcinogens and their metabolites. Of those toxic chemicals present in tobacco products and tobacco smoke, polycyclic aromatic hydrocarbons (PAH), tobacco-specific nitrosamines (TSNA), aromatic amines, aldehydes, and certain volatile organics likely contribute significantly to the carcinogenic activity. PAH and TSNA are the most heavily studied tobacco-related carcinogens.[8] They are found in both smoked as well as smokeless tobacco. In fact, much of the formation of carcinogenic compounds occurs during curing and processing. Seven TSNA have been identified in tobacco products: NNN, NNK, NNAL, NAB, NAT, iso-NNAL, and iso-NNAC. NNN and NNAL are the most potent, well-studied nitrosamines. NNAL is the metabolic product of NNK that is formed after a carbonyl reduction. It has been demonstrated in animal studies that NNAL is strongly associated with lung cancer regardless of its mode of administration.[9] NNN reproducibly induces head and neck tumors. This was demonstrated by subcutaneous administration of NNN in rats, leading to nasal tumors. When administrated through drinking water or liquid diet, NNN administration resulted in oral, esophageal, and nasal tumors.[10]

An animal study investigated the carcinogenic effects of NNN enantiomers.[11] In this study, it was demonstrated that S-NNN, when administered through drinking water, resulted in significantly higher rates of oral cavity tumors and esophageal tumors as compared to those receiving R-NNN. The S-NNN group had 4.5 oral cavity tumors per rat as compared to 0.25 per rat in those receiving the R enantiomer. The incidence of tumor development much higher when racemic mixture (S-NNN + R-NNN) was used (8 oral cavity tumors per rat). Thus, this study concluded that S-NNN is a powerful oral cavity carcinogen. Given that S-NNN is the predominant enantiomer in smokeless products, this data is especially concerning for smokeless tobacco users. The study also showed that, although R-NNN is not as potent a carcinogen alone, it appears to have synergistic co-carcinogenic effects with S-NNN.

PAH represent a diverse group of carcinogens which share multiple benzene rings and have demonstrated carcinogenic potential in animal studies.[2] BaP(benzo[*a*] pyrene) and 1-HOP serve as commonly used representatives of the group of compounds that make up PAH. Administration of BaP results in tumors of the stomach and/or colorectal tract.[12] Still, there is a general lack of PAH data pertaining to levels in head and neck cancer patients. Thus, we aim to add to the knowledge base regarding this particular carcinogen and its relationship with HNSCC.

## STUDY OF TOBACCO CARCINOGENS IN HNSCC

Two large prospective cohorts, the Shanghai Cohort Study and the Singapore Chinese Health Study have provided epidemiological data on the use of NNAL and NNN to predict the risk of lung and esophageal cancer, respectively. The Shanghai cohort consisted of 18,244 men enrolled from 1986 to 1989 who were between 45 and 64 years of age.[13] In the Singapore Chinese health study, 63,257 Chinese men and women were enrolled between 1993 and 1998.[14] These subjects were between 45 to 74 years of age. Each subject in both cohorts was interviewed using a structured questionnaire. Yuan et al. conducted a nested case control study on the data produced by these two prospective studies. A relationship was demonstrated between urinary NNAL levels and the development of lung cancer. It was concluded that, after adjusting for self-reporting smoking history, smokers in their highest tertiles of urinary total NNAL and cotinine exhibited 8.5-fold increased risk of lung cancer relative to smokers with comparable smoking history.[15] A similar study lead by Yuan et al. evaluated the role of NNN in the development of esophageal cancer.[13] Data from the Shanghai cohort was also analyzed and, after adjusting for smoking intensity and duration, found odds ratios of developing esophageal cancer in the second and third tertiles were 3.99 (1.25–12.7) and 17 (3.99–72.08), respectively. This, in combination with levels of glucuronidated carcinogens, suggests that both increased exposure and reduced detoxification are important consideration in determining tobacco-induced cancer risk.

The Singapore and Shanghai cohorts were successful in providing prospective epidemiological data associating TSNA with lung and esophageal cancer. To extend this

approach to HNSCC, a small matched control pilot study was conducted to understand the association between tobacco-related carcinogens and HNSCC.[16] In this study, urinary levels of 1-HOP, NNN, and NNAL were measured in smokers with a new diagnosis of HNSCC and compared to smokers without cancer. Levels of 1-HOP and NNN were elevated in the smokers with HNSCC compared to controls matched on multiple variables including age, gender, and self-reported cigarettes used per day. This suggested a difference in intrinsic carcinogen exposure among those smokers who went on to develop HNSCC. This was result in patients with head and neck disease suggested themes similar to that seen by Yuan et al. in studies of lung and esophageal cancer.

