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Chemical characterization of domestic oral tobacco products: Total nicotine, pH, unprotonated nicotine and tobacco-specific *N*-nitrosamines

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Abstract

In the United States, moist snuff has been studied more widely than other distinct categories of oral tobacco. In this study, we measured pH, moisture, nicotine (total and unprotonated), and tobacco-specific N’-nitrosamines (TSNAs) for other established (twist, loose leaf, plug, and dry snuff without pouch) and emerging oral tobacco products (dry snuff pouch, US-made snus, and dissolvable tobacco). Among the seven product categories, product pH ranged from 4.7 to 7.9, and total nicotine concentration spanned from 3.9 to 40.1 mg/g. The most readily absorbable form of nicotine (unprotonated nicotine) varied more than 350-fold, ranging from 0.01 to 3.7 mg/g. While the highest total nicotine concentrations were observed in twist products, snus and dissolvable tobacco had the highest unprotonated nicotine levels. Among all products, total TSNA concentrations ranged from 313 to 76,500 ng/g with dry snuff having the highest total TSNA concentrations. This study demonstrates the diversity among oral tobacco products and highlights the potential of these products to deliver a wide range of nicotine and carcinogenic TSNAs. Characterizing the chemical content of these products may be helpful in further understanding the risk of marketing these products to oral tobacco users and smokers as an alternative and discrete form of tobacco.

Keywords

Oral tobacco; Smokeless tobacco; Nicotine; Unprotonated Nicotine; pH; Tobacco-specific N’-nitrosamines

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1. Introduction

Oral tobacco products, also referred to as smokeless tobacco, are a highly diverse collection of non-combusted tobacco products that deliver nicotine when placed in the oral cavity. In 2011, 8.2 million Americans aged 12 or older were current (past month) users of smokeless tobacco (Substance Abuse and Mental Health Services Administration, 2012). Traditionally, oral tobacco products in the United States were available in two major forms: chewing tobacco and snuff (National Cancer Institute (NCI), 1992). Chewing tobacco, which contains tobacco, sweeteners (e.g., sugar or molasses) and flavorants (e.g., licorice or other additives), is available in three basic varieties: loose leaf, plug and twist (American Chemical Society (ACS), 2009 and World Lung Foundation (WLF), 2009; U.S. Department of Health and Human Services (USDHHS, 1986)). Typically, chewing tobacco users place a portion of the product between the cheek or lip and gum where it is either chewed or sucked. Snuff, a finely ground or pulverized tobacco product, is available as loose dry snuff, loose moist snuff or either form enclosed in tea bag-like pouches (NCI, 1992; International Agency for Research on Cancer (IARC), 2007). Dry snuff, also known as nasal or scotch snuff, consists of fermented and pulverized fire-cured tobacco that is typically held in the mouth or sniffed into the nose (3rd Int. Conf. on Smokeless Tobacco, 2002). Products containing dry snuff enclosed in a sachet (e.g., Taboka and Skoal Dry) are used by holding the pouch between the lip and gum or cheek (Stepanov et al., 2008). These products, however, are no longer sold in the US. Snus, a product based on snuff, which originated in Sweden, is now marketed in the United States. Swedish snus products are processed using steam pasteurized tobacco resulting in lower TSNA levels (Savage, 2007). Due to low salt content, the use of snus reduces salivation and the need for spitting (R.J. Reynolds Tobacco Company (RJRT), n.d.; Campaign for Tobacco-Free Kids, 2008).

For clarification, the term smokeless tobacco is used for tobacco products that are not burned during use; however, it should be noted that some smokeless products are exposed to smoke during fire-curing and can contain higher levels of compounds such as polycyclic aromatic hydrocarbons (PAHs), a class of compounds that are typically associated with cigarette smoke exposure (Stepanov et al., 2010; Hearn et al., 2013; Rodgman and Perfetti, 2009). In this study, we refer to all seven tobacco product categories (and moist snuff) as simply oral tobacco products (OTP) (See Table 1). Photographs of these tobacco product categories are found in Figure 1.

Recently, there has been renewed interest in oral tobacco products made from compressed tobacco that are designed to dissolve completely. These range in appearance from a small pellet to a “stick”, or “strip.” These products can be used more discretely than traditional oral tobacco products as there is no spitting and the tobacco portion is not discarded following use. However, there has been some concern over product safety issues resulting in additional health concerns such as infant and child choking or poisoning (Connolly et al., 2010).

