



Grass roots approach to control levels of carcinogenic nitrosamines, NNN and NNK in smokeless tobacco products

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ABSTRACT

Due to the extensive ban on public smoking, tobacco companies focused their business on new smokeless tobacco (SLT) products promoting them as a harm reduction strategy and a safer alternative to cigarettes. Two nitrosamines, N'-nitrosanornicotine (NNN) and nicotine-derived nitrosamine ketone (NNK), present in SLT, listed as group 1 human carcinogens by the International Agency for Research on Cancer, are found to be the prime agents for most of cancers in SLT users. This review illustrates the mechanism of cancer induction by NNN and NNK in humans along with factors influencing the formation of NNN and NNK at various stages of tobacco manufacturing. It reveals the high levels and wide variations of NNN and NNK found among the diverse variety of SLT products sold worldwide. According to a recent report by FDA- Centre for Tobacco Products, reducing levels of nitrosamines in SLT products could greatly enhance the quality of life by reducing mortality, morbidity and medical expenditures due to cancer. For the first time, grass root approaches to minimize the levels of NNN and NNK in tobacco, from plant growth to the finished products, have been systematically compiled as they have the potential to contribute to reducing tobacco related disease burden.

1. Introduction

Smokeless Tobacco (SLT) is the prime cause of potentially preventable global morbidity and mortality from cancer (Siddiqi et al., 2015). With growing regulatory pressure against cigarettes, particularly in the form of public smoking bans, big cigarette companies have re-framed their business by investing in SLT and nicotine delivery systems such as e-cigarettes. (Tomar et al., 2009; Rani et al., 2017). Tobacco companies are misleading the people by marketing SLT as a safe alternative to smoking (Rodu and Godshall, 2006a), spending about \$ 451 million annually on advertising and promotion of SLT products (Trade Commission, 2013). SLT is unburned tobacco, available for oral and nasal use. Oral tobacco can be sucked, chewed, spit, swallowed, dipped, gargled or applied to the gums or teeth while fine tobacco mixtures are inhaled into the nostrils as snuff (Bethesda et al., 2014; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2007). Product innovations make the sale and use of SLT very convenient, especially among youth (Sinha et al., 2014; Sinha et al., 2017a). The appearance of new flavored spitless products, portioned in ready-to-use small trendy pouches, and with varying levels of additives, such as areca nut or tonka beans, has attracted young people for initiating tobacco (Kostygina and Ling, 2016; Stepanov et al., 2008).

Food and Drug Administration (FDA) registration and listing data states that there are approximately 800 SLT products available in the

market including more than 500 moist snuff products, about 50 snus products, more than 60 dry snuff products, and over 120 chewing tobacco products (Siddiqi et al., 2015). The prevalence of SLT use varies by gender, ethnicity, age, educational attainment, and employment status (Siddiqi et al., 2017). More than 356 million people in 140 countries across the world are found to use SLT (Sinha et al., 2017b)). A global youth tobacco survey in 126 countries between 1999 and 2012 indicated rise in SLT use in young children in one-third of countries, which is a crucial indicator of the evolution of the tobacco epidemic (Ridgway et al., 2015). Due to great diversity in product types and forms like use of household informants and traditional custom-made mixture (Sinha et al., 2017b; Gupta et al., 2016), SLT usage exceeds cigarette smoking among both men and women in South Asian region (Sinha et al., 2016; Ansara et al., 2013; Drope et al., 2018).

Our recent global surveillance report showed that SLT is responsible for about 650 000 deaths worldwide with the South-East Asian region bearing the major share of the burden of about 88% (Sinha et al., 2018) with the majority (74%) occurring in India (Sinha et al., 2014). An estimated 258 million people use oral tobacco products in South-East Asia as compared to an estimated 8.1 million people in the United States (Stanfill et al., 2011). This could be due to the low cost of manufacturing and lower taxation rates on SLT products (Yadav et al., 2018). Further, owing to the non-existence of manufacturing standards, most of the products marketed as safe substitutes for smoking are

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actually harmful (Stepanov et al., 2015; Hecht et al., 2007). A qualitative review published in Lancet Oncology covers epidemiological studies on SLT use and risk of oral, esophagus, pancreas, and lung cancers (Boffetta et al., 2008). A WHO report on ‘warning about the dangers of tobacco’ revealed that, at present, tobacco usage causes nearly 6 million deaths per year worldwide and will continue to cause more than 8 million deaths annually by 2030 (WHO, 2011).

Though, no form of tobacco is safe and tobacco cessation practice is the best solution to tackle the tobacco menace but unfortunately, nicotine addiction is very difficult to break, even for those with a strong desire to quit. With the long term goal of tobacco and nicotine free world, the approach of tobacco harm reduction has gained momentum in recent years with the sole purpose to reduce the death and disease burden due to tobacco use. It involves the use of alternative sources of nicotine, or use of novel SLT products having low levels of toxic nitrosamines. According to a recent report by FDA-Centre for Tobacco Products, reducing levels of carcinogenic nitrosamines in SLT could greatly enhance the quality of life by reducing mortality, morbidity and medical expenditures due to cancer (Bennett and Fowler, 2017).