While the above data strongly suggest that tobacco is a prime etiological factor for several types of cancer, not all individuals exposed to tobacco will develop cancer. It is hypothesized that there is a complex interaction of exposures and predisposition which include genetic factors, carcinogen metabolism, excretion, and immunologic status that results in some individuals progressing along the carcinogenesis pathway while others are exposed to carcinogens but do not develop cancer. Biotransformation, detoxification, and elimination of carcinogens, together with DNA repair mechanisms and apoptotic pathways are the most important mechanisms of defense against carcinogenesis. One mechanism by which carcinogens exert toxic effects is through the binding of DNA to form DNA adducts. DNA adducts are formed when cancer causing agents bind and disrupt the double helical DNA structure. If left unrepaired, permanent mutation can occur. When binding and mutation occurs at critical genes (ie, tumor suppressor), the mutations can result in carcinoma. The formation of DNA adducts is central to the process of chemical carcinogenesis initiated by nitrosamines. DNA adducts are created by downstream byproducts following metabolic activation of TSNA. Binding of DNA releases HPB (4-hydroxy-1-[3-pyridyl]-1-butanone) which can be measured to quantify the level of adduct formation.

Our group has studied DNA adduct formation in the oral cavity among smokers with HNSCC.[17] We sought to understand whether levels of adduct formation may serve as an indicator of which smokers are at the highest risk of cancer, independent of carcinogen exposure. The study included 30 smokers with HNSCC and 35 cancer-free smokers who submitted buccal cell samples while still actively smoking. Univariate analysis of urinary NNAL and NNN, 1-HOP, cotinine, 3-hydroxycotinine/cotinine ratio (the nicotine metabolite ratio) were performed but did not reveal any statistically significant differences between groups. However, we did find significantly higher DNA adduct formation in the HSSCC cases. Multivariate analysis showed that smokers with HNSCC had significantly higher levels of DNA adduct formation as compared to controls when adjusted for multiple confounders. Thus, in spite of having similar exposures of nicotine and tobacco carcinogen exposures, our smokers with head and neck cancer demonstrate higher levels of DNA adduct formation. This information suggests that some smokers are more susceptible to carcinogens in tobacco products than others as manifested by higher DNA adduct formation. This could be related to inherent differences in adduct formation, DNA repair or a combination of both. Thus, measurement of these DNA adducts may play a role in the future by providing information on risk of HNSCC in smokers. The method to quantify this HPB-releasing DNA adduct was developed by members of our team and will be used in future studies of this type.[18] We eventually hope to use it this method as a screening tool to identify those who require the most intense efforts at tobacco cessation due to their extreme risk of HNSCC.

## USING TOBACCO CARCINOGENS TO STUDY EXPOSURE

The study of tobacco carcinogens can also be used to study tobacco exposure (as opposed to cancer risk). With regard to HNSCC, the relationship between self-reported tobacco use and the level of urinary tobacco carcinogen metabolites has been studied in a sample of patients.[19] This work examined the relationship between patient-reported smoking levels and the urinary carcinogen levels in smokers with HNSCC. The study found that, although it is the most commonly used assessment of tobacco exposure in the clinical setting, self-reported tobacco use does not predict actual carcinogen exposure. Instead, urinary cotinine levels correlate strongly with urinary carcinogen levels. Thus it was suggested that urinary cotinine be used in those cases where a more accurate exposure to tobacco is required (such as a preoperative assessment of wound healing capacity).[20] Finally, 1-HOP levels in this study were significantly associated with total NNN and total NNAL suggesting that smokers are exposed to these carcinogens proportionally.[19]

## USING TOBACCO CARCINOGEN RESEARCH TO INFORM PUBLIC POLICY

By 2020, it is estimated that the cancers most closely associated with exposure to tobacco will kill approximately 650 Americans every day, and 7800 people per day globally.[21] There are more than one billion active tobacco abusers in the world. Tobacco products sold in various parts of the world vary significantly in yields of nicotine and carcinogen level. Efforts aimed at banning some products have achieved a measure of success in some areas but less so in other areas of the world, including the United States where bans have not taken hold. Harm reduction, on the other hand, is another effective strategy for decreasing the impact of tobacco product use. This can be implemented through the regulation of tobacco product content which aims to reduce a user's exposure to carcinogenic material. Therefore, there is a need for regulatory policies to guide control of the carcinogen content in products and reduce the availability of these products which increase the risk of a variety of disease, including cancer.

