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TOBACCO: THE MONSTER

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ABSTRACT

In south-central Asia, oral cancer is the most common type of cancer. In India, 20/100000 population are affected by oral cancer which accounts for about 30% of all types of cancer. More than 5 people in India die every hour every day because of oral cancer and same number of people die due to cancer in oropharynx and hypo pharynx. Cancer registration is not compulsory in India, so the true incidence and mortality can't be measured as many cases are unrecorded and loses follow up. The low-income groups are the most affected group in India due to a wide exposure to risk factors such as tobacco chewing and insufficient exposure to newly diagnostic aids, resulting in a delay in reporting of oral cancer. Tobacco is consumed in different forms like Tobacco Squid, Pan, Gutka, smoked form. It is carcinogenic in whichever form it is consumed. If combined with alcohol, Arecanut and other environmental factors, it is more dangerous. The present review summarises the ingredients of Tobacco products and it's carcinogenic potential. This will be helpful for new generation who can be moulded to good oral habits.

KEYWORDS: Tobacco, Carcinogen, Head & Neck Squamous Cell Carcinoma (HNSCC), Nitrosamines, DNA adduct.

INTRODUCTION

In the long history of tobacco production, many different forms of tobacco have been developed. Largely, they include combustible and smokeless tobacco (SLT) products. According to the IARC, different form of combustible tobacco products used all over the world include cigarettes, cigars, cigarillos, bidis, chutta, kretek, and many others. Similarly, there are various SLT 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.[1] SLT is more prevalent in South Asian countries such as India. According to the GATS 2 survey, every fifth adult in India use SLT.[2] This number of tobacco users in India is equivalent to the entire US adult population. In south-central Asia, oral cancer is the most common type of cancer. In India, 20/100000 population are affected by oral cancer which accounts for about 30% of all types of cancer.[3] More than 5 people in India die every hour every day because of oral cancer and the same number of people die from cancer in oropharynx and hypopharynx.[4] Cancer registration is not compulsory in India, so the true incidence and mortality is not known as many cases are unrecorded and loses follow up. Low-income groups are the most affected group in India due to a wide exposure to risk factors such as tobacco chewing or smoking and insufficient exposure to newly diagnostic aids, resulting in a delay in reporting of oral cancer.

In contrast, in the US, cigarettes are the most popular form of tobacco used than others. Forty million American adults smoke cigarettes, 4.7 million middle and high school students use at least one tobacco product, including E-cigarettes.[5] Tobacco kills >80 Lakh people across the world with 70 lakh die due to direct consumption of tobacco, whereas 10 Lakh die due to second-hand smoking every year. On average, people who smoke have 10 years lesser life than people who have never smoked. Smoking causes about 20% of all cancers, about 30% of all cancer deaths in the United States. About 80% of lung cancers, about 80% of all lung cancer deaths are due to smoking. Smoking can also cause a number of other diseases and can damage nearly every organ in the body, including the lungs, heart, blood vessels, reproductive organs, mouth, skin, eyes, bones, lowers immune system also.[6] The present review summarises the ingredients of Tobacco products and it's carcinogenic potential and data can be used by regulatory bodies to make policies for tobacco control as well as provides idea about risk of cancer in tobacco users. This

will be helpful for the new generation who can be moulded to good oral habits.

History of Tobacco from Medicinal herb to Carcinogen

In the 1500s, tobacco was used as a medicinal herb as antidiarrheal, narcotic, emollient, and pain-relieving agent, applied locally to heal burns and ulcers. As tobacco use became more widespread, its abuse started. Franciscan monk Andre Thevet in Brazil reported that smoking of such leaves caused fainting and weakness which was well supported by Conrad.[7] 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 regulation of tobacco products was released in 1964 after the report concluded that cigarette smoking is a cause of lung cancer in men/women and laryngeal cancer in men and the most important cause of chronic bronchitis, emphysema and heart disease.[8] This report was a critical catalyst for action into tobacco research that has transpired over the ensuing 50-60 years.