This article reveals the high levels and wide variations of tobacco-specific nitrosamines, NNN and NNK, present among the diverse variety of SLT products currently sold worldwide and also illustrates the mechanism of their cancer induction in humans. The current review also discusses various factors influencing the formation of NNN and NNK at various stages of tobacco manufacturing. For the first time, grass root approaches to minimize the levels of NNN and NNK in tobacco from plant growth to the finished products have been systematically compiled, as they have the potential to contribute in reducing tobacco related disease burden.

2. Formation of tobacco-specific nitrosamines NNN and NNK and their cancer induction mechanism

Of the various TSNAs present in tobacco, two most dangerous nitrosamines, N'-nitrosornicotine (NNN) and nicotine-derived nitrosamine ketone (NNK) also called 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone have been identified and listed as group 1 human carcinogens by the International Agency for Research on Cancer (IARC) (Hecht, 2003; International Agency for Research on Cancer, 2012). Alkaloids, nicotine and nornicotine, are the key chemical precursors in the formation of NNN and NNK (Burton et al., 1992) (Fig. 1).

Freshly harvested tobacco contains negligible amount of TSNAs, however, they are mainly formed at the late yellowing and early

browning stage of tobacco leaves during final curing. NNN is formed by the nitrosation of alkaloid, nornicotine with the loss of a methyl group during curing of tobacco leaves. NNK is primarily formed through nitrosation of nicotine during the later stages of tobacco processing like curing and fermentation (Burton et al., 1992). The nitrosating agent is usually nitrite which is derived from the reduction of leaf nitrate by the action of microbes starting during curing. Reaction of nitrite with nicotine and nornicotine in the process of nitrosation produces NNN and NNK, respectively (Hecht, 1998). Substantial evidence supports site-specific concordance of the carcinogenic effects of NNN and NNK in animal and human epidemiological studies (Balbo et al., 2013; Stepanov and Hecht, 2005). Metabolically active nitrosamines induce deleterious mutations in oncogenes and tumor suppression genes by forming DNA adducts facilitating cancer cell growth, angiogenesis, migration, and invasion (Xue et al., 2014).

Various types of tobacco products, tobacco-related cancers and involvement of NNK and NNN in cancer have been recently reviewed by Xue and his coworkers (Xue et al., 2014). Binding of NNK and NNN to the nicotinic acetylcholine receptor (nAChR) promotes cancer cell proliferation by creating a micro environment for tumor growth (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2007). It is well established that NNN and NNK require cytochrome P-450 (CYP450) catalyzed metabolism to exert their carcinogenic effects (Hecht, 1998). Metabolic activation of NNN occurs by hydroxylation of the α -carbons to the nitroso moiety whereas hydroxylation of NNK occurs at the α -methyl or α -methylene carbon positions, resulting in the production of highly reactive pyridyloxobutyl (POB) diazonium ions and related species (Hecht et al., 2016) (Fig. 2). The NNN molecule has a chiral centre at 2' position, leading to two enantiomers (S)-NNN and (R)-NNN (Stepanov et al., 2013; Zhao et al., 2013). Generated reactive oxygen species (ROS) further react with DNA to form adducts that cause miscoding mutations, thereby initiating the carcinogenic process. If these mutations occur in crucial regions of specific genes, such as the RAS oncogene or the P53 tumor suppressor gene, the result can be the loss of mechanisms of healthy cellular growth control, and ultimately the development of cancer (Warnakulasuriya and Ralhan, 2007; Schick et al., 2017). Metabolism of (S)-NNN leads to more total adduct formation than does (R)-NNN in human systems, making it a powerful oral cavity carcinogen (Zarth et al., 2016).