With the passage of the Family Smoking Prevention and Tobacco Control Act, the Food and Drug Administration (FDA) now has the authority to establish product standards. This created a need for data that could help to inform standards of product content. A study evaluating varying levels of nicotine and TSNA in ST products as

well as patterns of use, demographic, and tobacco history to understand the extent of exposure to these carcinogens was recently performed.[22] ST users of brands varying in nicotine and TSNA content were recruited from three different regions in the US. A total of 391 subjects were recruited with sample data collected and analyzed on 359 subjects. Participants underwent two assessment sessions. During these sessions, demographic and ST use history information along with urine samples were collected to assess biomarkers of exposure and effect. During the time between assessments, ST users recorded the amount and duration of ST use on a daily basis using their diary cards. The study results revealed that levels of TSNA in ST products, independent of smokeless tobacco nicotine yields, played a significant role in carcinogen exposure levels. Thus, this work provided evidence for the FDA and other regulatory bodies that product standards for reducing levels of TSNA in ST products are necessary to decrease exposure to these toxicants and potentially to reduce risk for cancer.

In some cases, the tobacco industry has purported to initiate harm reduction through development of harm reducing products. One example of this is "snus", a smokeless product marketed as a safer alternative to standard tobacco. Existing epidemiological data suggests that the exclusive use of Swedish moist snuff (snus) is associated with a lower risk of cancer.[23] This is thought to be due to snus containing lower levels of TSNA. Based on this evidence, smokers have been encouraged to swap to the Swedish-type low nitrosamines snus to aid in harm reduction.[24] In India, a smokeless product known as Chaini Khaini is marketed as a snus equivalent. A study of Chaini Khaini was performed to determine its actual carcinogen content and whether it is similar to snus.[25] Stepanov et al. analyzed TSNA, nicotine, and unprotonated nicotine in samples of this product purchased in India. The authors demonstrated that Chaini Khaini actually had high levels of carcinogenic TSNA, contrary to its marketing. For purposes of comparison, the levels of TSNA were found to be second only to Sudanese toombak when considering Chaini Khaini in the context of other global smokeless products.[25] Data of this type has the potential to influence future policy efforts. In addition, it can be used to help educate current and potential users of this product.

In large part due to research similar to (and including) that described above, the FDA has recently taken steps to regulate the level of nitrosamines in smokeless tobacco products. The FDA has proposed a tobacco product standard that would establish a limit of NNN in finished smokeless tobacco products.[26] This important first step is a testament to the value of studying tobacco carcinogens in a variety of products. Based on the volume of literature documenting NNN levels in these products, the FDA found that regulating such content of smokeless tobacco would be appropriate in an effort to improve public health. The FDA has estimated that over the next 20 years following implementation of the proposed product standard, approximately 12,700 new cases of oral cancer and approximately 2200 oral cancer deaths would be averted in the US. Moreover, during that 20-year period, FDA estimates that approximately 15,200 life-years would be gained as a result of the proposed standard. To help further these regulatory efforts, research must continue to address tobacco products and the level of toxic constituents contained in them. For our part, this is reflected through continued work in this area in both the US and Asian countries (China, India) where tobacco use is highly prominent.

Possible results of regulation has been extensively discussed[27] to include:

1. Possible regulation of decreasing NNN and NNK levels should not lead to increase in levels of other harmful contents like PAH and etc.
2. All data summarized strongly suggest that a decrease in lung, oral cavity, and esophageal cancers although the exact time course of that decrease is difficult to predict due to multiple variables involved
3. Modified content in the new products should not encourage new users to start using under false the impression that they are safe while current users should still be encouraged to abstain. To accomplish this, post-marketing surveillance of regulated products is critical to ensure that the desired goals are being achieved.

In summary, tobacco use continues to be a prominent etiologic factor in the development of HNSCC. Because of this, efforts at reducing tobacco use and encouragement of cessation remain critical. In addition, the study of tobacco carcinogens in users and products has the ability to inform investigators about risk of cancer and relative danger associated with various products, respectively. Considering that worldwide tobacco use is associated with yearly mortality at levels of 10 times that seen with the collective nuclear explosions in Hiroshima and Nagasaki, these issues will remain an important focus of research in the effort to reduce harm associated with tobacco use.

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