Tobacco carcinogen and toxicant metabolites

Multiple carcinogens, toxicants have been identified in both combustible and SLT products.[1,9,10,11,12,13,14,15] More than 5300 compounds have been identified in tobacco smoke. Of those, there are >70 known carcinogens which have been described in cigarette smoke.[16] The common compound and the main reason for tobacco addiction is nicotine which is not a carcinogen but has been shown to act as a promoter of proliferation and survival in lung cancer cells.[17] In addition to nicotine, each puff of a cigarette/pinch of ST delivers a mixture of carcinogens. For example: Polycyclic aromatic hydrocarbons (PAHs), 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. Humans metabolize nicotine to cotinine/3′hydroxycotinine and their glucuronides. These metabolites along with several other compounds are excreted in urine.[15] Most of carcinogenic compounds are formed during curing/processing of Tobacco products.[18,19,20] Seven TSNA have been identified in tobacco products: NNN, NNK, NNAL, NAB, NAT, iso-NNAL, and iso-NNAC.

The most carcinogenic of these compounds are 4(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N ′-nitrosonornicotine (NNN). NNK is a highly effective carcinogen, metabolized in humans, leading to secretion of variety of compounds in urine such as 4(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (NNAL-Glucs).[21] The value of NNAL and NNAL-Glucs together represents total NNAL. Advantages of measuring urinary total NNAL include tobacco specificity, carcinogen exposure and consistent detection in those exposed to tobacco. **Smokers have been reliably shown to have higher levels of NNAL when compared to non-smokers**.[22] Studies of smoker's total level of NNAL demonstrated decreased levels upon reduction or cessation of smoking.[23,24]

A study was done on non-smokers who worked in restaurants/bars that permitted smoking. This study reported a statistically significant increase in urinary levels of total NNAL, nicotine and cotinine on working days compared to non-working days.[25] Thus, NNAL is a reliable indicator of tobacco exposure, even when the exposure is environmental/second-hand in nature.[26] NNK uptake was shown to be similar in users of regular, light, and ultra-light cigarettes.[27] Recent data have shown a linear relationship between NNAL levels in smokers and risk of the development of lung cancer.[28,29] Relative to the lowest tertile, risks associated with the second and third tertiles of total NNAL were higher, after adjustment for self-reported smoking history and urinary total cotinine. Smokers in the highest tertiles of urinary total NNAL and total cotinine exhibited an 8.5-fold increased risk for lung cancer as compared to smokers with comparable smoking history but possessing the lowest tertiles. This data suggests about possibility of **NNAL as a screening tool for those smokers who are at highest risk of developing lung cancer.**

NNK is felt to be related to the development of lung and pancreatic cancer whereas NNN is thought to be a cause of esophageal cancer.[30,31,32] It can be readily quantified in human urine by assaying for free, unchanged NNN and NNN-Gluc, the sum being referred to as total NNN. NNN and its metabolites are present in the urine of both smokers and SLT users. **The level of urinary total NNN was significantly higher in SLT users when compared to smokers.**[33]

Both NNK and NNN induce nasal tumors in laboratory animals.[34] NNAL is strongly associated with lung cancer regardless of its mode of administration found in animal study.[20] By subcutaneous administration of NNN in rats, leading to nasal tumors and through drinking water or liquid diet, NNN administration resulted in oral, esophageal, and nasal tumors.[35] Investigation of the carcinogenic effects of NNN enantiomers was done in an animal study.[36] It was demonstrated that S-NNN, when administered, resulted in significantly higher rates of oral cavity tumors and esophageal tumors as compared to those receiving R-NNN. The incidence of tumor development is much higher when racemic mixture (S-NNN+R-NNN) was used. Thus, this study concluded that **S-NNN is a powerful oral cavity carcinogen and is the predominant enantiomer in SLT products.** The study also showed that although R-NNN is not as a potent carcinogen alone, it appears to have synergistic co-carcinogenic effects with S-NNN.

