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The chemical composition of smokeless tobacco: A survey of products sold in the United States in 2006 and 2007

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ABSTRACT

Selected toxicant concentrations and other chemical measures have been determined for 43 U.S. smokeless tobacco products sold in 2006 and 2007. Products evaluated included moist snuff, dry snuff, loose leaf, plug, dissolvable and snus tobacco brands. Reference products available for scientific research purposes and eleven Swedish products were also evaluated and compared to the commercial products studied. Chemical endpoints determined included benzo[a]pyrene (B[a]P), N′-nitrosonornicotine (NNN), N′-nitrosoanatabine (NAT), N′-nitrosoanabasine (NAB), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N-Nitrosodimethylamine (NDMA), nitrite, cadmium, lead, arsenic, nickel, chromium, chloride, water, pH and nicotine. Different toxicant profiles were observed for the products studied, with snus tobacco brands generally containing relatively low concentrations of B[a]P and tobacco specific nitrosamines (TSNAs) compared to other moist snuffs. Smokeless tobacco reference product toxicant profiles were similar to corresponding commercial products, with the exception of the TSNA content of the dry snuff reference material. TSNA concentrations observed for all commercial products were lower than historically reported values, likely reflecting changes in product shelf life, tobacco curing practices and, possibly, product blend formulations during the last 20–30 years. The survey results summarized provide a temporal point of comparison with future data anticipated from FDA "harmful and potentially harmful constituents in tobacco products" reporting.

1. Introduction

It is generally accepted that no tobacco product is safe and that quitting tobacco use is the best way to eliminate risk. For those who choose to use tobacco, reducing exposure to tobacco toxicants is regarded as one possible approach to diminishing the health risks from such products. Toxicant profiles (i.e., the chemical toxicants and associated concentrations present in smokeless tobacco or cigarette smoke) are expected to vary both within a tobacco category (e.g., one commercial brand style vs. another brand style) and across tobacco product categories (e.g., smokeless tobacco vs. cigarettes). Therefore, the type of tobacco product used as well as the manner and frequency of use may significantly affect an individual's level of risk for serious disease.

Understanding the chemical composition associated with different types of smokeless tobacco, together with the specific chemical characteristics of individual commercial products, is the first step in assessing the potential toxicity of smokeless tobacco products. The development of such information, both on a product-category and product-specific basis, is consistent with recent calls from "the strategic dialogue on tobacco harm reduction" (Zeller et al., 2009), the Life Sciences Research Office (LSRO, 2008) and the WHO Study Group on Tobacco Products (WHO, 2012). Scientists and tobacco control advocates who participated in the dialog have suggested that information regarding the amounts of toxicants in tobacco products should be readily disclosed "by brand and brand subtype" in order to educate public health officials and regulatory policymakers.

Historically, a number of studies have reported the chemical composition of smokeless tobacco products sold in the United States. Generally, such studies have been limited both in terms of the number of analytes and the number of smokeless tobacco products evaluated. For example, since the 1980s, scientists from the U.S. Department of Agriculture and the American Health Foundation have investigated tobacco alkaloid levels, characterized selected toxicants and evaluated flavor components present in smokeless tobaccos (Chamberlain et al., 1988; Brunnemann and Hoffmann, 1992; Djordjevic et al., 1993; Hoffmann et al., 1995; Brunnemann et al., 2002). These studies addressed a relatively limited number of smokeless tobacco brands (typically ∼2–6). Smokeless tobacco brands have often been identified generically (e.g.,

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"Brand A") in these studies. More recent studies have also focused on either a limited number of smokeless products, often with emphasis on smokeless tobacco products introduced into the U.S. market in the last few years (Rodu and Jansson, 2004; McNeill et al., 2006; Stepanov et al., 2006; Hatsukami et al., 2007; Pappas et al., 2008; Richter et al., 2008; Stepanov et al., 2008, 2010; Klus et al., 2009).

Smokeless tobacco brands evaluated in this study include long established commercial brands, selected brands introduced more recently in the United States and several smokeless tobacco reference products intended for scientific research purposes. The established smokeless tobacco brands evaluated include moist snuff, dry snuff, loose leaf, plug and dissolvable tobacco brands sampled from the U.S. market in 2006 and 2007. The products selected for study represented a substantial portion of the total U.S. sales volume (generally ~50% or more), the principal manufacturers and the main pricing points (e.g., premium, value etc.) for each tobacco type in 2006/2007.

Several snus tobacco brands introduced in the U.S. are included in the study. "Snus" refers to a moist snuff tobacco product prepared by heat treating, rather than fermenting, the tobacco. Snus is composed of tobaccos selected for low toxicant content. Snus products may be refrigerated to maintain product quality. Since snus has been commercially available and popular in Sweden for decades, several Swedish snus brands were also studied for comparison purposes.

Smokeless tobacco reference products have been available for scientific research purposes for many years; however, there are relatively few published studies which have included these products in the study design. Reference products are prepared as a large "batch" at a single point in time, thus limiting the inherent variability of the product. Given their consistency, the inclusion of such products in research studies provides a unique means of evaluating the comparability of data generated in different studies, the consistency of data generated in laboratories over time and the differences which may occur when products are tested with more than one analytical method to determine a particular tobacco constituent. Moist snuff (2S3), dry snuff (1S2) and loose leaf reference (2S1) tobacco products are included in this work. This study assesses the relevance of these smokeless tobacco reference products to smokeless tobacco products sold in the U.S. in 2006 and 2007. Until recently, there has not been a snus reference product available. A CORESTA working group has recently prepared a set of smokeless tobacco reference products (CRP1–4) that include a snus reference product (CRP1) (http://www.tobacco.ncsu.edu/strp.html). Once reference value ranges are established for CRP1–4, it is anticipated that the new products will be widely used in smokeless tobacco research.

In June 2009, the U.S. Food and Drug Administration (FDA) assumed regulatory authority for tobacco products per the Family Smoking Prevention and Tobacco Control Act. Under the act, the FDA Center for Tobacco Products has established a list of harmful and potentially harmful constituents in tobacco products and tobacco smoke (FDA, 2012a). The list includes all of the tobacco constituents evaluated in this study, with the exceptions of chloride, NAT and NAB. Recently, the FDA Center for Tobacco Products has issued draft guidance for reporting harmful and potentially harmful constituents in tobacco products and tobacco smoke (FDA, 2012b,c). That guidance specifies six of the compounds determined in this work (arsenic, benzo[a]pyrene, cadmium, nicotine ("total and free"), NNK and NNN) for smokeless tobacco testing and reporting by brand and subbrand.