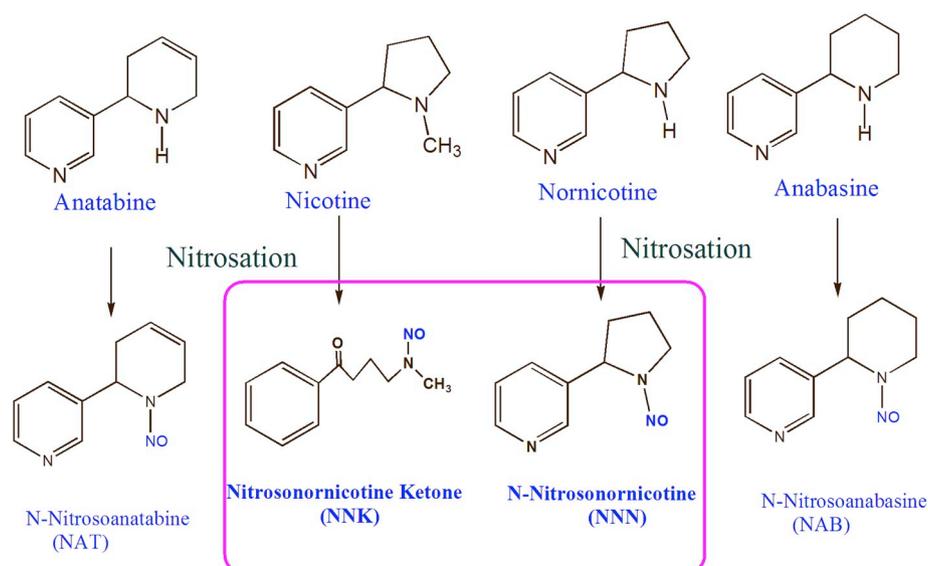


Fig. 1. Tobacco-specific carcinogenic nitrosamines (TSNAs) present in smokeless tobacco.

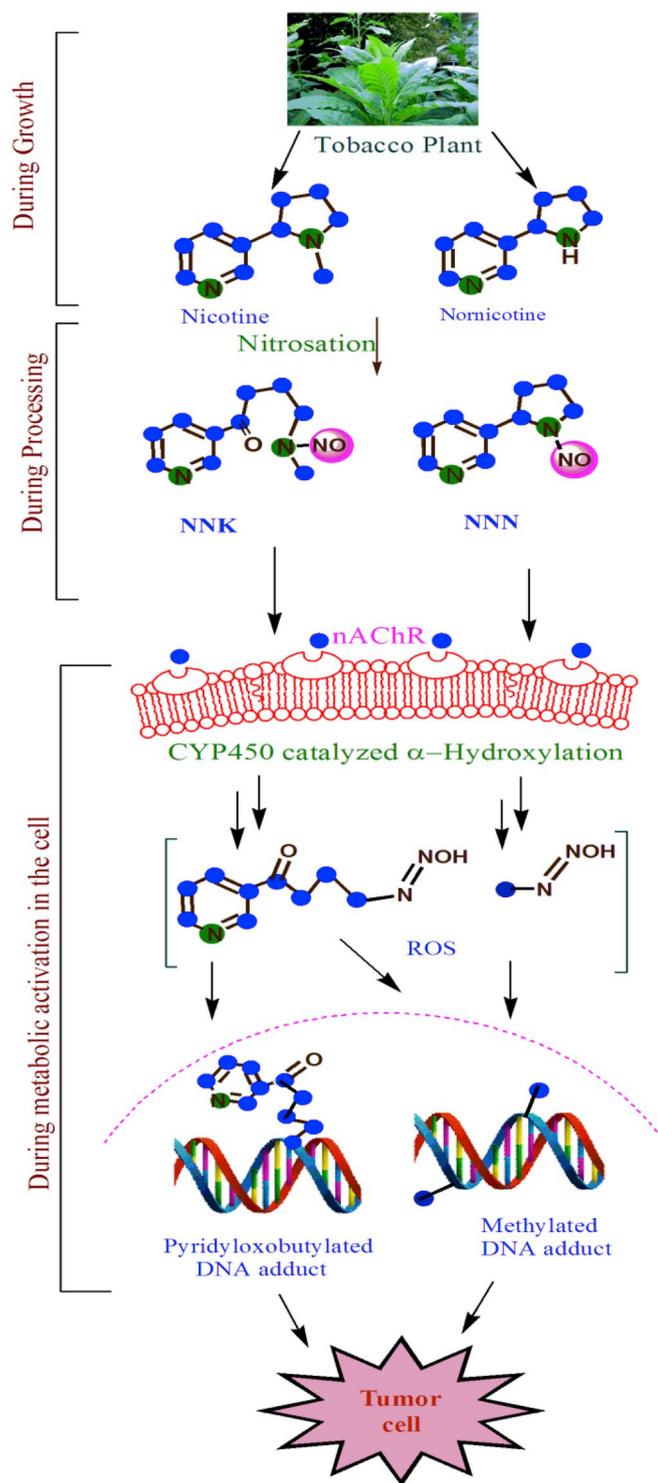


Fig. 2. Carcinogenic mechanism by N'-nitrosornicotine (NNN) and Nicotine-derived nitrosamine ketone (NNK). In the figure above, the role of Acetylcholine receptor (nAChR), reactive oxygen species (ROS) and Cytochrome P450 enzymes (CYP450) are shown.

3. Variation in NNN and NNK levels in SLT products sold worldwide: current scenario

It is well established now that NNK and NNN are the prime agents for oral, esophagus and pancreatic cancers in SLT users (International Agency for Research on Cancer, 2012). Difference in the magnitude of cancer risks due to SLT use across regions of the world has been found

to correlate highly with variation in the levels of NNN and NNK present in smokeless products (Hatsukami et al., 2015; WHO Technical Report Series 955, 2010).