Another group of carcinogens is the PAHs[37,38] which are not tobacco-specific and commonly detected in polluted air, water, engine exhaust, broiled foods and in tobacco products. For example: BaP (benzo[a] pyrene) and 1-HOP(1-Hydroxy Pyrene) Administration of BaP results in tumors of the stomach and/or colorectal tract.[39] Various markers of PAH exposure are **PAH-DNA adducts** and 1-HOP. Several studies have quantified 1-HOP in the urine of people exposed to PAH.[40,41] Sensitive, accurate and precisely high assays for quantifying 1-HOP are available.[42] Urinary levels are generally 2-3 times higher in smokers when compared to non-smokers.[43,24,32] Still, there is a general lack of PAH data pertaining to levels in HNSCC patients.

Acetaldehyde and formaldehyde are tobacco smoke constituents that are associated with HNSCC in both laboratory animals and humans.[44,45] Acetaldehyde is found widely in the environment and is known to be genotoxic, carcinogenic.[29] It causes mutations, micronuclei, aneuploidy in mammalian cells and mutations in bacteria.[45,46] Levels of acetaldehyde in cigarette smoke typically range from 500-1000 micrograms/cigarette.[45] One mechanism by which carcinogens exert toxic effects is through binding of DNA to form DNA adducts. After this HPB (4-hydroxy-1-[3-pyridyl]-1-butanone) is released which can be measured to quantify the level of adduct formation.[47,48] A method of quantifying acetaldehyde-DNA adduct formation was developed and validated.[49]

Alcohol has synergistic effect with tobacco in the development of HNSCC and is also an independent risk factor for HNSCC. Thus, acetaldehyde DNA adduct levels may reflect the impact of both tobacco and alcohol use in patients with HNSCC. Individuals who are deficient in their ability to metabolize acetaldehyde due to polymorphisms in the aldehyde dehydrogenase gene leading to acetaldehyde accumulation and who nevertheless consume significant amounts of alcohol are at higher risk for HNSCC. The IARC, therefore, concluded that acetaldehyde associated with alcoholic beverages is carcinogenic to humans.[50] Formaldehyde is genotoxic/cytotoxic due to formation of DNA adducts that plays important roles in its carcinogenicity.[9]

Using tobacco carcinogens to study exposure: The study of tobacco carcinogens can be used to study tobacco exposure. With regard to HNSCC, the relationship between self-reported tobacco use and the level of urinary tobacco carcinogen metabolites has been studied.[51] Tobacco-specific biomarkers, such as those derived from nicotine or TSN secreted in urine, are felt to be a superior gauge of tobacco product exposure when compared to user-reported "cigarettes/day" estimates. This is because each tobacco user consumes his/her product of choice in different manner resulting in wide variability of actual exposure for a given amount of tobacco "used." For example, some smokers inhale more deeply, smoke more puffs, smoke cigarettes more completely than other smokers, thereby getting a higher dose of carcinogen/toxicant per cigarette. Techniques to quantify uptake of nicotine and NNK metabolites have been refined and described.[20, 21, 52, 53, 54, 32] Urinary cotinine levels has strong correlation with urinary carcinogen levels.

Urinary cotinine can be used in those cases where a more accurate exposure to tobacco is required such as a preoperative assessment of wound healing capacity.[55] 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.[51] Further work has demonstrated the feasibility and value of studying total NNN in urine.[45] Biomarkers of non-tobacco specific compounds include 1-HOP in urine, acetaldehyde-DNA and formaldehyde-DNA adducts in leukocytes.[15, 31, 56]

Exposure versus Risk: Metabolites such as NNAL and NNN are useful in measuring carcinogen exposure as they correlate with levels of nicotine/cotinine. However, when the levels are corrected for cotinine, cigarettes/day and years of smoking, NNAL, NNN levels are elevated in those patients at greater risk for lung cancer. Therefore, these 2 metabolites are likely markers of exposure and risk whereas cotinine level indicates exposure alone. Further research in patients with lung cancer/HNSCC has the potential to further elucidate the nature of the metabolites discussed in this review as markers of exposure and/or markers of risk.