In addition to the identification of harmful and potentially harmful constituents in tobacco and tobacco smoke, the FDA Center for Tobacco Products has completed other activities which demonstrate their interest in the chemical composition of smokeless tobacco. Specifically, the Center has conducted a workshop on tobacco product analysis which included presentations on new reference products and potential methods of analysis for smokeless tobacco products (FDA, 2012d). Also, on March 1, 2012, the Tobacco Products Scientific Advisory Committee (TPSAC) submitted their final report and recommendations to FDA regarding dissolvable tobacco products (FDA, 2012e). As part of that evaluation, TPSAC reviewed available information related to dissolvable tobacco, including information on the chemical composition of dissolvable and other forms of smokeless tobacco (FDA, 2012f).

The chemical analysis schema applied in this work was intended as a "starting point," as no scientific consensus exists regarding the most significant toxicants in smokeless tobacco products. Studies that elucidate the chemical compositions of smokeless tobacco to identify the toxicants present in such products continue to be an area of active research (Rainey et al., 2011; Grimm and Lauterbach, 2011a,b,c). The tobacco constituents determined in this study represent several different chemical classes. Benzo[a]pyrene (B[a]P), N′-nitrosonornicotine (NNN), N′-nitrosoanatabine (NAT), N′-nitrosoanabasine (NAB), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N-Nitrosodimethylamine (NDMA), nitrite, cadmium, lead, arsenic, nickel and chromium were selected for study, as these chemical endpoints are among the most consistently cited tobacco toxicants. Chloride, water, pH and nicotine determinations were also conducted to further describe the chemical composition of the tobacco products studied.

2. Materials and methods

2.1. Smokeless tobacco product categories

A wide range of smokeless tobacco products are sold in the United States. Some have been commercially available for more than a hundred years and others have been introduced into the market only recently. Smokeless tobacco products differ in many respects, including the types of tobacco used in the product, physical characteristics, methods of use, duration of use and moisture content, among others. Smokeless tobacco products can be broadly categorized as chewing tobaccos, snuff tobaccos and dissolvable tobaccos. Modern chewing tobacco is produced in three forms (loose leaf, plug and twist) and may include additives such as licorice, corn syrup, molasses, saccharin, humectants and preservatives. Snuff is produced in both dry and moist forms. While traditionally a fermented tobacco product, heat-treated snuffs have been recently introduced. Dissolvable tobacco products are also a more recent addition to the marketplace. Chemical analysis results for U.S. smokeless tobacco are summarized in this work on an individual product basis and by product category. Products are categorized according to tobacco type and tax designation as dissolvable, loose leaf, plug, moist snuff or dry snuff tobaccos. A description of each product category follows. A schema summarizing how these smokeless tobacco categories fit into the larger group of smokeless tobacco categories found around the world, together with additional product descriptions, may be found in a smokeless tobacco glossary prepared by CORESTA at (http://www.coresta.org/Reports/CSTS_Smokeless-Tobacco-Glossary.pdf).

2.1.1. Loose leaf tobacco

Loose leaf tobacco is cured and sweetened like plug tobacco, but sold loose in bags rather than in plug form. Traditionally, loose leaf chewing tobacco generally is made from air-cured, cigar-leaf tobaccos grown in Pennsylvania and Wisconsin. It consists of stripped and processed tobacco leaves that are stemmed, cut or granulated and loosely packed to form small strips of shredded tobacco. Consumers either chew the tobacco or "dip" as with snuff, tucking tobacco between the gum and jaw, usually toward the back of the mouth.

2.1.2. Plug tobacco

Plug chewing tobacco is made by pressing together cured tobacco leaves in a sweet (often molasses-based) syrup. Plug chewing tobacco consists of cured burley, bright and cigar tobaccos wrapped in tobacco and pressed together into bricks. Depending on its moisture content, plug tobacco can be firm (less than 15% moisture) or moist (15% or greater moisture). Consumers cut or bite off a piece of the plug to chew. While chewing, plug tobacco may also be held in the cheek or lower lip as it is consumed over several hours.

2.1.3. Moist snuff

Fermented moist snuff is the form of moist snuff traditionally sold in the U.S. Fermented moist snuff, or dipping tobacco, is made primarily from dark air and dark fire-cured tobaccos with moisture content that is typically near 50% of the product weight. Snuff products with $\geqslant$25% moisture content are categorized as moist snuff in this work. Tobaccos are aged, cut, fermented and flavored to produce moist snuff. Moist snuff tobacco cut sizes generally range from ~7 mm for long cut, to 1 mm for mid cut, and down to granules slightly larger than coffee grounds for fine cut. A small amount (a "pinch" or "dip") is placed between the lip or cheek and gum and typically is held in the mouth for 30 min or longer. While moist snuff is most frequently used in loose form, pouched moist snuff is gaining popularity.

Heat treated moist snuff is made from air-cured tobacco. Heat treated moist snuff is typically called "snus," the Swedish word for snuff. Snus is finely ground tobacco that is placed in the mouth. The main ingredients in snus are tobacco, water, salt and flavorings. Snus tobaccos are selected to have very low levels of certain undesirable natural components or toxicants. Snus is sold in two major forms in Sweden, loose and portioned. Portioned snus is contained in prepackaged pouches for ease of use. Heat treated moist snuff products introduced in the U.S. have been pouched products. Most snus sold in Sweden is refrigerated at the point of purchase.

2.1.4. Dry snuff

Dry snuff is a generic term for smokeless tobacco products with low moisture content, typically ~10%. Snuff products with less than 25% moisture content are categorized as dry snuff in this work. Traditionally, dry snuff has consisted of fermented, finely-ground fire-cured tobacco from Kentucky and Tennessee that

Table 1
Smokeless tobacco products samples from the U.S. marketplace.