Nicotine dependence and the subsequent long-term exposure to various harmful tobacco constituents pose significant health risks. Oral tobacco use has been associated with hypertension, heart disease, bone loss around teeth and receding gums, leukoplakia and various cancers (Centers for Disease Control and Prevention (CDC), 2009; IARC, 2007). Tobacco-specific *N*-nitrosamines (TSNAs) include N'-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosoanatabine (NAT), N'-nitrosoanabasine (NAB), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). TSNAs are one of the more potent classes of carcinogens in oral tobacco products formed during the growing, curing, fermentation, and aging of tobacco, and have been linked to cancers of the oral and nasal cavity, kidney, bladder and pancreas (IARC, 2007; NCI, 2003; Rivenson et al., 1988). Fire-cure OTPs can have significant levels of carcinogenic PAHs (Stepanov et al., 2010). Nicotine absorption across oral mucosa is highly dependent upon product pH, which influences the proportion of total nicotine present in the unprotonated form. Compared with protonated nicotine, nicotine in its unprotonated state passes through epithelium much more readily and results in larger and more rapid increases in blood nicotine levels (Tomar and Henningfield, 1997; Fant et al., 1999; Richter and Spierto, 2003; CDC, 1999; Davis and Curvall, 1999). Many heavy metals in the tobacco lamina reflect the growing soil content or fertilizer used and may also increase health risks from exposure particularity for those metals like cadmium which tend to bio-accumulate (Pappas et al., 2008). Because much of the previous work on OTP has focused primarily on moist snuff, we saw a need to expand available data on other product categories.

Previous studies have measured the levels of nicotine and TSNAs in select groups of domestic (IARC, 2007; Richter et al., 2008; Stepanov et al., 2008; Hearn et al., 2013) or international (IARC, 2004; Stepanov et al., 2005; Stanfill et al., 2011) products. Our study examines the levels of pH, total nicotine, unprotonated nicotine, and TSNAs in 29 products representing seven distinct categories of domestic oral tobacco products. These findings may aid other researchers and public health advocates with current information on a wider range of oral tobacco products.

2. Materials and Methods

2.1 Chemicals

Nicotine (purity ≥99%), purchased from Sigma Chemical Co. (St. Louis, MO) was diluted in isopropanol (Tedia; Fairfield, OH) to prepare stock standards. Quinoline (purity ≥97%, Aldrich Chemical Co. (Milwaukee, WI) served as the internal reference for all nicotine analyses. Sodium hydroxide (2N NaOH) was purchased from Red Bird Service (Batesville, IN). HPLC-grade (purity, 99.8%) methyl *tert*-butyl ether (MTBE) was purchased from Spectrum Chemical Mfg. (Gardena, CA). Research grade Helium (purity, 99.9999%) for GC/MS (gas chromatography/mass spectrometry) analysis was obtained from AirGas, Inc. (Jacksonville, FL). All chemicals were screened and used without further purification. Standards and samples were weighed with an analytical balance (Sartorius AG; Göttingen, Germany) to an accuracy of ± 0.01 mg.

2.2 Tobacco Samples

The oral tobacco products were purchased or acquired between May 2007 and May 2009. Camel Orbs was purchased at a retail store in Portland, Oregon. Taboka samples were provided by Phillip Morris USA. All other oral tobacco products were purchased locally in the metropolitan Atlanta, Georgia area. Quality Control (QC) materials included moist snuff purchased from retail stores in Atlanta, Georgia and 2S3 moist snuff reference tobacco provided by the Tobacco Analysis Laboratory (North Carolina State University; Raleigh, NC). The oral tobacco samples were placed in re-sealable plastic bags and stored at −70°C before testing to inhibit formation of TSNAs. Samples were mixed in accordance to standard protocol (Federal Register, 1999).

2.3 Sample Preparation

2.3.1 Measurement of total moisture content and product pH—Total moisture content was determined by the weight difference of the fresh and dried tobacco after drying the tobacco at 99°C for 3 h (Federal Register, 1999). Moisture content measurements provide a means of calculating products values on a wet weight or a dry weight basis. Duplicate pH measurements were performed by suspending 2.0 g of tobacco product in 20 mL deionized distilled water, using a standard pH electrode and Pinnacle M545P pH meter (Nova Analytics Corporation, Woburn, MA) as described in the revised protocol (Federal Register, 2009). An additional sample of tobacco product was prepared in 10 mL deionized distilled water according to the previous protocol for pH comparison (Federal Register, 1999). The pH meter was calibrated daily with standard pH buffers: 4.01, 7.00, and 10.01. For each oral tobacco sample, pH readings at 5, 15, 30, and 60 min were measured and averaged. Due to limited sample quantity, dissolvable tobacco pH measurements were made with 1.00 g of oral tobacco in 10 mL deionized distilled water.

It should be noted that the standard pH protocol measures pH of a 2.0 g oral tobacco sample dispersed in 10mL water (Federal Register, 1999). A 2.0g sample of a very low moisture product prepared in 10 mL water produced a thick paste-like consistency that could not be adequately stirred and measured. Doubling the water volume (20 mL) produced a slurry more amenable to analysis. A previous comparison of pH measurements in several oral tobacco products including low moisture products with 10 mL or 20 mL volumes of water showed only slight differences (Federal Register, 2009). The pH differences between the 10 mL or 20 mL preparations in this study were on the order of ±1.0%. The largest change in pH as a function of aqueous volume was the dry snuff (no pouch) products with a maximum 3.1% difference. Products with higher pH (> 7.0) showed little or no change with the additional water volume.