Study of Tobacco Carcinogens in HNSCC

Increased exposure and reduced detoxification of NNAL and NNN are important consideration in determining tobacco-induced cancer risk.[57,58] All individuals exposed to tobacco will not develop cancer. It is due to complex interaction of exposures and predisposition which include genetic factors, carcinogen metabolism, excretion and immunologic status. Biotransformation, detoxification, elimination of carcinogens, together with DNA repair mechanisms, apoptotic pathways are the most important mechanisms of defence against carcinogenesis. DNA adducts are formed when cancer causing agents bind to the double helical DNA structure and disrupt it. If left unrepaired, permanent mutation can occur affecting critical gene result in carcinoma. DNA adducts are created by downstream by products after 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**. A group has studied DNA adduct formation in the oral cavity among smokers with HNSCC.[47] 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 and will be used in future studies of this type[48] to find out extreme risk cases of HNSCC.

Importance of Tobacco Carcinogen Research

A better understanding of tobacco carcinogen dose, metabolism, and DNA adduct formation provides an opportunity to identify those tobacco users who are at greatest risk for HNSCC for prevention/early detection and may be specifically targeted for smoking cessation efforts. Disease diagnosed earlier would require less intense treatment, low treatment cost, less hospital time and fewer procedures. Tobacco metabolites also acts as prognostic markers.

Using tobacco carcinogen research to make public policy: There are >one billion active tobacco users in the world. Efforts aimed at banning some products and for decreasing the impact of tobacco product use. This can be implemented through regulation of tobacco product content which aims to reduce a user's exposure to carcinogenic material. This created a need of data that could help to inform standards of tobacco product content. A study evaluating varying levels of nicotine and TSNA in SLT products as well as patterns of use, demographic and tobacco history to understand the extent of exposure to carcinogens was conducted by Ferlay et. Al. (2012).[59] This provided evidence for the Food and Drug administration (FDA) authority and other regulatory bodies that product standards for reducing levels of TSNA in SLT products are necessary to decrease exposure to these toxicants and to reduce risk for cancer.

Tobacco industry has tried to initiate harm reduction through development of harm reducing products. One example of this is "Snus" (SLT 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[60] due to 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.[61] In India, a SLT product known as Chaini-Khaini is marketed as a Snus equivalent but a study of Chaini-Khaini indicates that it actually has high levels of carcinogenic TSNA. 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 SLT.[62] Data of this type has the potential to influence future policy efforts and can be used to educate current and potential users of this product.

The FDA has proposed a tobacco product standard that would establish a limit of NNN in finished SLT products.[63] 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, approximately 2200 oral cancer deaths would be averted in the US and approximately 15,200 life-years would be gained.

CONCLUSION

Tobacco use continues to be a prominent etiologic factor in the development of HNSCC. 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 and acts as potential tools for prevention and screening for HNSCC. Unstudied potential role of tobacco carcinogen and toxicant metabolites and DNA adducts as biomarkers of tumour behaviour and prognosis warrants investigators to further characterize HNSCC in tobacco user.

REFERENCES

1. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans Tobacco smoke and involuntary smoking. IARC monographs on the evaluation of carcinogenic risks to humans, 2004; 83: 1–1438.
2. Asma S, Mackay J, Song SY, et al. The GATS Atlas. Atlanta: CDC Foundation, 2015.
3. Sankarnarayanan R, Ramadas K, Thomas G, Muwonge R, Thara S, Mathew B et. al. Lancet, 2005; 365(9475): 1927-33.
4. Gupta B, Ariyawardana A, Johnson N W. Oral cancer in India continues in epidemic proportions: evidence base and policy initiatives. Int Dent J, 2013; 63(1): 12-25.
5. The Health Consequences of Smoking-50 Years of Progress: A Report of the Surgeon General. Atlanta, 2014.
6. American Cancer Society. Health Risks of Smoking Tobacco: American Cancer Society Medical and editorial content team; October28, 2020.
7. Gesner C. Epistolarum Medicinalium, Libri 111. Zurich, 1577.
8. Surgeon General's Advisory Committee on Smoking and Health. Smoking and Health: Report of the Advisory Committee to the Surgeon General of the Public Health Service. Washington: US Deptartment of Health, Education, and Welfare, Public Health Service: Washington, DC, 1964.
9. IARC. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Lyon (France). Tobacco smoking, 1986.
10. IARC., editor. IARC. Smokeless Tobacco and Tobacco-Specific Nitrosamines. Lyon, 2007; 57-86.
11. Hoffmann D, Adams JD, Lisk D, Fisenne I, Brunnemann KD. Toxic and carcinogenic agents in dry and moist snuff. J Natl Cancer Inst, 1987; 79: 1281–1286. [PubMed: 3480379]
12. Hoffmann D, Hoffmann I, El-Bayoumy K. The less harmful cigarette: a controversial issue. A tribute to Ernst L. Wynder. Chem Res Toxicol, 2001; 14: 767–790.
13. Swauger JE, Steichen TJ, Murphy PA, Kinsler S. An analysis of the mainstream smoke chemistry of samples of the U.S. cigarette market acquired