Brand	Manufacturer[a]	Tobacco type	Moist/dry	Package type	Tobacco form	U.S. price segment	Year sampled	
							2006	2007
Ariva	A	Dissolvable	Dry	Box	Pieces	Premium		X
Stonewall Natural	A	Dissolvable	Dry	Box	Pieces	Premium		X
Beech-Nut Chewing Tobacco	B	Loose leaf	n/a	Pouch	Loose	Premium	X	
Hawken Wintergreen	C	Loose leaf	n/a	Tin	Cut	Premium		X
Lancaster Premium Chewing Tobacco	D	Loose leaf	n/a	Pouch	Loose	Premium		X
Levi Garrett Chewing Tobacco	C	Loose leaf	n/a	Pouch	Loose	Premium	X	
Red Man Chewing Tobacco	I	Loose leaf	n/a	Pouch	Loose	Premium	X	
Red Man Golden Chewing Tobacco	I	Loose leaf	n/a	Pouch	Loose	Premium	X	
Stoker Chew Apple Chewing Tobacco	B	Loose leaf	n/a	Pouch	Loose	Premium		X
Taylor's Pride	C	Plug	n/a	Pouch	One Cut	Premium	X	
Catch Dry Eucalyptus	E	Snuff	Dry	Tin	Pouch	Premium	X	
Catch Dry Licorice	E	Snuff	Dry	Tin	Pouch	Premium	X	
Skoal Dry	F	Snuff	Dry	Tin	Pouch	Premium		X
Taboka	G	Snuff	Dry	Tin	Pouch	Premium		X
Taboka Green	G	Snuff	Dry	Tin	Pouch	Premium		X
Bruton Scotch Snuff	F	Snuff	Dry	Can	Powder	Premium	X	
Dental Sweet Snuff	C	Snuff	Dry	Can	Powder	Premium	X	
Levi Garrett Snuff	C	Snuff	Dry	Can	Powder	Premium		X
Railroad Mills Plain Scotch Snuff	D	Snuff	Dry	Can	Powder	Premium		X
Red Seal Sweet Snuff	F	Snuff	Dry	Can	Powder	Premium		X
Camel Frost	H	Snuff	Moist	Tin	Pouch	Premium	X	X
Camel Original	H	Snuff	Moist	Tin	Pouch	Premium	X	X
Camel Spice	H	Snuff	Moist	Tin	Pouch	Premium	X	X
Cooper Long Cut Wintergreen	D	Snuff	Moist	Tin	Long-Cut	Value		X
Copenhagen	F	Snuff	Moist	Tin	Fine Cut	Premium	X	
Copenhagen Long Cut	F	Snuff	Moist	Tin	Long Cut	Premium	X	
Copenhagen Pouches	F	Snuff	Moist	Tin	Fine Cut	Premium	X	
General Loose	E	Snuff	Moist	Tin	Coarse	Premium	X	
General Original Portion	E	Snuff	Moist	Tin	Pouch	Premium	X	
General White Portion	E	Snuff	Moist	Tin	Pouch	Premium	X	
Grizzly Long Cut Wintergreen	C	Snuff	Moist	Tin	Long Cut	Value	X	X
Husky Fine Cut Natural	F	Snuff	Moist	Tin	Fine Cut	Value		X
Kayak Long Cut Wintergreen	D	Snuff	Moist	Tin	Long Cut	Value		X
Kodiak Premium Wintergreen	C	Snuff	Moist	Tin	Long Cut	Premium	X	X
Longhorn Long Cut Wintergreen	I	Snuff	Moist	Tin	Long Cut	Value		X
Red Seal Fine Cut Natural	F	Snuff	Moist	Tin	Fine Cut	Value		X
Renegades Wintergreen	I	Snuff	Moist	Tin	Pouch	Value		X
Skoal Fine Cut Original	F	Snuff	Moist	Tin	Fine Cut	Premium	X	
Skoal Long Cut Cherry	F	Snuff	Moist	Tin	Long Cut	Premium	X	
Skoal Long Cut Mint	F	Snuff	Moist	Tin	Long Cut	Premium	X	
Skoal Long Cut Straight	F	Snuff	Moist	Tin	Long Cut	Premium	X	
Skoal Long Cut Wintergreen	F	Snuff	Moist	Tin	Long Cut	Premium	X	
Timberwolf Long Cut Wintergreen	I	Snuff	Moist	Tin	Long Cut	Value		X

E = Swedish Match, F = U.S. Smokeless Tobacco Co., G = Philip Morris USA, H = R.J. Reynolds Tobacco Co, I = Swedish Match (Pinkerton Tobacco Co).
[a] Manufacturer key: A = Star Scientific, B = National Tobacco Co., C = American Snuff Co., D = Swisher International Inc.

Table 2
Smokeless tobacco products sampled from the Swedish marketplace.

Brand	Manufacturer[a]	Tobacco type	Moist /dry	Package type	Tobacco form	Year sampled	
						2006	2007
Catch Dry Cassis Menthol	A	Snuff	Dry	Tin	Pouch	X	
Catch Dry Eucalyptus	A	Snuff	Dry	Tin	Pouch		X
Catch Dry Vanilla Coffee	A	Snuff	Dry	Tin	Pouch	X	
Wise Citrus & Menthol	A	Snuff	Dry	Tin	Pouch		X
General Onyx	A	Snuff	Moist	Tin	Pouch		X
Gustavus Original	B	Snuff	Moist	Tin	Pouch	X	
Nick and Johnny Stark	A	Snuff	Moist	Tin	Pouch		X
Rocker Black	D	Snuff	Moist	Tin	Pouch		X
Rocker Silver	D	Snuff	Moist	Tin	Pouch		X
Oliver Twist Original	C	Twist	n/a	Tin	Pellet		X
Picannell Original	A	Twist	n/a	Tin	Pellet		X

[a] Manufacturer key: A = Swedish Match, B = Gustavus, C = House of Oliver, D = Rocker.

was intended to be sniffed up the nose or placed in the oral cavity like moist snuff. Modern dry snuff includes pasteurized products intended for use in the oral cavity.

2.1.5. Dissolvable tobacco

Most forms of smokeless tobacco require removal of the tobacco from the oral cavity after the user is finished with the product in order to discard it. Recently, products have been developed and introduced commercially in the U.S. that are spitless like snus, but are intended to be dissolved completely in the oral cavity. Dissolvable tobacco products consist of finely milled tobacco, flavors and binders. When used, dissolvable tobacco products are placed in the mouth and allowed to dissolve.

2.1.6. Smokeless tobacco products tested

Forty-three brands of smokeless tobacco commercially available in the United States were evaluated in this study (Table 1). Products selected for study included moist snuff (both fermented and heat-treated, i.e., "pasteurized"), dry snuff, loose leaf tobacco, plug tobacco and dissolvable. All principal manufacturers of smokeless tobacco sold in the United States today were represented by the products selected for study. Most brands evaluated were well established in the U.S. marketplace, while others were introduced to the market more recently. All products were purchased in either March–April 2006 or April–June 2007.

Table 3
Smokeless tobacco reference products composition.