2.3.2 Quantification of total and unprotonated nicotine—The nicotine analysis procedure used in the study was described by Stanfill et al., 2009. Briefly, that method involves adding a 5-mL of 2N NaOH and 50 mL of extraction solution (methyl tert-butyl ether (MTBE) containing quinoline (internal standard)) to a 1-g tobacco sample. Sample vials were shaken on an orbital shaker at 160 rpm for 2 h. The resulting extract was transferred to a 2-mL autosampler vial. For analysis, 1-µl from each vial was injected into an Agilent 6890 gas chromatograph /5973N Mass Selective Detector (MS) operated in selected ion monitoring mode. A flow rate of 1.7 ml/min was used on an Ultra2 GC column (25m × 0.32mm × 0.52µm) for separation. The inlet temperature was maintained at 230° C. The oven conditions were 175° C initially, hold for 1 min, ramp at 5° C/min to 180° C, followed by a ramp at 35° C/min to 240° C with a total run time of 3.7 min. Analyses were made in triplicate. Plotting relative response factors (nicotine quantification ion area/quinoline ion area) against nicotine concentrations produced a calibration curve used to quantify total nicotine. The percentage of nicotine present in the unprotonated form was calculated using tobacco product pH and the pK_a value of the pyrollic nitrogen of nicotine (8.02), which is substituted into the Henderson-Hasselbalch equation (Federal Register, 1999). The total amount of unprotonated nicotine was calculated by multiplying the percentage of nicotine in the unprotonated form by total nicotine.

2.3.3 Quantification of Tobacco-specific N-nitrosamines—Triplicate measurements were performed on each of the tobacco-specific *N*-nitrosamines. The oral tobacco samples were ground and about 0.25 g of each sample was placed in an amber extraction vial and spiked with ^{13}C-labeled TSNA internal standards. Samples were then extracted with 10 mL of 100 mM aqueous ammonium acetate buffer using a Lab-line shaker (Melrose Park, IL) operated at 250 rpm for one hour. Each extract was filtered with a 0.45-µm nylon syringe filter. Analytical separation was performed on an Agilent 1100 type high performance liquid chromatography system (Agilent Corporation, Santa Clara, California), using an Xterra C18 MS reversed phase column (Waters Corporation, Milford, MA). Eluent A (aqueous phase) was 5 mM ammonium acetate solution; whereas, eluent B (organic phase) was a mixture of 95% acetonitrile and 5% water with 5mM ammonium acetate content. The column temperature was kept at 60° C and the flow rate was a constant 1.0 mL/min. The eluent gradient was as follows: initial organic phase concentration was 5% for one minute and then increased to 35% at two minutes elapsed time. This plateau of 35% component B was held for 3 minutes, and then decreased to 5% for 1 minute. Finally, 5% organic phase was held for two more minutes to allow for column equilibration before the next injection. The detector was an API 4000 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA), working in electrospray, positive polarity - multiple reaction monitoring (MRM) mode. All chromatographic data were processed using Analyst®_1.5 software from AB Sciex. Mass spectrometry transitions and settings as well as a detailed description of the HPLC method are found in the paper published by Wu et al. (2003).

3. Results and Discussion

In the present study, we examined the major categories of domestic oral tobacco products marketed in the United States (Table 1) excluding moist snuff. The moisture, pH, and the concentrations of total nicotine, unprotonated nicotine, and five TSNA compounds for 40 top-selling brands of moist snuff (loose and pouched varieties) have been reported elsewhere (Richter et al., 2008). One brand in the study by Richter and other CDC scientists was a non-tobacco herbal product (Oregon Mint Snuff). The mean values expressed in our comparison exclude that product. Data for moisture, pH, and the concentrations of total nicotine and unprotonated nicotine are also reported in IARC Monograph 89; however, TSNA values are not reported (IARC, 2007).

In terms of moisture content, products analyzed in this study were drier (3.87 – 29.5%) than moist snuff brands measured previously. Percent moisture in moist snuff ranged from 44.5 to 54.5% with exception of two brands (27.4 – 33.0%) (Richter et al., 2008). In our current study, the products examined had pH values ranging from 4.73 to 7.88 (Table 2) with a mean value of pH 7.56. Plug (pH 5.10 – 5.95), loose leaf (pH 5.64 – 5.98), twist (pH 4.73 – 5.77), and traditional non-pouch dry snuff (pH 5.71 – 6.25) products analyzed had acidic pH resulting in a small percentage (0.05–1.68%) of the total nicotine being present as unprotonated nicotine. Pouched dry snuff (pH 6.42 – 7.24), snus (pH 7.55 – 7.70), and dissolvables (pH 7.23 – 7.88) had slightly acidic to slightly alkaline values ranging from pH 6.04 – 7.88 resulting in a higher percentage (2.45 – 41.9%) of total nicotine being present as unprotonated nicotine. The pH for moist snuff products ranged from 5.54 to 8.62 (mean, 7.73) with seven brands having values equal or greater than pH 8.00, resulting in the percentage of total nicotine present as unprotonated nicotine from 0.30% to 79.9 % (Richter et al., 2008).

