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Chemical and toxicological characteristics of conventional and low-TSNA moist snuff tobacco products

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HIGHLIGHTS

- The first study to apply multidimensional data analysis to determine the similarity and differences in features of chemicals and cancer risks among the most popular moist snuff products worldwide.
- Identification of clearly different toxicant constituents through multidimensional data approach for a wide range of chemicals.
- Differences in toxicant levels when expressed on a dry weight basis, but less variation when results are normalized for nicotine content.
- Higher cancer risk estimates for dry weight determinations than nicotine normalized determination.

GRAPHICAL ABSTRACT

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ABSTRACT

Use of smokeless tobacco products (STPs) is associated with oral cavity cancer and other health risks. Comprehensive analysis for chemical composition and toxicity is needed to compare conventional and newer STPs with lower tobacco-specific nitrosamines (TSNAs) yields. Seven conventional and 12 low-TSNA moist snuff products purchased in the U.S., Sweden, and South Africa were analyzed for 18 chemical constituents (International Agency for Research on Cancer classified carcinogens), pH, nicotine, and free nicotine. Chemicals were compared in each product using Wilcoxon rank-sum test and principle component analysis (PCA). Conventional compared to low-TSNA moist snuff products had higher ammonia, benzo[a]pyrene, cadmium, nickel, nicotine, nitrate, and TSNAs and had lower arsenic in dry

Abbreviations: CPDB, California's; CPF, carcinogenic potency factor; DMSO, dimethyl sulfoxide; EPA, Environmental Protection Agency; FDA, Food and Drug Administration; HNSCC, head and neck squamous cell carcinoma; HPHCs, harmful and potentially harmful constituents; IARC, International Agency for Research on Cancer; LOD, limit of detection; LOQ, limit of quantification; MN, micronucleus; NAB, *N*′-Nitrosoanabasine; NAT, N′-Nitrosoanatabine; NDMA, *N*′-nitrosodimethylamine; NNK, nicotine-derived nitrosamine ketone; NNK, 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*′-Nitrosonornicotine; NRU, Neutral Red Uptake; PAHs, polycyclic aromatic hydrocarbons; PCA, principle component analysis; SD, standard deviation; STPs, smokeless tobacco products; TSNAs, tobacco-specific *N*-nitrosamines.

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weight content and per mg nicotine. Lead and chromium were significantly higher in low-TSNA moist snuff products. PCA showed a clear difference for constituents between conventional and low-TSNA moist snuff products. Differences among products were reduced when considered on a per mg nicotine basis. As one way to contextualize differences in constituent levels, probabilistic lifetime cancer risk was estimated for chemicals included in The University of California's carcinogenic potency database (CPDB). Estimated probabilistic cancer risks were 3.77-fold or 3-fold higher in conventional compared to low-TSNA moist snuff products under dry weight or under per mg nicotine content, respectively. *In vitro* testing for the STPs indicated low level toxicity and no substantial differences. The comprehensive chemical characterization of both conventional and low-TSNA moist snuff products from this study provides a broader assessment of understanding differences in carcinogenic potential of the products. In addition, the high levels and probabilistic cancer risk estimates for certain chemical constituents of smokeless tobacco products will further inform regulatory decision makers and aid them in their efforts to reduce carcinogen exposure in smokeless tobacco products.

1. Introduction

Smokeless tobacco products (STPs) have been increasingly promoted in recent years by major tobacco companies as an alternative to smoking (Hatsukami et al., 2007), and the global market has grown considerably (Delnevo et al., 2014). STPs includes chewing tobacco (loose leaf, plug, or twist), snuff (moist and dry), and dissolvable (lozenges, sticks, strips, and orbs) (IARC, 2007). On the U.S. market, conventional and low-TSNA moist snuff products are most popular forms of STPs (Stepanov et al., 2008a; Xue et al., 2014). Moist snuff products are sold as small pouches or powder and are held in the mouth between the lip or cheek and gum or sniffed up the nose, rather than smoking (Stepanov et al., 2008a). Tobacco companies are marketing these products with colorful packages and sweet flavors, which make them more attractive to youth, young adults, and women as a substitute for smoking (Adkison et al., 2014).