between 1995 and 2000. Regul Toxicol Pharmacol, 2002; 35(2 Pt 1): 142–156. [PubMed: 12052000]
14. Hecht, SS. Etiology of cancer: tobacco. In: DeVita, VT.Lawrence, TS., Rosenberg, SA., editors. Cancer: Principles and Practice of Oncology. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins, 2008; 147-155.
15. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nat Rev Cancer, 2003; 3: 733–744. [PubMed: 14570033]
16. Khariwala SS, Hatsukami D, Hecht SS. Tobacco carcinogen metabolites and DNA adducts as biomarkers in head and neck cancer: potential screening tools and prognostic indicators. Head Neck, 2012; 34: 441–447.
17. Tsurutani J, Castillo SS, Brognard J, et al. Tobacco components stimulate Akt-dependent proliferation and NFkappaB-dependent survival in lung cancer cells. Carcinogenesis, 2005; 26: 1182–1995. [PubMed: 15790591]
18. Hecht SS, Hoffmann D. Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. Carcinogenesis, 1988; 9: 875–884. [PubMed: 3286030]
19. Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. Chem Res Toxicol, 1998; 11: 559–603. [PubMed: 9625726]
20. Spiegelhalder B, Bartsch H. Tobacco-specific nitrosamines. Eur J Cancer Prev., 1996; 5(Suppl 1): 33–38.
21. Carmella SG, Han S, Fristad A, Yang Y, Hecht SS. Analysis of total 4-(methylnitrosamino)-1-(3pyridyl)-1-butanol (NNAL) in human urine. Cancer Epidemiol Biomarkers Prev., 2003; 12(11 Pt 1): 1257–1261. [PubMed: 14652291]
22. Hecht SS. Progress and challenges in selected areas of tobacco carcinogenesis. Chem Res Toxicol, 2008; 21: 160–171. [PubMed: 18052103]
23. Hecht SS, Murphy SE, Carmella SG, et al. Effects of reduced cigarette smoking on the uptake of a tobacco-specific lung carcinogen. J Natl Cancer Inst., 2004; 96: 107–115. [PubMed: 14734700]
24. Carmella SG, Chen M, Han S, et al. Effects of smoking cessation on eight urinary tobacco carcinogen and toxicant biomarkers. Chem Res Toxicol, 2009; 22: 734–741. [PubMed: 19317515]
25. Tulunay OE, Hecht SS, Carmella SG, et al. Urinary metabolites of a tobacco-specific carcinogen in nonsmoking hospitality workers. Cancer Epidemiol Biomarkers Prev., 2005; 14: 1283–1286. [PubMed: 15894687]
26. Hecht SS, Carmella SG, Le KA, et al. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides in the urine of infants exposed to environmental tobacco smoke. Cancer Epidemiol Biomarkers Prev., 2006; 15: 988–992. [PubMed: 16702381]
