Viewpoint

Development of comprehensive data repository on chemicals present in smokeless tobacco products: Opportunities & challenges

Smokeless tobacco (SLT) products are consumed by 356 million people in 140 countries around the world. Consumption of SLT products has been estimated to account for about 0.65 million deaths per year. Worldwide, SLT products are consumed in varying forms ranging from simple cured tobacco to processed products with many chemical ingredients and additives. Variations occur with the geographical location, type of tobacco plant, additives, flavouring agents, processing and curing methods. Published reports highlight the chemical changes that a tobacco plant undergoes until the formation of final SLT product. This creates product-wise and brand-wise variations in different SLT products.

Chemical profiling of SLT products has been attempted by some researchers. A vital role of various chemicals has been indicated in the adverse health effects reported with SLT use. Based on their carcinogenicity in experimental animals and humans, the International Agency for Research on Cancer (IARC) has classified SLT products as group 1 carcinogen (Carcinogenic to humans). Two of the chemicals found abundantly in SLT products, namely N’-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) belonging to the class of tobacco-specific nitrosamines (TSNAs) have also been classified in this group. NNN and NNK are formed by the nitrosation of nicotine, the major tobacco alkaloid. Nicotine is available in SLT products in predominantly two forms as follows: protonated and unprotonated. Unprotonated/free nicotine, the amount of which varies according to the pH of the SLT product, can cross cell membranes readily. Studies to estimate the pH and amount of total and free nicotine of various SLT products have been conducted. However, a major drawback is the lack of comprehensive analysis of SLT products on a periodic basis. Most of the previous studies have been independent projects undertaken by different research groups focusing on one or two classes of chemical compounds only. These studies depict variations in the amount of chemicals, pH and free nicotine content of SLT products. Thus, the need of the hour is to develop a one-stop repository compiling data on chemical analysis of SLT products from all the published studies. This will provide initial data for establishing priorities in research on chemical profiling of SLT products.

Opportunities in the field of chemical profiling of smokeless tobacco products

Available studies and reports on the chemical profiling of SLT products can be used to collect initial data. Data on pH, moisture and free nicotine are also available from many reliable sources. After compiling the available data, information about the physicochemical properties and protein targets of the chemicals can be obtained from freely available chemical databases such as PubChem, ChemSpider, BindingDB and ChEMBL. Further open source technologies, such as Linux, Apache, MySQL, PHP/Perl, cascading style sheets, HTML, JavaScript and Data Tables can be exploited for the development of online repository. The repository can then be equipped with built-in modules for data upload, simple search and browse options along with dashboards for different stakeholders.

Challenges towards development of repository on smokeless tobacco products

Although the literature is replete with studies on SLT products, a major bottleneck is the availability and distribution of the data in an organized fashion to allow for further analysis. Furthermore, the available studies cater to potent carcinogens (such as TSNAs...
and polyaromatic hydrocarbons) with the scarcity of information about other chemical compounds\(^3\,^5\). One of the frequently used additives in SLT products, areca nut has been classified as a group 1 carcinogen\(^6\). Flavouring agents and other additives of SLT products have not been researched well. Moreover, the evolution of SLT products with the ever-increasing demand is making it difficult for researchers to study every SLT product in detail. Another significant challenge likely to be faced in the development of a repository of SLT product constituents is the absence of a standardized protocol of testing of these products and hence, the lack of comparability between studies.

**Conclusion**

Consumption of SLT products is a growing menace taking lives of millions of people worldwide. Evaluating the severity of the health effects of SLT products is a complex task mainly because of the variations in the products, their chemical composition and the differences in the mode of intake of these products. To effectively reduce the harm associated with SLT products, an important milestone will be the development of an online repository of chemical constituents of SLT products, as a one-stop information source on the carcinogenicity, physicochemical properties, protein targets and structural diversity of these chemicals. The repository will also provide initial data for developing regulations, guidelines and policies on the chemical composition of SLT products. Toxicokinetic studies can be utilized for identification of potentially harmful functional groups. The identified harmful groups can be replaced or removed to reduce the harm caused due to SLT products.