Seeing the carcinogenicity of NNN and NNK, third report of WHO Study Group On Tobacco Product Regulation, 2010 recommended the regulatory limit for maximal total concentration of NNN and NNK as less than $2 \mu\text{g/g}$ dry weight of tobacco (WHO Technical Report Series 955, 2010). A comprehensive analysis of 53 international tobacco product samples from 9 countries in 2011 showed wide variation in NNN and NNK levels depending on product type, patterns of use across the world (Stanfill et al., 2011). Though, a downward trend is seen in NNN and NNK levels in cigarettes over the past 10 years (Appleton et al., 2013), a very recent global surveillance across 113 countries from 5 WHO regions indicates high levels of carcinogenic TSNA levels present in diverse oral SLT tobacco products (Siddiqi et al., 2015). IARC Monograph on the "Evaluation Of Carcinogenic Risks To Humans", 2007 compiled concentration data of TSNA in the mainstream smoke of commercial cigarettes and SLT products sold across the world. [7] International comparison of the TSNA levels per unit dose showed that levels of NNN and NNK are much higher in SLT products as compared to the cigarette smoke. Mainstream cigarette smoke contains NNK and NNN in the range of $0.006\text{--}1.74 \mu\text{g/g}$ and $0.004\text{--}2.83 \mu\text{g/g}$, respectively worldwide. However, diverse SLT products sold across the world showed the levels of NNK between 0.019 and $7870 \mu\text{g/g}$ and NNN in between 0.080 and $3080 \mu\text{g/g}$.

Fig. 3 represents comparative data on levels of NNN and NNK in selected popular SLT products available worldwide along with the recommended permissible limits ($\mu\text{g/g}$ dry weight). Maximum concentrations of NNN and NNK content for Toombak product from Sudan are found to be 3085 and $7870 \mu\text{g/g}$ respectively (not shown in Fig. 3) which are extremely higher than most of the products sold worldwide (Ahmed and Mahgoob, 2007). Dry zarda and khaini sold in India also contain alarmingly high levels of NNN and NNK (Stanfill et al., 2011). This could be the reason that in India and Sudan, more than 50% of oral cancers are attributable to SLT products use (Bhisey, 2012). Chewing tobacco with betel quid and areca nuts along with reverse smoking accounts for high incidence of oral cancer in India and Sri Lanka (Niaz et al., 2017). Apart from Toombak, average value for NNN ranged from 0.09 to $76.9 \mu\text{g/g}$; while, NNK value ranged from 0.04 to $28.4 \mu\text{g/g}$ in selected SLT products (Fig. 3). Moist snuff sold in US has maximum levels of NNN and NNK as 42.55 and $9.95 \mu\text{g/g}$, respectively which are very much outside the permissible levels (Richter et al., 2008). Thus, as seen in Fig. 3, an almost 70-fold variation is seen in NNN and NNK content among brands across the world. Prevalence of SLT use in North Africa, Central and Western Asia, Latin America and Caribbean is generally very low as compared to cigarette smoking, so very limited data of SLT products in that region is available regarding the levels of nitrosamines (Sreeramareddy and Pradhan, 2015). However, SLT products sold in this region include imported U.S. moist snuff (Mexico), chimó (Venezuela), and rapé (Brazil) (Keith et al., 2017). High levels of NNN and NNK are reported in chimó which is a paste-like extract from cooked tobacco leaves, mixed with sodium bicarbonate and flavorings such as brown sugar, vanilla (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2007; Stanfill et al., 2011) (Fig. 3).

For the protection of the public health, recently for the first time FDA has proposed a tobacco product standard rule, which states that the mean level of NNN in any batch of finished SLT product should not exceed $1.0 \mu\text{g/g}$ of tobacco (on a dry weight basis) at any time through the product's labelled expiration date (Berman and Hatsukami, 2018). Though, a US based survey showed a general decrease in NNN levels ($0.64\text{--}12.0 \mu\text{g/g}$) in some novel SLT products in US market from 2004 to 2015 (Ammann et al., 2016), however, the concentrations of NNN and NNK in majority of popular SLT products sold worldwide continue to exceed the permissible limits (Hecht, 2014). It is noteworthy that recommended mean level of sum NNN and NNK of less than $2.0 \mu\text{g/gm}$ in finished SLT product is achievable using technology available to the