Composition	Moist snuff (2S3)	Dry snuff (1S2)	Loose leaf (2S1)
Dark fire cured tobacco (%)	25.63	22.75	
Air cured tobacco (%)	7.80		
Fire cured virginia tobacco (%)		19.66	
Wisconsin air cured tobacco (%)			17.40
Penn. air cured tobacco (%)			15.47
Air cured stems (%)		33.03	
Flue cured stems (%)		15.20	
Burley stems (%)	3.72		
Crushed burley stems (%)			5.80
Sodium carbonate (%)	0.72		
Sodium chloride (%)	7.32	0.36	1.60
Moisture (%)	54.81	9.00	23.48
Glycerin (%)			3.75
Sucrose (%)			23.01
Dextrose (%)			1.70
Maltose (%)			1.30
Other corn syrup solids (%)			6.21
Sodium propionate (%)			0.28

In addition to U.S. smokeless tobacco products, eleven products available for purchase in Sweden (Table 2) and three reference smokeless tobacco products (Table 3) were included in the study for comparison. Upon receipt, all tobacco samples were stored in their original packaging at −8 °C until shipment to a contract laboratory for constituent testing. Samples were shipped to the contract laboratory under ambient conditions.

2.2. Smokeless tobacco reference products

Three smokeless tobacco reference products, 2S3 moist snuff, 1S2 dry snuff and 2S1 loose-leaf chewing tobacco, were obtained from the North Carolina Agricultural Research Service. The reference products were obtained by contacting the Tobacco Analytical Lab Crop Science Department of North Carolina State University in Raleigh, North Carolina. Each smokeless tobacco reference product was manufactured at one point in time as a single "batch." Table 3 summarizes the composition of each product. After manufacturing, the products were stored at −18 °C until shipment for analysis.

2.3. Test methods

Labstat International ULC (Kitchener, Ontario) conducted all analyses under contract to R.J. Reynolds Tobacco Company. The analytical test methods applied consisted of methods specified by Health Canada for regulatory testing and reporting purposes (nicotine, metals, benzo[a]pyrene, tobacco specific nitrosamines, whole tobacco pH), AOAC INTERNATIONAL official methods (moisture, chloride) or Labstat test methods (nitrite, NDMA). Triplicate

Table 4
Test method limits of detection (LOD) and limits of quantitation (LOQ).

Analyte	Limit of detection		Limit of quantitation	
	2006	2007	2006	2007
Nicotine (μg/g) dry wt	75.0	75.0	250.0	250.0
B[a]P (ng/g) as received	0.042	0.042	0.141	0.141
NNN (ng/g) as received	54.0	21.6	180.0	72.0
NAT (ng/g) as received	63.9	25.6	213.0	85.2
NAB (ng/g) as received	31.0	12.4	103.0	41.2
NNK (ng/g) as received	81.5	32.6	272.0	109.0
Nickel (ng/g) dry wt	39.3	39.3	131.0	131.0
Lead (ng/g) dry wt	39.9	39.9	126.0	126.0
Cadmium (ng/g) dry wt	43.3	43.3	144.0	144.0
Chromium (ng/g) dry wt	11.9	11.9	39.7	39.7
Arsenic (ng/g) dry wt	25.0	25.0	60.0	60.0
Nitrite (μg/g) as received[a]	4.72	0.57	15.70	1.89
NDMA (ng/g) as received[a]	3.54	1.17	11.70	3.90
Chloride (wt.%) as received	n/a	n/a	0.03	0.03

[a] 2007 limits also apply to Camel Snus samples tested in 2006.

determinations were performed with each test method for each analyte. Changes were made to some test methods during the study to improve sensitivity and to reduce limits of quantitation and detection (Table 4).

It should be noted that the extraction procedures applied are intended to provide quantitative assessment of the toxicant concentrations present in the products studied. The extraction procedures applied do not provide test results indicative of what smokeless tobacco users may be exposed to when using tobacco products, beyond providing an estimate of maximum potential exposure.

2.3.1. Determination of tobacco specific nitrosamines (TSNA) in whole tobacco

N-nitrosonornicotine, 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone, N-nitrosoanatabine and N-nitrosoanabasine were determined according to Health Canada Official Method T-309 (Canada, 2000a,b). During the study, the procedure was modified to increase sensitivity by reducing the final sample volume from 5 mL to 2 mL.

2.3.2. Determination of alkaloids in whole tobacco

Nicotine was determined according to Health Canada Official Method T-301 (Canada, 2000a,b).

2.3.3. Determination of Ni, Pb, Cd, Cr and As in whole tobacco

Nickel, lead, cadmium, chromium and arsenic were determined according to Health Canada Official Method T-306 (Canada, 2000a,b). Microwave digestion was applied to prepare smokeless tobacco samples for inductively coupled atomic emission spectroscopy (ICP-AES). Lead and arsenic quantitation was achieved by interpolation according to the relevant calibration curves. To compensate for observed sample matrix effects, cadmium, chromium and nickel quantitation was achieved via standard addition, a modification to the official method.

2.3.4. Determination of benzo[a]pyrene (B[a]P) in whole tobacco

Benzo[a]pyrene was determined according to Health Canada Official Method T-307 (Canada, 2000a,b).

2.3.5. Determination of whole tobacco pH

Tobacco pH was determined according to Health Canada Official Method T-310 (Canada, 2000a,b).

2.3.6. Determination of moisture in tobacco

Tobacco moisture was determined according to AOAC INTERNATIONAL Official Method 966.02 via a gravimetric procedure (AOAC INTERNATIONAL, 2000a).

2.3.7. Determination of chlorides in tobacco

Chloride was determined according to AOAC INTERNATIONAL Official Method 963.05 via a potentiometric procedure (AOAC INTERNATIONAL, 2000b).

2.3.8. Determination of nitrite in tobacco

Nitrite was determined by a spectrophotometric method adapted from the AOAC INTERNATIONAL Official Method for the determination of nitrite in cured meats (AOAC INTERNATIONAL, 2000c). For smokeless tobacco samples, 0.5 g of tobacco was extracted with 80 °C type 1 water, using sonication in a heated bath. The extract was brought to volume and filtered to remove solids from solution. An aliquot of the extract was treated with sulfanilamide and N-(1-naphthyl)ethylenediamide dihydrochloride (NED) reagents in 15% acetic acid to form color with any nitrite present in solution. The concentration of nitrite present in the extract was determined by measuring absorbance at 540 nm. During the study, the procedure was modified to increase sensitivity by reducing the final sample volume from 50 mL to 10 mL and by extending the calibration curve to lower concentrations.