In this study, we observed that total nicotine concentrations ranged from 3.90 to 40.1 mg/g. Two dissolvable products (3.90 – 4.09 mg/g) and two twist products (30.1 – 40.1 mg/g) (Table 2) were outside of the range observed for total nicotine in moist snuff brands, which ranged from 4.42 to 25.03 mg/g tobacco with a mean of 11.9 mg/g (Richter et al., 2008). Comparison of tobacco products on the basis of total nicotine alone is misleading because unprotonated nicotine is the form of nicotine that is most readily absorbed. For this reason, products with low pH and high nicotine, such as twist and dry snuff or some moist snuff products (Richter et al., 2008) can contain very low unprotonated nicotine values. Calculated total unprotonated nicotine concentrations for the seven oral tobacco categories ranged from ~0.01 to 3.69 mg/g. The ranges of unprotonated nicotine for the moist snuff product category is much wider (range 0.01 – 7.81 mg/g; mean 3.8 mg/g (Richter et al., 2008). Among the established product categories examined in this study, twist chewing tobacco generally had lower levels of unprotonated nicotine than did the emerging product categories (e.g., snus, dissolvables) that are more frequently advertised (Federal Trade Commission (FTC), 2009; Campaign for Tobacco-Free Kids, 2008; Rogers et al., 2010). The calculated mean total unprotonated nicotine for the oral tobacco product categories in this study ranged from ~0.03 mg/g (plug tobacco) to 2.94 mg/g (snus tobacco), a 100-fold difference. In addition, the values found in Table 2 were comparable to the data ranges found in the 2004 Massachusetts Department of Public Health (MDPH) Smokeless Tobacco Data Base (IARC, 2007).

A comparison of mean values of total moisture, total nicotine, pH, and unprotonated nicotine was made using either 10 mL or 20 mL of water by oral tobacco product type (Table 3). When comparing the different types of tobacco measured for pH at 10 mL and 20 mL, it is clear that there is little or no difference in the reported values for all tobacco types except for dry snuff and loose leaf for pH values. Dry snuff was the only product that exhibited a noticeable shift in reported unprotonated nicotine values (from 0.13 to 0.18 mg/g). These findings support the use of a 20ml water volume to ensure that pH is measured in a well-mixed solution, rather than a slurry, even when tobacco products are dry, highly absorptive or difficult to stir.

Products with a given brand name can be formulated differently over time. In 2006, Skoal Bandits, a pouched moist snuff product popular among beginning users, changed the packaging lid of two flavor products, Mint and Wintergreen, to a new chevron-style design. The unprotonated nicotine concentrations of these Skoal Bandits (Mint and Wintergreen) products with the new packaging (4.77 and 3.81 mg/g, respectively) were approximately 10–12 times higher than the previous package design (0.37 and 0.39 mg/g, respectively) (Table 4). In this case, the change in unprotonated nicotine was due to both total nicotine (an increase of 4.74 and 6.32 mg/g) and pH (a 0.8 and 1.1 pH unit increase) in the products (Table 4). In the current regulated environment, changes in product design resulting in shifts in unprotonated nicotine must be reported to the US Food and Drug Administration, Center for Tobacco Products, which has authority to determine whether permitting the marketing of products with changes such as this are appropriate for the protection of public health.

The levels of TSNAs in the oral tobacco products were examined (Table 5). The NNK levels ranged from 49 to 14,600 ng/g and the NNN levels spanned from 74 to 31,300 ng/g. The highest NNK and NNN levels were measured in dry snuff products (Dental Mild Scotch Snuff and Tube Rose Sweet Scotch Snuff) while much lower NNK and NNN levels were measured in Stonewall Wintergreen (49 ng/g and 94 ng/g), Ariva Wintergreen (52 ng/g and 77 ng/g) and Ariva Java (54 ng/g and 74 ng/g), respectively. The levels of NNAL in this study ranged from below the detectable limits to 1050 ng/g. A dry snuff brand (Dental Mild Scotch Snuff) contained the highest concentration of NNAL. Mean total TSNA levels increased across oral tobacco product categories examined in this study following the trend: dissolvables < snus < dry snuff pouch < loose leaf < twist < plug < dry snuff. Previously reported mean total TSNA levels for moist snuff (Richter et al., 2008) are higher than values for plug but lower than dry snuff products included in this study. The TSNA content found in this study was also comparable to the Stepanov et al., 2008 tobacco-specific nitrosamine levels when calculated on a dry weight basis.

It is possible that the ordering of the product categories could change over time or the mean values could shift if a larger number of brands were tested. The limited number of samples examined is a limitation of this study. The samples included do not represent all brands currently marketed. Most of the tobacco products were purchased from local metro Atlanta, GA retail stores (with the exception of Taboka and Camel Orbs) and may not reflect regional differences. Our results are based on replicate analysis of only a few product packages; therefore, the product results may not reflect the variation of lots over time.

4. Conclusion

This study supports the assertion that all oral tobacco products, taken together, should not be thought of as a single, homogeneous category of products, rather there is a wide variation in pH, nicotine, and TSNAs as a whole and even within sub-categories (IARC, 2007; Stanfill et al., 2011). Tobacco products are not equal in their potential to deliver addictive nicotine and carcinogenic TSNAs. The results of these analyses support other reports that certain newly emerging forms of oral tobacco (e.g., snus and dissolvable) products contain lower TSNA levels than some established forms of oral tobacco (e.g., dry snuff, twist, loose leaf, and plug) products, indicating it is possible to manufacture oral tobacco products with lower levels of this class of potent carcinogens. Unfortunately, lowering only one particular class of harmful chemicals may have no impact on the levels of other harmful constituents such as polycyclic aromatic hydrocarbons, phenols, or heavy metals (not analyzed in this study). Traditional oral tobacco product categories, in general, contained higher amounts of TSNAs.