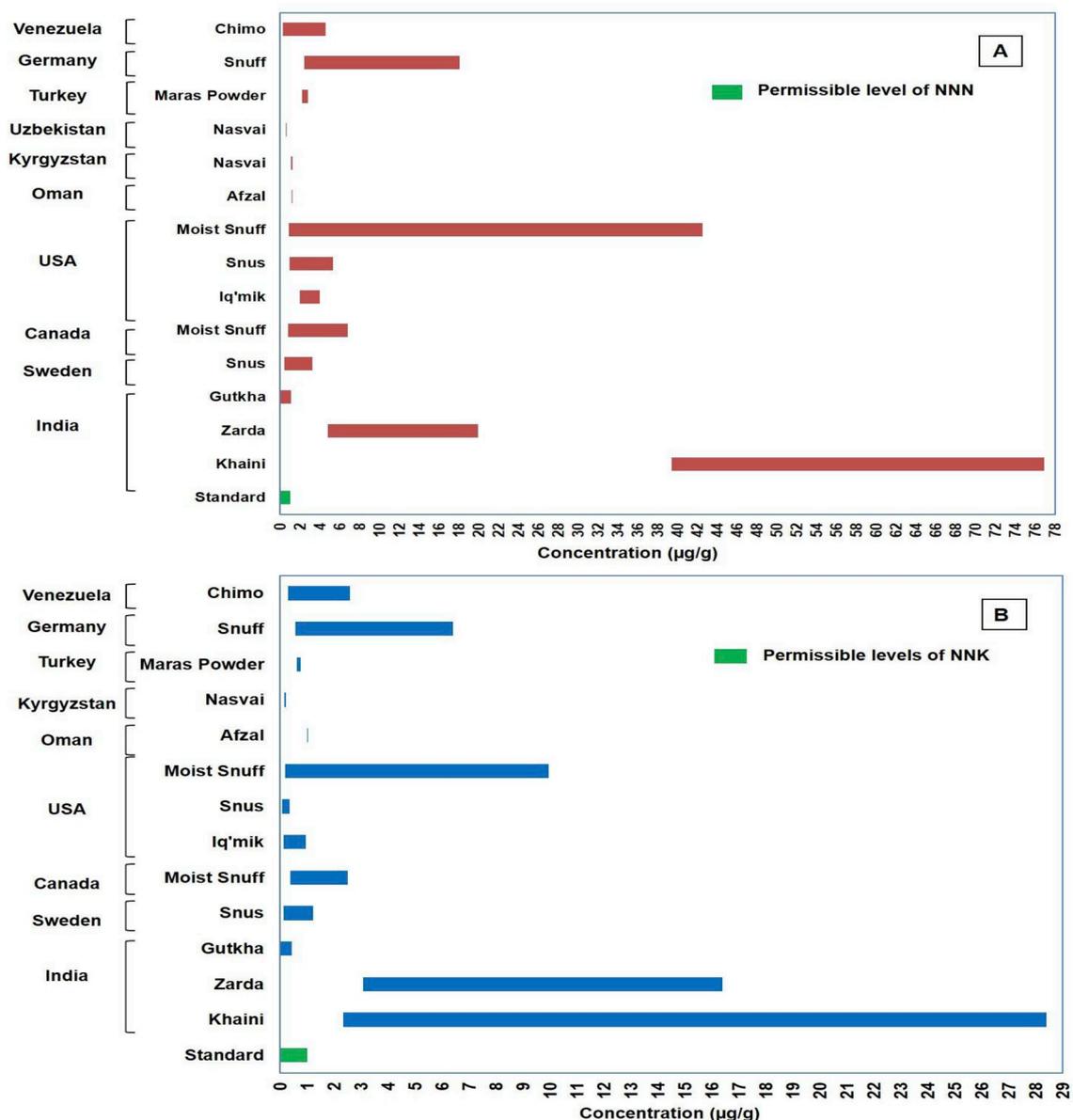


Fig. 3. Comparative levels of carcinogenic nitrosamines, NNN(A) and NNK(B) present in selected smokeless tobacco products (SLT) available worldwide. NNN and NNK levels for Toombak are not shown here. (Ref: WHO-FCTC Report on Global smokeless tobacco control policies and their implementation, 2017, NICPR, Noida).

major tobacco companies in the world, yet they do not wish to apply it to their popular SLT brands (Osterdahl et al., 2004; Hecht et al., 2011).

4. Factors influencing formation of NNN and NNK at various stages and their harm reduction measures

Levels of NNN and NNK in tobacco are governed by numerous factors like plant genetics, growth factors, cultivation practices, processing, blending and storage conditions (Niaz et al., 2017). In Sweden, careful manipulation of these factors has led to a decrease in the levels of carcinogenic nitrosamines by about 85% over a 20-year period (Gray et al., 2008; Djordjevic et al., 1993a; Borgerding et al., 2012). But NNN values for most US SLT brands ranged from 3.76 to 6.86 as compared to 1.66 for Swedish snus which has lowest NNN content than any other SLT product in the world (Osterdahl and Slorach, 1983). A nine member panel of medical experts from the Delphi group assessed the mortality risks of low-nitrosamine SLT products like snus and concluded that such products are associated with at least a 90% reduction in the risk associated with smoking cigarettes (Levy et al., 2004;

Stepanov et al., 2006). The regulatory environment around harm reduction using comparatively safer nicotine alternatives has been continuously evolving across the globe. (Fagerström and Bridgman, 2014) However, it is important that the approach should not increase population prevalence of tobacco dependence or increase the risk of adolescent misuse or abuse. Any promotion or marketing efforts of this approach should provide consistent tobacco cessation messages and eventually help to quit smoking and eliminate SLT use.