2.3.9. Determination of N-Nitrosodimethylamine (NDMA) in whole tobacco

The method for determination of N-Nitrosodimethylamine (NDMA) was developed by Labstat International ULC (Kitchener, Ontario) based on a tobacco TSNA method reported by Risner and Wendelboe (1994). NDMA was extracted from a 1 g tobacco sample using 50 mL of citrate–phosphate buffer containing L-ascorbic acid (55 mM citric acid, 90 mM sodium phosphate dibasic, 20 mM L-ascorbic acid; pH range 4–5) to extract the volatile nitrosamines (VNA) and prevent artifact formation. The sample was extracted for 30 min on a wrist-action shaker to totally saturate the tobacco and extract the VNA. After extraction, N-Nitrosodi-n-hexylamine (NDHA), the internal standard, and 800 μl iso-octane were added to the extraction solution. The entire contents of the flask were transferred to a column containing hydromatrix (Varian Chem Elut CE20100). After allowing the packing to absorb the aqueous solution, the VNA were eluted with approximately 300 mL dichloromethane until 250 mL of eluent is collected. The eluent was concentrated by rotary evaporation to approximately 0.8 mL (approximately 150 rpm; bath temperature 40 °C; 450 mmHg) and made to a final volume of 1 mL with dichloromethane. N-Nitrosodimethylamine (NDMA) quantitation was achieved by gas chromatography-thermal energy analysis (GC-TEA; furnace temperature 500–550 °C dependent on specific instrument sensitivity) using a 30 m × 0.32 mm × 1.0 μm Stabilwax-DB fused silica capillary column temperature programmed from 50 °C to 210 °C (run time 35 min). During the study, the procedure was modified to increase sensitivity by increasing the sample size from 1 g to 3 g.

3. Results and discussion

3.1. Tobacco specific nitrosamine (TNSA) results

TSNAs are considered by many to be the most harmful toxicants present in smokeless tobacco (Brunnemann and Hoffmann, 1992). Animal studies conducted by scientists at the former American Health Foundation and by others have evaluated the carcinogenic potential of both individual TSNAs and some forms of smokeless tobacco (Hoffmann et al., 1992; Schwartz et al., 2010). Results from such studies have led the National Cancer Institute to state in a fact sheet provided to the public (http://www.cancer.gov/cancertopics/factsheet/Tobacco/smokeless) that "Chewing tobacco and snuff contain 28 carcinogens (cancer-causing agents). The most harmful carcinogens in smokeless tobacco are the tobacco-specific nitrosamines (TSNAs)."

Based on the reported biological activity of TSNAs, a substantial body of research has been conducted to understand the role of tobacco production, curing, processing and storage on nitrosamine levels in smokeless tobacco. For example, Hecht et al. (1977) have studied the role of fertilization, curing and tobacco stalk position on NNN levels in tobacco. Andersen et al. (1989, 1993) and Djordjevic et al. (1993) have characterized the effects of temperature and tobacco moisture content on TSNA formation during storage. Burton et al. (1989) and Djordjevic et al. (1989a) have studied the role of tobacco curing conditions on TSNA formation in burley and flue-cured tobaccos. Brunnemann et al. (2002) have investigated differences in oral snuff brands when purchased in different geographical regions. Tobacco cultivar differences have been investigated to determine if burley tobacco breeding lines can be developed with reduced levels of TSNA (Miller, 2008). Such reports have led tobacco growers and manufacturers to make changes in the tobacco

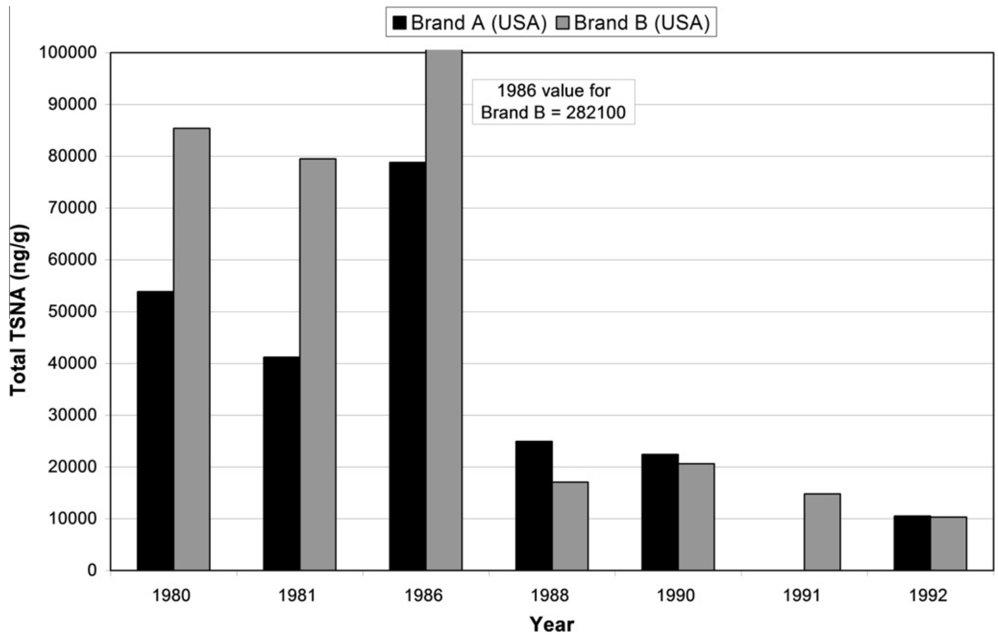

Fig. 1. Change in TSNA levels for two U.S. oral snuff products from 1980 to 1992 (adapted from Djordjevic et al., 1993).