**Conflicts of Interest:** None.

**References**

1. Sinha DN, Gupta PC, Kumar A, Bhartiya D, Agarwal N, Sharma S, *et al.* The poorest of poor suffer the greatest burden from smokeless tobacco use: A study from 140 countries. *Nicotine Tob Res* 2017; December 22. doi 10.1093/ntr/nt3e276.
2. Sinha DN, Suliankatchi RA, Gupta PC, Thamarangsi T, Agarwal N, Parascandola M, *et al.* Global burden of all-cause and cause-specific mortality due to smokeless tobacco use: Systematic review and meta-analysis. *Tob Control* 2018; 27 : 35-42.
3. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. *Smokeless tobacco and some tobacco-specific N-nitrosamines.* Vol. 89. Lyon, France, International Agency for Research on Cancer: IARC Monograph on the Evaluation of Carcinogenic Risks to Humans; 2007.
4. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Betel-quid and areca-nut chewing and some areca-nut derived nitrosamines. *IARC Monogr Eval Carcinog Risks Hum.* Lyon, France, International Agency for Research on Cancer 2004; 85 : 1-334.
5. National Cancer Institute and Centers for Disease Control and Prevention, *Smokeless tobacco and public health: A global perspective.* Bethesda, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health, National Cancer Institute; NIH Publication No. 14-7983, 2014.
6. Wagner KA, Huang CB, Melvin MS, Ballentine R, Meruva NK, Flora JW, *et al.* Gas chromatography-mass spectrometry method to quantify benzo[α]Pyrene in tobacco products. *J Chromatogr Sci* 2017; 55 : 677-82.
7. Stepanov I, Gupta P, Parascandola M, Yershova K, Jain V, Dhumal G, *et al.* Constituent variations in smokeless tobacco purchased in Mumbai, India. *Tob Regul Sci* 2017; 3 : 305-14.
8. Food and Drug Administration. Tobacco product standard for N-nitrosornicotine level in finished smokeless tobacco products. *Fed Regist* 2017; 82 : 8004-53.
9. Hegde V, Nanukuttan A. Comparison of nicotine concentration and pH of commercially available smokeless tobacco products. *J Oral Res Rev* 2017; 9 : 21.
10. Houas I, Teyeb H, Rochina-Maro A, Douki W, Najjar MF, Gaha L, *et al.* Comparison of mineral contents in three different tobacco formulations. *Biomed Environ Sci* 2017; 30 : 52-8.
11. Jain V, Garg A, Parascandola M, Chaturvedi P, Khariwala SS, Stepanov I, *et al.* Analysis of alkaloids in areca nut-containing products by liquid chromatography-tandem mass spectrometry. *J Agric Food Chem* 2017; 65 : 1977-83.
12. Stepanov I, Hecht SS, Ramakrishnan S, Gupta PC. Tobacco-specific nitrosamines in smokeless tobacco products marketed in India. *Int J Cancer* 2005; 116 : 16-9.
13. Song MA, Marian C, Brasky TM, Reisinger S, Djordjevic M, Shields PG, *et al.* Chemical and toxicological characteristics of conventional and low-TSNA moist snuff tobacco products. *Toxicol Lett* 2016; 245 : 68-77.
14. Bhartiya D, Kumar A, Kaur J, Kumari S, Sharma AK, Sinha DN, *et al.* In silico study of toxicokinetics and disease association of chemicals present in smokeless tobacco products. *Regul Toxicol Pharmacol* 2018; 95 : 8-16.
15. Siminszky B, Gavilano L, Bowen SW, Dewey RE. Conversion of nicotine to nornicotine in *Nicotiana tabacum* is mediated by CYP82E4, a cytochrome P450 monooxygenase. *Proc Natl Acad Sci U S A* 2005; 102: 14919-24.

16. Pickworth WB, Rosenberry ZR, Gold W, Koszowski B. Nicotine absorption from smokeless tobacco modified to adjust pH. *J Addict Res Ther* 2014; 5: 1000184.

17. Centers for Disease Control and Prevention (CDC). Determination of nicotine, pH, and moisture content of six U.S. commercial moist snuff products – Florida, January-February 1999. *MMWR Morb Mortal Wkly Rep* 1999; 48: 398-401.

18. Richter P, Hodge K, Stanfill S, Zhang L, Watson C. Surveillance of moist snuff: Total nicotine, moisture, pH, un-ionized nicotine, and tobacco-specific nitrosamines. *Nicotine Tob Res* 2008; 10: 1645-52.

19. Richter P, Spierto FW. Surveillance of smokeless tobacco nicotine, pH, moisture, and unprotonated nicotine content. *Nicotine Tob Res* 2003; 5: 885-9.

20. Stanfill SB, Oliveira da Silva AL, Lisko JG, Lawler TS, Kuklenyik P, Tyx RE, *et al.* Comprehensive chemical characterization of rapé tobacco products: Nicotine, un-ionized nicotine, tobacco-specific N’-nitrosamines, polycyclic aromatic hydrocarbons, and flavor constituents. *Food Chem Toxicol* 2015; 82: 50-8.

21. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, *et al.* PubChem substance and compound databases. *Nucleic Acids Res* 2016; 44: D1202-13.

22. Pence HE, Williams A. ChemSpider: An online chemical information resource. *J Chem Educ* 2010; 87: 1123-4.

23. Liu T, Lin Y, Wen X, Jorissen RN, Gilson MK. BindingDB: A web-accessible database of experimentally determined protein-ligand binding affinities. *Nucleic Acids Res* 2007; 35: D198-201.

24. Gaulton A, Bellis LJ, Bento AP, Chambers J, Davies M, Hersey A, *et al.* ChEMBL: A large-scale bioactivity database for drug discovery. *Nucleic Acids Res* 2012; 40: D1100-7.