27. Hecht SS, Murphy SE, Carmella SG, et al. Similar uptake of lung carcinogens by smokers of regular, light, and ultralight cigarettes. Cancer Epidemiol Biomarkers Prev., 2005; 14: 693–698. [PubMed: 15767351]
28. Yuan JM, Koh WP, Murphy SE, et al. Urinary levels of tobacco-specific nitrosamine metabolites in relation to lung cancer development in two prospective cohorts of cigarette smokers. Cancer Res., 2009; 69: 2990–2995. [PubMed: 19318550]
29. US Dept. of Health and Human Services. Report on carcinogens. 11. Research Triangle Park, NC, 2004; III-1-III-3.
30. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nat Rev Cancer, 2003; 3: 733–744. [PubMed: 14570033]
31. Hecht SS, Hoffmann D. The relevance of tobacco-specific nitrosamines to human cancer. Cancer Surv, 1989; 8: 273–294. [PubMed: 2696581]
32. Hecht SS. Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. Carcinogenesis, 2002; 23: 907–922. [PubMed: 12082012]
33. Stepanov I, Hecht SS. Tobacco-specific nitrosamines and their pyridine-N-glucuronides in the urine of smokers and smokeless tobacco users. Cancer Epidemiol Biomarkers Prev, 2005; 14: 885– 891. [PubMed: 15824160]
34. Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. Chem Res Toxicol, 1998; 11: 559–603. [PubMed: 9625726]
35. Koppang N, Rivenson A, Reith A, Dahle HK, Evensen O, Hoffmann D. A study of tobacco carcinogenesis XLVIII. Carcinogenicity of N'-nitrosonornicotine in mink (Mustela vison). Carcinogenesis, 1992; 13: 1957–1960.
36. Balbo S, James-Yi S, Johnson CS, O'Sullivan MG, Stepanov I, Wang M, Bandyopadhyay D, Kassie F, Carmella S, Upadhyaya P, Hecht SS (S)-N'-Nitrosonornicotine, a constituent of smokeless tobacco, is a powerful oral cavity carcinogen in rats. Carcinogenesis, 2013; 34: 2178–2183.
37. Luch, A. Polycyclic aromatic hydrocarbon-induced carcinogenesisan introduction. Luch, A., editor. London: Imperial College Press, 2005; 1-18.
38. Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst, 1999; 91: 1194–1210. [PubMed: 10413421]
39. Diggs DL, Huderson AC, Harris KL, Myers JN, Banks LD, Rekhadevi PV, Niaz MS, Ramesh A, Polycyclic aromatic hydrocarbons and digestive tract cancers: a perspective. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 2011; 29: 324–357.
40. Jongeneelen FJ, Anzion RB, Leijdekkers CM, Bos RP, Henderson PT. 1-hydroxypyrene in human urine after exposure to coal tar and a coal tar derived product. Int Arch Occup Environ Health, 1985; 57: 47–55. [PubMed: 4077281]
41. Hansen AM, Mathiesen L, Pedersen M, Knudsen LE. Urinary 1-hydroxypyrene (1-HP) in