Table 5
Tobacco specific nitrosamine levels observed for different tobacco product categories (all values are expressed on a dry weight basis).[a]

Tobacco type	Products tested	NNN[b] (ng/g)	NAT (ng/g)	NAB (ng/g)	NNK (ng/g)	Total TSNA (ng/g)
Products from U.S. market						
Dissolvable	2	107 (<76–139)[c]	175 (113–236)	<43[d]	<114	399 (<346–<451)[e]
Loose leaf	7	1798 (662–2853)	908 (503–1316)	121 (<53–179)	523 (<140–898)	3350 (<1491–5208)
Plug	1	5053	1702	353	1230	8388
Moist Snuff	23	4058 (659–12,770)	3947 (487–13,908)	387 (63–2221)	1394 (250–6761)	9786 (1840–35,660)
Dry Snuff	10	5535 (814–14,424)	4844 (570–16,124)	577 (<46–3023)	2522 (<121–7387)	14,768 (<1750–40,958)
Products from Swedish market						
Moist Snuff	5	736 (601–885)	586 (422–754)	104 (<78–<195)	275 (<206–<516)	1701 (<1307–<2176)
Dry Snuff	4	585 (<78–843)	447 (<92–588)	89 (<44–<131)	280 (<118–<345)	1402 (<332–<1803)
Twist	2	1229 (1140–1318)	1284 (622–1945)	70 (<61–80)	186 (<137–236)	2769 (<2059–<3480)

[a] Data for individual products are summarized in Appendices A and B.
[b] Mean (minimum–maximum) values found for the products tested in each category. For products that were tested in both 2006 and 2007, the average values for the two years were used in the assignment of category means and ranges.
[c] For analyte responses reported as BLQ (below limit of quantitation) or BLD (below limit of detection), the reported limit of quantitation or limit of detection was used to determine the observed category mean and minimum/maximum range. As such, values that include products with either BLQ or BLD responses are overestimates. When BLQ or BLD values define the range minimum, the values are preceded by "<". TSNA limits of quantitation and detection were reported for tobacco products on an "as received basis." BLQ and BLD values were converted to a dry weight basis based upon the moisture content of each product.
[d] When more than one product was tested in a category and a range is not reported, all values were either BLQ or BLD.
[e] Values are preceded by "<" if any individual TSNA used to calculate the total TSNA level was reported as BLQ or BL.

varieties cultivated for commercial use, as well as changes to tobacco curing, processing and storage practices. Consistent with the changes, smokeless tobacco TSNA levels have decreased substantially since 1980, e.g., TSNA levels in two brands of moist snuff declined by more than 70% from 1980 to 1992 (Fig. 1).

A recent report by Fisher et al. (2011) has provided detailed information regarding the impact of tobacco agronomic and curing practices on TSNA formation in dark fire-cured tobacco, a type of tobacco prevalent in moist snuff brands. The report also describes approaches to help prevent the formation of TSNAs during the tobacco fermentation process and product storage. Results presented for three moist snuff products (no brand identification information provided) demonstrate that TSNAs in commercial brands have continued to decline since 1997, reaching a relatively constant level since 2005. Total TSNA values reported by Fisher et al. are consistent with the average moist snuff total TSNA values found in this work (Table 5).

Although TSNAs are perhaps the most widely studied group of toxicants in smokeless tobaccos, there have been no extensive market surveys previously reported which provide a broad benchmark of TSNA levels in U.S. smokeless tobacco products. While such surveys have been conducted for smokeless tobacco products in other countries, e.g., TSNA levels for 32 smokeless tobacco products marketed in India were reported in 2005 (Stepanov et al., 2005), studies reported for U.S. products have typically included a limited number of smokeless products (Schwartz et al., 2010) and products are often identified generically (i.e., "Brand A") rather than by marketed brand designation (Brunnemann et al., 2002). TSNA levels for the individual smokeless tobacco products surveyed in this work are summarized in Appendices A and B.

Table 5 summarizes TSNA levels found for U.S. and Swedish products by category. U.S. smokeless tobacco products are categorized in the table according to tobacco type and tax designation as dissolvable, loose leaf, plug, moist snuff or dry snuff tobaccos. Large differences in mean total TSNA level are evident for the different categories of U.S. smokeless tobacco with observed TSNA concentrations spanning approximately two-orders of magnitude. Mean total TSNA concentrations are rank-ordered as follows: dry snuff > moist snuff > plug > loose leaf ≫ dissolvable. Within a smokeless tobacco category, total TSNA levels can also vary by more than an order of magnitude, e.g., the moist snuff and dry snuff categories.

More subtle differences are also evident among products within a category with respect to the relative extent to which individual TSNAs contribute to the observed total TSNA level. For some products, the extent to which NNN and NNK (the TSNAs generally considered to be the most biologically active) contribute to the total TSNA level is greater than for other products. For the moist snuff and dry snuff categories, the lowest total TSNA levels are found for snus tobacco brands recently introduced in the U.S., consistent with expectations for a snuff tobacco product prepared (a) by heat treating, rather than fermenting, the tobacco and (b) composed of tobaccos selected for low toxicant content. Total TSNA levels found for the snus products studied, those that define the lower end of the U.S. moist snuff and dry snuff TSNA concentration ranges summarized in Appendix A, are in agreement with total TSNA levels found for snus tobaccos from the Swedish market found in Appendix B and reported by Klus et al. (2009).

Comparison of TSNA levels observed for U.S. smokeless tobacco products (Table 5) with reference smokeless tobacco products (Table 10) suggests that the 2S3 reference moist snuff and the 2S1 reference loose leaf tobacco are each generally consistent with smokeless tobacco products sold in the U.S. in 2006 and 2007. While the individual reference products may be above or below the mean values observed in this survey, each reference was within the range of TSNA concentrations observed for the particular type of smokeless tobacco. The 1S2 reference dry snuff greatly exceeded all TSNA levels observed for dry snuff products commercially available in the U.S. in 2006 and 2007. Since the 1S2 dry snuff was produced a number of years ago, this finding emphasizes the downward change in smokeless tobacco TSNA levels in recent years.

3.2. Results from the determination of selected metals

Human exposure to toxic trace metals occurs from a variety of sources that include diet, the environment, vitamin and dietary supplements, tobacco and tobacco smoke, among others. Metals are incorporated into tobacco from naturally occurring soil components and from materials that are added to the soil, e.g., fertilizers, pesticides and soil conditioners (Westcott and Spincer, 1974; Rickert and Kaiserman, 1994; Stephens et al., 2005). Environmental factors such as airborne contaminants and water pollution also contribute to the metal content of tobacco (Rickert and Kaiserman, 1994). Accumulation of metals in the tobacco plant has been found to vary from one tobacco variety to the next and for different stalk positions within a tobacco variety (Westcott and Spincer, 1974; Wagner and Yeargan, 1986; Lugon-Moulin et al., 2006). Growing region and temporal variation within a fixed geographical region have also been reported to affect the metals content of tobacco (Westcott and Spincer, 1974; Rickert and Kaiserman, 1994; Lugon-Moulin et al., 2006; McNeill et al., 2006). In addition to tobacco type, environmental conditions and agronomic practices, the amount of metals found in tobacco depends upon the analytical method applied for the measurement (Scherer and Barkemeyer, 1983).