In the following section, factors affecting the formation of nitrosamines in tobacco have been discussed and how the use of modified agricultural, production, manufacturing and storage practices can potentially reduce the level of harmful carcinogens, NNN and NNK in SLT products.

- **During Growth:** Concentration of NNK and NNN in tobacco is found to depend on production year, climate, country of origin, agricultural practices including seed selection and nitrogen fertilization regimes (Lewis, 2018).
 - a. *Tobacco variety type:* Levels of NNN and NNK are higher in Burley

- and dark flue-cured tobacco type than the bright tobacco variety due to the presence of high amount of specific alkaloid precursors (Burton et al., 1994). Difference in NNN and NNK levels among varieties are mainly due to their differential capacities in absorbing, reducing, and assimilating nitrate. *Nicotiana tabacum* and *Nicotiana rustica* tobacco species contain high levels of NNK and NNN in green leaves. So Bright and flue-cured Virginia tobacco varieties of tobacco are to be grown.
- b. **Seed type:** Seed lots of commercial viable tobacco varieties like Burley, generally have high conversion, thus have potential for high TSNA formation. So, selecting low converter Burley tobacco seed varieties i.e. having low propensity of conversion of nicotine to normicotine from one generation to another will help (Gunduz et al., 2016). Apart from naturally occurring low nicotine tobacco variety (flue-cured), genetic engineering approaches can be used to develop new tobacco varieties with improved trait combinations and reduced nicotine levels in cured tobacco leaves (Lewis, 2018).
 - c. **Weather:** Levels of NNN and NNK in tobacco plants can vary from inter-year, intra-year within a farm depending on weather conditions like rainfall and temperature (Burton et al., 1994). TSNA levels increase with increasing maturity at harvest time mainly due to accumulation of more alkaloids in the later harvested tobacco (Fisher et al., 2012).
 - d. **Fertilizer:** Higher NNN and NNK levels are found in tobacco crops which are fertilized with nitrogen rich fertilizers (Chamberlain and Chortyk, 1992). During tobacco growth, more nitrogen is incorporated in tobacco leaves in the form of nitrate (Hoffmann et al., 1979). Burley tobacco requires more nitrogenous fertilizer to achieve an economically viable yield, thus high levels of nitrate accumulate in the leaves leading to increase the levels of TSNA in the cured product. Therefore, fertilizers with low nitrogen content must be used for growth of tobacco plants as a harm reduction measure.
- **Curing techniques:** During curing, humidity, air flow, temperature and the fuel used for heating the tobacco influence the extent to which the NNN level changes (Lusso et al., 2014). Studies have shown that curing of tobacco leaves by flue and fire-curing results in higher NNN levels than air-curing method. Due to higher temperature during curing, the biological and chemical reactions are enhanced (Staaft et al., 2005). Nitrogen oxide (NOx) gases formed by burning of wood increases nitrosation of tobacco alkaloids. Air curing method is commonly used by cigarette manufacturers but most SLT products are flue and fire-cured leading to high levels of NNN and NNK in them (Chamberlain and Chortyk, 1992). Therefore, methods of air or sun curing must be employed for SLT products too, for the reduction of nitrosamine content.
 - **During production:**
 - a. **Fermentation:** Fermentation is used to impart flavors to tobacco during production. Nitrate reducing bacteria like *Staphylococcus*, and *Cornu bacterium*, present in the leaves or in manufacturing environment from non-sterilized equipment surfaces, may contribute to the formation of NNN and NNK (Tyx et al., 2016). Some fungi and yeast population also allows more conversion of nicotine into normicotine, thereby increasing NNN and NNK content in tobacco (Di Giacomo et al., 2007; Han et al., 2016). Instead of fermentation, heat treatment or pasteurization can be implied to decrease or eliminate the population size of micro-organisms, including those that form and release nitrite. Seeding with non-nitrite-forming ‘starter cultures’ bacteria (Fisher et al., 2012), use of nitrite scavengers and cleaning or sterilizing equipments between fermentation batches are some other measures that need to be utilized (Rutqvist et al., 2011).
 - b. **pH control:** The pH of unprocessed tobacco is generally slightly acidic with less than 5% free nicotine. During SLT production, various alkaline agents like ammonia, ammonium carbonate are added to raise pH thus increasing the amount of free nicotine which is more easily absorbed but also a precursor for NNN formation (Bhisey, 2012). SLT products like Tabaka and Marlboro snus sold in US, have low pH values and thus have less free nicotine thus having reduced addictive and carcinogenic potential (Stepanov et al., 2008).
 - c. **Humectants:** Humectants such as glycerol and propylene glycol can be added to tobacco products to facilitate processing of the cured tobacco leaf, retain moisture with lower water activity which tends to thwart microbial growth and increase shelf life (Rainey et al., 2013; Rodgman, 2013).
 - d. **Additives:** Application of a nicotine reducing agent like glucose oxidase during growth or compounds like ferulic acid during curing helps in reducing nitrosamines levels (Fernando et al., 2013; Thomas et al., 2010). As bicarbonate and carbonate salts of sodium are more water soluble than traditional potassium or ammonium carbonate, use of their water solution facilitates the production (Rutqvist et al., 2011).
 - **Storage condition:** Humidity and high temperature play significant role in determining levels of NNN and NNK while storing tobacco (Wang et al., 2016).
 - a. **Tobacco leaves:** Tobacco leaves are often stored on farms for up to 3 months prior to sale to the tobacco product manufacturers who then usually store them for an additional 18 months before culminating in the final product (Shi et al., 2013). Contents of TSNA in Burley and sun-cured tobacco increases significantly by storing them in high temperature condition for one year under natural storage environment as their nitrate content is 10 times more than flue-cured tobacco. High water content along with insufficient air movement support micro-organism growth causing microbial enzymes to reduce more nitrates to nitrite, thus increasing NNN and NNK levels (Rutqvist et al., 2011).
 - b. **Finished SLT product:** As SLT products ‘age’, the water content changes, leading to bacterial growth (Rutqvist et al., 2011). Storing wet or high moisture SLT products at temperature 37 °C or above can markedly increase the levels of TSNA formation (Wang et al., 2017). Further, pH and nicotine content can decrease causing NNN and NNK levels to rise (Andersen et al., 1989). Enormous increase in the levels of NNN and NNK is seen in ambient air-exposed moist SLT products during storage at 24 °C for 52 weeks in comparison to the non exposed counterpart (Andersen et al., 1991).
 - c. **Packaging:** NNN and NNK levels increase in products maintained at 37 °C in individual tins rather than log wrapped for 24 weeks. Less dramatic increases were reported for 10-can plastic wrapped logs stored under the same conditions (Djordjevic et al., 1993b). Storage in large can logs at 37 °C and 85% relative humidity (RH) resulted in an increase of NNN and NNK. Product pack or pouch size also plays an important role in levels on NNN and NNK (Stepanov et al., 2012). Further, SLT products sold in larger ‘round tin version’ packs have higher moisture content than those of the “slim pack” versions (Richter et al., 2008).