environmental and occupational studies–a review. Int J Hyg Environ Health, 2008; 211: 471–503. [PubMed: 18222724]
42. Carmella SG, Le KA, Hecht SS. Improved method for determination of 1-hydroxypyrene in human urine. Cancer Epidemiol Biomarkers Prev., 2004; 13: 1261–1264. [PubMed: 15247141]
43. Hecht SS, Yuan JM, Hatsukami D. Applying tobacco carcinogen and toxicant biomarkers in product regulation and cancer prevention. Chem Res Toxicol, 2010; 23: 1001–1008. [PubMed: 20408564]
44. IARC. Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-Ol. Lyon, FR, 2006.
45. IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Lyon, France. Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide (part two), 1999; 318-335.
46. IARC. Allyl compounds, aldehydes, epoxides and peroxides. iarc monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol. 36. Lyon, France, 1985; 101-132.
47. Khariwala SS, Carmella SG, Stepanov I, Fernandes P, Lassig AA, Yueh B, Hatsukami D, Hecht SS Elevated levels of 1-hydroxypyrene and N'-nitrosonornicotine in smokers with head and neck cancer: a matched control study. Head Neck, 2013; 35: 1096–1100.
48. Khariwala SS, Ma B, Ruszczak C, Carmella SG, Lindgren B, Hatsukami DK, Hecht SS, Stepanov I High level of tobacco carcinogen-derived dna damage in oral cells is an independent predictor of oral/head and neck cancer risk in smokers. Cancer Prev Res (Phila), 2017; 10: 507–513.
49. Chepiga TA, Morton MJ, Murphy PA, et al. A comparison of the mainstream smoke chemistry and mutagenicity of a representative sample of the US cigarette market with two Kentucky reference cigarettes (K1R4F and K1R5F). Food Chem Toxicol, 2000; 38: 949–962. [PubMed: 11039328]
50. Chen L, Wang M, Villalta PW, et al. Quantitation of an acetaldehyde adduct in human leukocyte DNA and the effect of smoking cessation. Chem Res Toxicol, 2007; 20: 108–113. [PubMed: 17226933]
51. Wang M, Cheng G, Villalta PW, Hecht SS. Development of liquid chromatography electrospray ionization tandem mass spectrometry methods for analysis of DNA adducts of formaldehyde and their application to rats treated with N-nitrosodimethylamine or 4-(methylnitrosamino)-1-(3pyridyl)-1-butanone. Chem Res Toxicol, 2007; 20: 1141–1148. [PubMed: 17676814]
52. Khariwala SS, Carmella SG, Stepanov I, Bandyopadhyay D, Nelson HH, Yueh B, Hatsukami DK, Hecht SS Self-reported Tobacco use does not correlate with carcinogen exposure in smokers with head and neck cancer. Laryngoscope, 2015; 125: 1844–1848.
53. Carmella SG, Akerkar S, Hecht SS. Metabolites of the tobacco-specific nitrosamine 4(methylnitrosamino)-1-(3-pyridyl)-1-butanone in smokers' urine. Cancer Res., 1993; 53: 721–724. [PubMed: 8428352]
54. Carmella SG, Han S, Villalta PW, Hecht SS. Analysis of total 4-(methylnitrosamino)-1-(3pyridyl)-1-butanol (NNAL) in smokers' blood. Cancer Epidemiol Biomarkers Prev., 2005; 14(11 Pt 1): 2669– 2672. [PubMed: 16284395]
55. Hukkanen J, Jacob P III, Benowitz NL. Metabolism and disposition kinetics of nicotine. Pharmacol Rev., 2005; 57: 79–115. [PubMed: 15734728]
56. Cheng G, Shi Y, Shana SJ, et al. Reactions of formaldehyde plus acetaldehyde with deoxyguanosine and DNA: formation of cyclic deoxyguanosine adducts and formaldehyde crosslinks. Chem Res Toxicol, 2003; 16: 145–152. [PubMed: 12588185]
57. Marin VP, Pytynia KB, Langstein HN, Dahlstrom KR, Wei Q, Sturgis EM. Serum cotinine concentration and wound complications in head and neck reconstruction. Plast Reconstr Surg, 2008; 121: 451–457.
58. Yuan JM, Knezevich AD, Wang R, Gao YT, Hecht SS, Stepanov I. Urinary levels of the tobacco-specific carcinogen N'-nitrosonornicotine and its glucuronide are strongly associated with esophageal cancer risk in smokers. Carcinogenesis, 2011; 32: 1366–1371.
59. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer, 2015; 136: E359–E386.
60. Hatsukami DK, Stepanov I, Severson H, Jensen JA, Lindgren BR, Horn K, Khariwala SS, Martin J, Carmella SG, Murphy SE, Hecht SS Evidence supporting product standards for carcinogens in smokeless tobacco products. Cancer Prev Res (Phila), 2015; 8: 20–26.
61. Gartner CE, Hall WD, Vos T, Bertram MY, Wallace AL, Lim SS. Assessment of Swedish snus for tobacco harm reduction: an epidemiological modelling study. Lancet, 2007; 369: 2010–2014.
62. Foulds J, Ramstrom L, Burke M, Fagerstrom K. Effect of smokeless tobacco (snus) onsmokingandpublichealthinSweden. TobControl, 2003; 12: 349–359.
63. Stepanov I, Gupta PC, Dhumal G, Yershova K, Toscano W, Hatsukami D, Parascandola M. High levels of tobacco-specific N-nitrosamines and nicotinein Chaini Khaini, a product marketed assnus. Tob Control, 2015; 24: e271–274.