Table 6 summarizes metals levels found for U.S. and Swedish products by category. U.S. smokeless tobacco products are categorized in the table according to tobacco type and tax designation as dissolvable, loose leaf, plug, moist snuff or dry snuff tobaccos. Differences in mean total metals levels are evident for the different categories of U.S. smokeless tobacco with mean aggregate concentrations (i.e., the summation of observed mean cadmium, arsenic, nickel, chromium and lead concentrations) spanning more than a twofold range. Mean total metals levels are rank-ordered as follows: dry snuff (7368 ng/g dwb) > moist snuff (5239 ng/g dwb) > dissolvable (3786 ng/g dwb) ≈ plug (3534 ng/g dwb) > loose leaf (2985 ng/g dwb). However, this rank-ordering of smokeless tobacco categories is not consistently observed for individual metals. For example, while dissolvable tobacco products, on average, contained lower levels of cadmium and lead compared to the other tobacco categories surveyed, levels of chromium for the dissolvable category were greater than for all other smokeless tobacco categories, except dry snuff. In addition, levels of individual metals within a smokeless tobacco category typically vary more than twofold and by as much as approximately sixfold for nickel in the dry snuff category.

Within the moist snuff and dry snuff categories, the lowest metals levels are generally found for snus tobacco brands in the U.S., consistent with expectations for a snuff tobacco product composed of tobaccos selected for low toxicant content. Metals values found for the snus products studied, those that generally define the lower end of the U.S. moist snuff and dry snuff concentration ranges in Table 6, are also in good agreement with metals levels found for snus tobaccos from the Swedish market. The moist snuff and snus metals levels agree well with those reported by Klus et al. for U.S.

Table 6
Metals levels observed for different tobacco product categories (on a dry weight basis).[a]

Tobacco type	Products tested	Cadmium (ng/g)	Arsenic (ng/g)	Nickel (ng/g)	Chromium (ng/g)	Lead (ng/g)
Products from U.S. market[b]						
Dissolvable	2	361 (251–471)	126 (72–181)	1368 (807–1928)	1729 (1418–2040)	202 (181–223)
Loose leaf	7	599 (469–811)	105 (74–157)	1102 (648–1410)	946 (585–1432)	332 (227–424)
Plug	1	681	149	1331	1009	364
Moist Snuff	23	1052 (355–1871)	214 (108–312)	2047 (1322–2836)	1607 (877–2285)	319 (180–474)
Dry Snuff	10	879 (356–1794)	179 (70–312)	3077 (1223–7540)	2838 (1184–5740)	395 (179–791)
Products from Swedish market[b]						
Moist Snuff	5	467 (362–615)	111 (78–160)	1932 (1182–2781)	1260 (870–1822)	197 (157–244)
Dry Snuff	4	449 (278–672)	160 (96–325)	1779 (1545–2318)	1857 (955–4452)	357 (198–737)
Twist	2	966 (436–1496)	211 (115–307)	1456 (770–2141)	841 (726–956)	444 (386–501)

[a] Data for individual products are summarized in Appendices C and D.
[b] Mean (minimum–maximum) values found for the products tested in each category. For products that were tested in both 2006 and 2007, the average values for the two years were used in the assignment of category means and ranges.

and Swedish markets (2009). Metals values observed in the U.S. plug tobacco sample are similar to values observed for twist tobacco samples from the Swedish market.

Comparison of metals levels observed for U.S. smokeless tobacco products (Table 6) with reference smokeless tobacco products (Table 10) suggests that the 2S3 reference moist snuff, the 1S2 reference dry snuff and the 2S1 reference loose leaf tobacco are each generally consistent with smokeless tobacco products evaluated in this study. While the individual reference products may be above or below the mean values observed in this survey, each reference was within the range of metals concentrations observed for the particular type of smokeless tobacco, with three exceptions. Lead values determined for the 1S2 dry snuff were greater than values observed for the U.S. dry snuff products studied. Similarly, chromium and lead values determined for the 2S1 loose leaf were greater than values observed for the U.S. loose leaf products studied.

There have been very few reports of metals levels in U.S. smokeless tobacco products. Hoffmann et al. (1987) reported cadmium and lead values for five U.S. moist snuff products and three U.S. dry snuff products in 1987. Pappas et al. (2008) reported levels for eight metals (arsenic, barium, beryllium, cadmium, chromium, cobalt, lead and nickel) in 16 moist snuff products and one loose leaf product (Hawken Wintergreen) mistakenly classified as a moist snuff in 2008. In addition, Rickert et al. (2009) reported metals levels in 19 moist snuff products and two chewing tobacco products (a loose leaf and a plug) sold in Canada and manufactured in the U.S. in 2008.

Comparison of cadmium values (Table 6) to values reported by Hoffmann et al. (1987) suggests that moist snuff and dry snuff cadmium levels have remained relatively constant for more than two decades. However, comparison of reported lead values suggests that moist snuff lead values have dropped substantially during that time, while dry snuff lead values have remained relatively constant. Environmental sources of lead, such as the use of leaded gasoline, have also declined substantially during the time period of interest. While the rationale for such an observed difference in moist and dry snuffs is unclear from our findings, one possibility is that environmental sources of lead have historically contributed more significantly to moist snuff than to dry snuff products.

Metals levels for the individual smokeless tobacco products surveyed in this work are summarized in Appendix C. Values summarized in Appendix C generally agree well with values reported by Pappas et al. (2008), with some exceptions. The range of chromium and lead values reported by Pappas et al. exceeds the corresponding ranges reported in this work, primarily driven by the chromium and lead values found by Pappas et al. for the Redwood Regular moist snuff brand. The U.S. snus products surveyed in this work generally contained levels of cadmium, chromium, lead and nickel below the ranges reported by Pappas et al., excluding the Hawken Wintergreen loose leaf values which were misclassified as moist snuff in that work.

Arsenic and lead values reported by Rickert et al. (2009) for smokeless tobacco products manufactured in the United States and sold in Canada agree well with values found in this work (Table 6), suggesting that product formulations are similar for products in each market. However, cadmium, chromium and nickel results reported by Rickert et al. for U.S. manufactured products are generally less than values found in this work. Direct comparison of metals values found for two styles of Copenhagen moist snuff and four styles of Skoal moist snuff common to each study suggest that cadmium, chromium and nickel values reported by Rickert et al. are ~10%, ~40% and ~40% lower than values found in this work, respectively. On its face, this is a surprising finding given that all metals determinations were conducted in the same laboratory and according to the same Health Canada Official Method. A likely source of the observed difference for these three metals is the quantitation technique used in this work. As described earlier in the test methods section (i.e., Section 2.3.3), cadmium, chromium and nickel quantitation was achieved via standard addition, a modification to the official method, to compensate for observed sample matrix effects. Confirmation of this analytical difference as the source of bias between the two studies is not possible, since reference smokeless tobacco samples were not reported by Rickert et al.