Low-TSNA moist snuff products sold in the recent within Sweden, the US, and elsewhere are distinguished from conventional moist snuff products because they are produced using different curing methods that greatly reduce TSNAs, namely air- and sun-cured in contrast to conventional moist snuff STPs that tent to include blends in fire-cured tobacco (Foulds et al., 2003; Rutqvist et al., 2011). In addition to these differences, low-TSNA moist snuff products contain pasteurized tobacco and generally refrigerated immediately after production to help minimize of the risk of the formation of TSNAs and other toxicants in the product during storage, whereas conventional moist snuff products are mostly fermented, allowing continued formation of TSNAs (Foulds and Furberg, 2008; Osterdahl and Slorach, 1983; Twombly, 2010).

Although the use of the STPs is considered less harmful than smoking for exclusive users because STPs do not yield combustion products when used, they still contain a large number of chemicals and carcinogens (IARC, 2007). By The Food and Drug Administration (FDA) under The Family Smoking Prevention and Tobacco Control Act of 2009 (Tobacco Control Act or TCA), a tobacco product including the STPs standard, what is put into STPs, has been established (Section 907)

(http://www.fda.gov/TobaccoProducts/GuidanceComplianceRegulatoryInformation/ucm263053.htm). Moreover, The FDA Center for Tobacco Products current Research Priorities include the study of smokeless tobacco toxicity (https://prevention.nih.gov/tobacco-regulatory-science-program/research-priorities), and has established a list of harmful and potentially harmful constituents (HPHCs) in tobacco including STPs (http://www.fda.gov/downloads/TobaccoProducts/GuidanceComplianceRegulatoryInformation/UCM297981.pdf). Nine constituents in STPs are currently in enforcement discretion to require reporting for industry (http://www.fda.gov/downloads/TobaccoProducts/GuidanceComplianceRegulatoryInformation/UCM297981.pdf).

Scientific evidences have shown that health risks associated with snus use are lower than those associated with cigarette smoking (Lewin et al., 1998; Schildt et al., 1998; Ye et al., 1999), but it should be noted that there are some population studies for adverse effects of STPs use (Hecht et al., 2007; Luo et al., 2007; Martin et al., 1999; Zhou et al., 2013). A large study found that exposure levels of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in STPs users ($n = 182$) were comparable to those in cigarette smokers ($n = 420$) (Hecht et al., 2007). Clinical outcomes for oral leukoplakia showed association with use of U.S. conventional moist snuff products including Copenhagen, Skoal, and Kodiak (Martin et al., 1999). In a case-control study, individuals who reported 10 or more years of STPs use had a significantly increased risk of head and neck squamous cell carcinoma (HNSCC), suggesting adverse effects of long-term use of STPs(Zhou et al., 2013). Another study showed that use of Swedish snus could heighten a user's risk for pancreatic cancer (Luo et al., 2007).

According to The International Agency for Research on Cancer (IARC), approximately 30 known or probable human carcinogens in STPs are identified (IARC, 2007). A number of studies have reported chemical composition of STPs worldwide, but the majority of the studies have limited on dry snuff, chewing tobacco, plug, tobacco pellets, conventional moist snuff, or Swedish snus (Hoffmann and Djordjevic, 1997; IARC, 2007; Lawler et al., 2013; Rodu and Jansson, 2004). Recently, some studies have reported large variation in the levels of some toxicants in low-TSNA moist snuff products; however, these studies have mainly focused on a limited range of toxicants such as polycyclic aromatic hydrocarbons (PAHs) (Stepanov et al., 2008a; Stepanov et al., 2010) and TSNAs (Lawler et al., 2013; Stepanov et al., 2008a).

Smokeless tobacco is classified as a known human carcinogen by IARC, and various constituents are similarly classified (http://monographs.iarc.fr/). Currently, there is no way to relate a particular level of a chemical constituent in STPs to cancer risk. The University of California's Carcinogenic Potency Database (CPDB) provides a carcinogenic potency from systematic and unifying analysis of chronic and long-term animal cancer tests in literature through 2001 and by the National Cancer Institute/National Toxicology Program through 2004, which can be used to contextualize the magnitude for differences among risk by individual constituents. This approach has been done for cigarette smoke by Fowles and Dybing (Fowles and Dybing, 2003) using the US Environmental Protection Agency (EPA)'s cancer potency values, analogous to what is available from the CPDB (USEPA, 1991), and probabilistic lifetime cancer risk for selected Swedish snus and moist snuff products were estimated by Ayo-Yusuf and Connolly using CPDB's carcinogen potency values (Ayo-Yusuf and Connolly, 2011). These databases somewhat assign cancer risks based on experimental animal studies and safety quotients to account for uncertainty. Thus, while they are a means to compare