Conversely, some of the newly emerging products have higher nicotine and their design may make them attractive to youth (Connolly et al., 2010). This publication together with recent publications addressing toxic constituents of moist snuff, iq'mik and a wide variety of international oral tobacco products, gives us a more comprehensive understanding of the diversity that exists among oral tobacco products domestically and globally (Connolly et al., 2010; Stepanov et al., 2010; Pappas et al., 2008; Richter et al., 2009; Hearn et al., 2013; Stanfill et al., 2011). It is important for consumers, public health decision makers, and researchers to understand the variability that exists in domestic oral tobacco products. Small differences in some of the products' harmful or potentially constituents may or may not contribute to overall reduced harm. At present the only proven means to reduce health risks associated with tobacco use is by never starting to use or quitting early in life.

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Highlights

*This study reports pH, nicotine, and tobacco-specific *N*-nitrosamines in seven categories of oral tobacco products. *Among these samples, newer forms of oral tobacco contained lower TSNA levels than more established forms of oral tobacco. *Our data suggests that it is possible to manufacture oral tobacco products with lower levels of potent carcinogens.* Some newer forms of oral tobacco have designs that may be more attractive to youth and also have higher nicotine levels. *Understanding nicotine and TSNA content may help evaluate risks to smokeless users and smokers using these products.

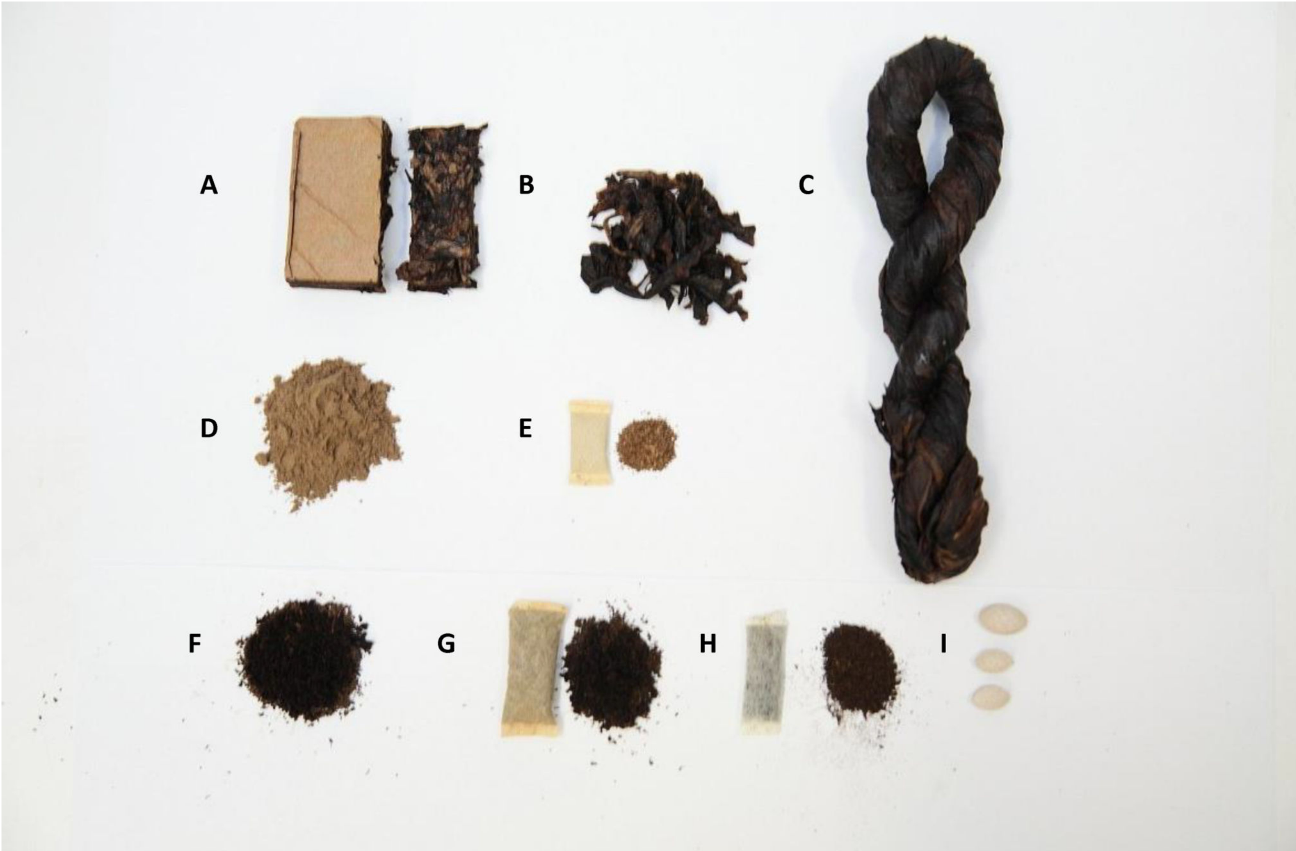

Figure 1.