Fig. 4 depicts the detailed measures to reduce the levels of carcinogenic nitrosamine in tobacco at various stages.

Further, regulation of standardization of all SLT products under the WHO-FCTC and US FDA by reducing attractiveness and/or toxicity can have a long lasting impact on public health. However, lawsuits have failed to motivate most tobacco companies to manufacture low TSNA ‘reasonable alternative design’ products to protect the public from major cancer risks associated with SLT products (Berman and Hatsukami, 2018).

5. Road ahead

A total of 180 countries covering about 87% of the global tobacco

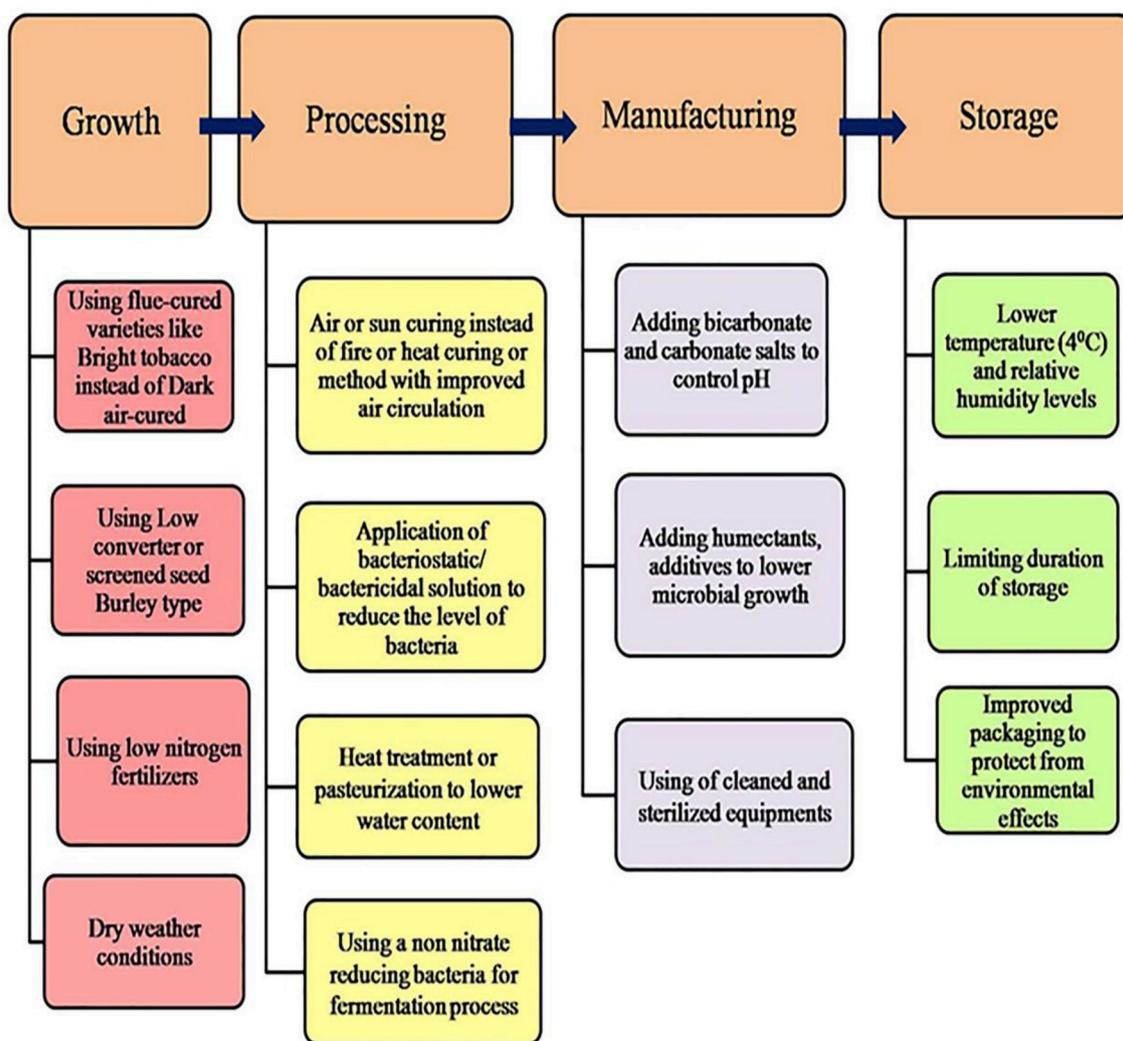


Fig. 4. Recommended measures to be taken for the reduction of NNN and NNK levels at various stages in smokeless tobacco production.

burden, have legally signed an evidence-based international treaty for the regulation of the contents for all tobacco products and their disclosures under WHO Framework Convention on Tobacco Control (FCTC) (WHO Library Cataloguing-in-Publication Data, 2016). However, due to high heterogeneity of SLT products across the world, firm implementations of the WHO FCTC with respect to SLT are currently lacking (Siddiqi et al., 2017; WHO, 2014).

Tobacco manufacturers must disclose the amount of NNN and NNK in all their commercial SLT products. As high temperature and humidity increases the levels of NNN and NNK in finished tobacco products stored for longer time, labels of all SLT products must contain a manufacturing code, expiration date and storage conditions along with all its constituents and their associated health hazards (Borgida et al., 2015). This will educate tobacco users about the variations of nitrosamines, especially NNN and NNK levels within a class of SLT products and their carcinogenic properties.

Scientific evidence-based evaluation of SLT constituents is crucial to reduce the burden of SLT induced fatal diseases (Berman et al., 2015).

Though, a Tobacco Laboratory Network (TobLabNet) has been established by WHO in South Asia to regulate and provide testing of contents and emissions of cigarettes, but such product specific analysis is now made available for SLT owing to wide range of products available in the South Asian market (Siddiqi et al., 2016). There is also a lack of relevant laboratory expertise to standardize analysis procedures for NNN and NNK present in SLT products (WHO Study Group on Tobacco

Product Regulation, 2015). To initiate the process, Ministry of Health and Family Welfare, Government of India has recently established three National Tobacco Testing Laboratories (NTTL) in India with the sole purpose of monitoring analytical testing and regulatory mechanisms for all tobacco products (Mehrotra et al., 2018b). Persistence of high degree of SLT use, especially among youth, necessitates the implementation of FDA's proposed rule of establishing a tobacco product standard for NNN in finished SLT products, as early as possible (Mehrotra et al., 2018a).

In conclusion, apart from public awareness, a strong framework and built is required to monitor the levels of NNN and NNK in all SLT product sold in the market, worldwide. First-ever compilation of the global progress made in implementing SLT control policies has been done by the Indian Council of Medical Research (ICMR)-National Institute of Cancer Prevention and Research (NICPR) (Mehrotra et al., 2017). It is high time that the tobacco companies around the world should realize the carcinogenic affects of tobacco specific nitrosamines and must utilize the advance technologies to reduce the concentration of NNN and NNK (< 2 µg/g dry weight). Applying grass roots approach to lower the levels of carcinogenic nitrosamines at various stages of tobacco production could be a beneficial strategy, especially for South Asian countries which are facing the greatest burden of tobacco specific disease. However, the sole purpose of this article is to create public awareness to reduce the death and disease burden due to tobacco use and the authors do not intent to support harm reduction strategy as an

alternate to tobacco cessation as no form of tobacco or nicotine delivery system could be safe.

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Declaration of interest

Authors declare no conflict of interest.

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Transparency document

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