Photographs of eight categories of oral tobacco product that have been sold in the USA

Note. (A) Plug; (B) Loose Leaf; (C) Twist; (D) Dry Snuff (loose); (E) Dry Snuff (pouch); (F) Moist Snuff; (G and H) Snus; and (I) Dissolvable.

Table 1

Description of oral tobacco product (OTP) types available in the United States.

Type	Common examples	Description	Content	Characteristics
Plug[a]	Levi Garrett, Days O Work	Tobacco leaves pressed into bricks or cakes	Burley, bright or cigar tobacco	Sugar content: approximately 24%. Plug available as moist plug (>15% moisture) or firm plug (<15% moisture)
Loose leaf[a]	Red Man, Beechnut	Shredded tobacco leaves	Air-cured, cigar leaf tobaccos from Pennsylvania and Wisconsin	High sugar content (approx. 35%)
Twist[a]	Cumberland, Mammoth Cave	Dried tobacco leaves twisted into a 'rope'	Dark and air-cured leaf tobacco and treated with tar-like tobacco leaf extracts	Normally no added sweetener or flavorings
Dry snuff (loose)[b]	Tube Rose, Navy	Finely ground or pulverized tobacco	Fermented fire-cured tobacco from Kentucky and Tennessee	Moisture content is < 10%; available in two varieties, "sweet" and "salty"; this type of snuff can be used in the mouth or sniffed into the nose
Dry snuff (pouch)[b]	Skoal Dry, Taboka	Finely ground or pulverized tobacco enclosed in pouch	Fermented fire-cured tobacco from Kentucky and Tennessee	Very low moisture levels; available in cinnamon, spice, frost varieties, etc.
Moist snuff[b,c]	Copenhagen, Skoal	Ground or minced tobacco	Air-cured or fire-cured tobaccos	Moisture content ranges from 10%–55%; may be highly flavored with wintergreen, mint, apple, berry, bourbon, etc.
Snus	Camel Snus, Marlboro Snus	Tobacco enclosed in a pouch	Steam-cured and pasteurized tobacco	Pouches typically 6–15 per pack
Dissolvable	Ariva, Camel Orbs	Pellet made from compressed tobacco	May contain 100% Virginia tobacco	Typically 15–20 per pack

Note: USDHHS, 1986; IARC, 2007; 3rd Int. Conf. on Smokeless Tobacco, 2002; RJRT, n.d.; Star Scientific, Inc., 2007.

[a]Loose leaf, plug, and twist are collectively known as chewing tobacco.

[b]Snuff may refer to moist or dry snuff.

[c]This product type was not analyzed in this study.

Table 2

Levels of pH, total moisture, total nicotine (mg/g) and unprotonated nicotine (%, mg/g) in seven categories of oral tobacco products marketed in the United States.

Tobacco Product Type	Brand	Manufacturer	Total moisture[a] (%)	pH[b]		Total nicotine[c] (mg/g, wet)		Unprotonated Nicotine (%)	Unprotonated Nicotine (mg/g, wet)
				Mean	(SD)	Mean	(SD)		
Plug	Days O Work	Swedish Match	15.0	5.10	(0.01)	9.35	(0.33)	0.12	0.01
	Sun Cured	American Snuff[d]	16.4	5.17	(0.02)	15.1	(0.45)	0.14	0.02
	Levi Garrett	American Snuff	21.7	5.92	(0.01)	5.12	(0.06)	0.78	0.04
	Taylor's Pride Plug Chew	American Snuff	20.2	5.95	(0.03)	5.15	(0.09)	0.85	0.04
Loose Leaf	Beech-Nut Chew	National Tobacco	22.0	5.64	(0.03)	6.97	(0.19)	0.42	0.03
	Taylor's Pride Chew	American Snuff	20.6	5.89	(0.05)	4.87	(0.13)	0.73	0.04
	Red Man Chew	Swedish Match	23.2	5.98	(0.02)	7.04	(0.08)	0.90	0.06
Twist	Good Money	R.C. Owen	16.8	4.73	(0.02)	30.1	(0.43)	0.05	0.02
	Cumberland	American Snuff	19.3	5.75	(0.05)	21.6	(0.39)	0.53	0.12
	Futurity	R.C. Owen	8.98	5.77	(0.02)	40.1	(0.10)	0.56	0.22
Dry Snuff	Tube Rose Sweet Scotch	American Snuff	6.13	5.71	(0.02)	14.9	(0.25)	0.48	0.07
	Dental Mild Scotch	American Snuff	5.79	5.99	(0.01)	20.2	(0.35)	0.93	0.19
	Navy Sweet Scotch	Swisher	6.78	6.02	(0.01)	17.0	(0.15)	0.98	0.17
	RailRoad Mills Sweet Scotch	Swisher	7.46	6.02	(0.00)	18.1	(0.29)	0.99	0.18
	Carhart's Choice Sweet	USSTC[e]	6.43	6.25	(0.03)	17.9	(0.16)	1.68	0.30
Dry Snuff (Pouch)	Taboka	Philip Morris USA	6.15	6.42	(0.14)	14.0	(0.18)	2.45	0.34
	Skoal Dry Cinnamon	USSTC	5.97	6.78	(0.07)	10.5	(0.34)	5.46	0.57
	Skoal Dry Menthol	USSTC	6.57	7.22	(0.14)	11.4	(0.49)	13.8	1.57
	Skoal Dry Regular	USSTC	6.04	7.24	(0.02)	10.8	(0.49)	14.1	1.52
Snus	Camel Snus Frost	R.J. Reynolds	27.6	7.55	(0.03)	9.99	(0.06)	25.1	2.51
	Camel Snus Spice	R.J. Reynolds	20.5	7.64	(0.00)	8.97	(0.11)	29.3	2.63
	Camel Snus Original	R.J. Reynolds	29.5	7.70	(0.02)	11.3	(0.19)	32.6	3.69
Dissolvable[f]	Stonewall Wintergreen	Star Scientific	6.08	7.23	(0.03)	8.34	(0.22)	14.1	1.17
	Stonewall Java	Star Scientific	4.64	7.34	(0.03)	8.74	(0.11)	17.2	1.50
	Stonewall Natural	Star Scientific	3.87	7.39	(0.03)	8.60	(0.11)	19.1	1.64

Tobacco Product Type	Brand	Manufacturer	Total moisture[a] (%)	pH[b]		Total nicotine[c] (mg/g, wet)		Unprotonated Nicotine (%)	Unprotonated Nicotine (mg/g, wet)
				Mean	(SD)	Mean	(SD)		
	Ariva Java	Star Scientific	5.00	7.46	(0.02)	5.70	(0.02)	21.4	1.22
	Ariva Wintergreen	Star Scientific	4.56	7.57	(0.05)	5.81	(0.08)	26.4	1.53
	Camel Orbs Mellow	R.J. Reynolds	5.80	7.80	(0.05)	4.09	(0.13)	37.7	1.54
	Camel Orbs Fresh	R.J. Reynolds	6.15	7.88	(0.05)	3.90	(0.12)	41.9	1.64

[a]Based on a single measurement; sample weight: 2.0000 ± 0.0005 g.

[b]n = 2.

[c]n = 3.

[d]Previously known as Conwood.

[e]US Smokeless Tobacco Company.

[f]Due to limited sample quantity for dissolvable tobacco, pH measurements were made by measuring 1.0000 ± 0.0005 g of tobacco in 10 mL deionized distilled water and total moisture content was accurately weighed to 1.5000 ± 0.0005 g. Measurements of pH in 1.00 g in 10 mL deionized distilled water were found to be statistically equivalent to 2.00 g in 20 mL deionized distilled water.

Table 3

Mean values of total moisture, total nicotine, pH, and unprotonated nicotine using either 10 mL or 20 mL of water for each domestic oral tobacco product type.

Tobacco Type	Number of brands	Total moisture (%)	Total nicotine (mg/g, wet)	pH		Unprotonated Nicotine (mg/g, wet)	
		Mean	Mean	Mean		Mean	
				10 mL	20 mL	10 mL	20 mL
Dry snuff	5	6.52	17.6	5.86	5.99	0.13	0.18
Loose leaf	3	21.9	6.29	5.74	5.82	0.04	0.04
Twist	3	15.0	30.6	5.34	5.39	0.10	0.11
Plug	4	18.3	8.68	5.48	5.55	0.03	0.03
Dry snuff (pouch)	4	6.18	11.7	6.94	6.98	1.08	1.14
Snus	3	25.9	10.1	7.64	7.64	2.97	3.01

Table 4

Levels of pH, total nicotine (mg/g), and unprotonated nicotine (%, mg/g) found in two flavors (Wintergreen (WG) and Mint) of Skoal Bandits before (August 2004) and following brand repackaging (December 2006).

Smokeless brand	Purchase Date	pH (Mean ± SD)	Total nicotine (mg/g, wet) (Mean ± SD)	Unprotonated Nicotine (%)	Unprotonated Nicotine (mg/g, wet)
Skoal Bandits Wintergreen[1]	January 1999	6.85 ± 0.07	7.11 ± 0.22	6.40	0.45
Skoal Bandits Wintergreen[2]	August 2004	6.84 ± 0.00	6.23 ± 0.09	6.20	0.39
Skoal Bandits Wintergreen[2]	December 2006	7.66 ± 0.00	12.55 ± 0.17	30.3	3.81[3]
Skoal Bandits Mint[2]	August 2004	6.72 ± 0.00	7.64 ± 0.10	4.80	0.37
Skoal Bandits Mint[2]	December 2006	7.82 ± 0.03	12.38 ± 0.14	38.6	4.77[4]

[1] Data taken from: *Morbidity and Mortality Weekly Report*, 48:398–401. Mint Skoal Bandit and Copenhagen snuff data was triplicate measurements of packages purchased from four locations in Florida (n=12) between January–February 1999.

[2] Nicotine and pH data were for triplicate measurements from a single package.

[3] The weight of a Wintergreen (WG) Skoal Bandits pouch as 0.65 g (triplicate). When unprotonated nicotine is expressed on a per pouch basis, the amount of unprotonated nicotine per pouch for Mint Skoal Bandits is approximately 3.10 mg/pouch.

[4] The weight of a Mint Skoal Bandits pouch as 0.65 g (triplicate). When unprotonated nicotine is expressed on a per pouch basis, the amount of unprotonated nicotine per pouch for Mint Skoal Bandits is approximately 2.47 mg/pouch.

Table 5

Levels of five tobacco-specific *N*-nitrosamines found in 29 brands representative of seven types of oral tobacco marketed in the United States.

Tobacco Product Type	Brand	NAB[a] (ng/g, wet)		NAT (ng/g, wet)		NNK (ng/g, wet)		NNN (ng/g, wet)		NNAL (ng/g, wet)		Total TSNAs (ng/g, wet) Sum
		Mean[b]	(SD)[c]	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	
Plug	Days O Work	30	(10)	762	(79)	340	(28)	2920	(900)	41	(1.1)	4090
	Conwood Sun Cured	69	(10)	1520	(260)	844	(226)	3130	(260)	11	(11)	5580
	Levi Garrett	199	(29)	1330	(100)	941	(78)	5140	(90)	140	(49)	7750
	Taylor's Pride Plug Chew	183	(6.1)	1400	(100)	803	(83)	4640	(350)	188	(44)	7210
Loose Leaf	Beech-Nut Chew	23	(2.3)	563	(28)	300	(32)	1640	(60)	21	(0.9)	2550
	Taylor's Pride Chew	76	(17)	796	(72)	306	(22)	2830	(240)	90	(80)	4100
	Red Man Chew	16	(2.5)	351	(27)	238	(23)	942	(22)	20[d]	(23)	1550
Twist	Good Money	55	(5.0)	1430	(280)	309	(26)	828	(83)	41	(8.4)	2670
	Cumberland	168	(11)	1660	(70)	556	(28)	2460	(170)	104	(5.1)	4950
	Futurity	54	(5.6)	1310	(90)	350	(28)	871	(74)	n.d.[e]	(−)	2590
Dry Snuff	Tube Rose Sweet Scotch	1840	(40)	29100	(600)	12600	(200)	19000	(960)	442	(67)	63000
	Dental Mild Scotch	1630	(70)	28000	(800)	14600	(1590)	31300	(4890)	1050	(170)	76500
	Navy Sweet Scotch	253	(4.9)	4210	(300)	3000	(100)	8100	(320)	544	(30)	16100
	RailRoad Mills Sweet Scotch	300	(18)	4590	(140)	3820	(80)	9080	(410)	669	(72)	18500
	Carhart's Choice Sweet	124	(11)	2710	(100)	1340	(100)	6120	(200)	47	(14)	10300
Dry Snuff (Pouch)	Taboka	11	(3.3)	474	(29)	84	(3.6)	933	(28)	20	(13)	1520
	Skoal Dry Cinnamon	42	(2.8)	727	(20)	80	(4.8)	929	(96)	n.d.	(−)	1780
	Skoal Dry Menthol	46	(2.7)	708	(8.3)	121	(12)	973	(48)	n.d.	(−)	1850
	Skoal Dry Regular	39	(2.4)	659	(30)	113	(8.9)	945	(62)	3.1	(1.4)	1760
Snus	Camel Snus Frost	28	(1.3)	265	(37)	146	(13)	425	(53)	20	(3.0)	884
	Camel Snus Spice	28	(8.5)	259	(35)	84	(22)	369	(59)	21	(12)	761
	Camel Snus Original	26	(10)	251	(32)	140	(58)	389	(111)	20	(14)	826
Dissolvable	Stonewall Wintergreen	10	(0.2)	218	(9.3)	49	(5.4)	94	(5.8)	n.d.	(−)	371
	Stonewall Java	11	(1.3)	251	(8.0)	63	(3.5)	103	(17)	n.d.	(−)	428
	Stonewall Natural	11	(1.5)	247	(4.0)	73	(6.4)	117	(8.5)	n.d.	(−)	448
	Ariva Java	7.0	(1.4)	178	(11)	54	(5.2)	74	(6.8)	n.d.	(−)	313

Tobacco Product Type	Brand	NAB[a] (ng/g, wet)		NAT (ng/g, wet)		NNK (ng/g, wet)		NNN (ng/g, wet)		NNAL (ng/g, wet)		Total TSNAs (ng/g, wet) Sum
		Mean[b]	(SD)[c]	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	
	Ariva Wintergreen	8.0	(0.8)	176	(3.8)	52	(1.4)	77	(14)	n.d.	(−)	313
	Camel Orbs Mellow	15	(0.4)	176	(15)	147	(7.1)	189	(3.7)	5.6	(1.4)	533
	Camel Orbs Fresh	17	(0.4)	194	(26)	202	(4.4)	193	(8.7)	5.8	(1.8)	612

[a] *N*‘-nitrosoanabasine (NAB); *N*‘-nitrosoanatabine (NAT); 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK); *N*‘-nitrosonornicotine (NNN); 4-(methylnitrsoamino)-1-(3-pyridyl)-1-butanol (NNAL).

[b] n = 3 (Mean).

[c] standard deviation (SD).

[d] n = 2 (one sample was non-detected).

[e] n.d. = non-detected.