3.3. Results from the determination of other selected analytes

Other analytes evaluated in this study include benzo[a]pyrene, nitrite, N-Nitrosodimethylamine and chloride (Table 7).

3.3.1. Benzo[a]pyrene results

Benzo[a]pyrene, a polycyclic aromatic hydrocarbon, is a probable human carcinogen. Tobacco may be exposed to trace levels of B[a]P while in the field or during tobacco curing (Brunnemann and Hoffmann, 1992). While growing, the plant may be exposed to B[a]P as an environmental pollutant. Tobacco may also be exposed to B[a]P during curing practices that involve wood smoke. As such, it is expected that the B[a]P content of a smokeless tobacco product will be dependent upon the tobacco blend characteristics.

Differences in mean B[a]P levels are evident for the different categories of U.S. smokeless tobacco surveyed in this study, with mean response spanning more than two orders of magnitude. Mean B[a]P levels are rank-ordered as follows: moist snuff > dry

Table 7
Other GothiaTek® analyte levels and chloride results observed for different tobacco product categories (all values are expressed on a dry weight basis).[a]

Tobacco type	Products tested	B[a]P (ng/g)	Nitrite (μg/g)	NDMA (ng/g)	Chloride (mg/g)
Products from U.S. market[b]					
Dissolvable	2	0.4 (0.3–0.4)	4.9 (3.7–6.1)	4.1[c] (4.0–4.1)	2.2 (2.2–2.2)
Loose leaf	7	3.5 (1.2–8.0)	5.3[c] (2.7–6.3)	4.3[c] (1.6–5.0)	20.7 (18.1–22.0)
Plug	1	5.4	6.1[c]	15.1[c]	19.0
Moist Snuff	23	61.6 (0.6–193.0)	113.5[c] (0.9–1299)	14.6[c] (2.4–39.8)	95.7 (31.9–127)
Dry Snuff	10	30.5 (0.7–118.0)	9.3[c] (0.6–43.6)	32.7[c] (4.2–222.0)	23.1 (2.0–72.2)
Products from Swedish market[b]					
Moist Snuff	5	1.9 (0.3–4.1)	4.6[c] (1.2–9.0)	13.4[c] (6.7–24.5)	67.7 (58.5–79.5)
Dry Snuff	4	1.2 (0.8–1.6)	3.3[c] (0.6–6.0)	6.2[c] (0.7–15.0)	47.0 (0.6–63.0)
Twist	2	45.1 (1.8–88.5)	3.8[c] (2.4–5.2)	5.3[c] (4.9–5.7)	54.2 (4.3–104)

[a] Data for individual products are summarized in Appendices E and F.
[b] Mean (minimum–maximum) values found for the products tested in each category. For products that were tested in both 2006 and 2007, the average values for the two years were used in the assignment of category means and ranges.
[c] For analyte responses reported as BLQ (below limit of quantitation) or BLD (below limit of detection), the reported limit of quantitation or limit of detection was used to determine the observed category mean and minimum/maximum range. As such, values that include products with either BLQ or BLD responses are overestimates.

snuff ≫ plug ≈ loose leaf > dissolvable (Table 7). Within the moist and dry snuff categories, the lowest B[a]P levels are found for the U.S. snus tobacco brands studied (Appendix E). Snus tobaccos are ~50- to 200-fold lower in B[a]P than are traditional moist and dry snuff tobaccos. One exception is noted in the dry snuff category, as Dental Sweet dry snuff is similar to snus tobaccos in that category. Swedish moist and dry snuff product B[a]P levels agree well with snus product levels found in the U.S.

Comparison of B[a]P levels observed for U.S. smokeless tobacco products (Table 7) with reference smokeless tobacco products (Table 10) suggests that the 2S3 reference moist snuff, the 1S2 reference dry snuff and the 2S1 reference loose leaf tobacco are each generally consistent with traditional smokeless tobacco products sold in the U.S., but are much greater than levels found for snus tobaccos.

Smokeless tobacco B[a]P values have been reported by several authors during the last 20 years. Hoffmann et al. (1987) reported B[a]P values for leading moist and dry snuff tobaccos. McNeill et al. (2006) reported B[a]P levels for a U.S. moist snuff and a dissolvable tobacco product while surveying smokeless tobacco products in the U.K. Stepanov et al. (2008) reported B[a]P values for selected new and traditional U.S. smokeless tobacco products. Rickert et al. (2009) reported B[a]P values for a number of moist snuff products, a loose leaf tobacco and a plug tobacco, all of which were manufactured in the U.S. and marketed in Canada. Comparison of B[a]P values from this work with available literature values suggest general agreement for the various types of smokeless tobacco studied, although some individual U.S. moist snuff values found in this work exceed previously reported values. Stepanov et al. (2010) reported 23 polycyclic aromatic hydrocarbons in moist snuff and snus in the U.S. market products. B[a]P results from that study are in limited agreement with this work. For those products that represent the upper range of response observed in this work, results reported by Stepanov et al. are in general agreement. For those products that represent the lower range of response observed in this work, results reported by Stepanov et al. are greater.

3.3.2. Nitrite results

Nitrite is of interest when evaluating the chemistry of a smokeless tobacco product because its potential to act as a nitrosating agent either post-manufacture of the product or *in vivo*. Differences in mean nitrite levels are evident for the different categories of U.S. smokeless tobacco surveyed in this study, with mean concentrations spanning approximately a 20-fold range. Mean nitrite levels are rank-ordered as follows: moist snuff ≫ dry snuff > plug ≈ loose leaf ≈ dissolvable (Table 7). However, it should be noted that two moist snuff products with observed nitrite levels of 975 and 1299 μg/g dry weight, respectively, drive the observed mean value for the moist snuff category (Appendix E). The other 21 moist snuff products surveyed were all below 100 μg/g dry weight, with 17 of the 21 products below 20 μg/g dry weight. Within the moist and dry snuff categories, snus tobacco brands are among the lowest levels observed, consistent with the suggestion by Stepanov et al. (2008) that "relatively low levels of nitrite and nitrate in the new smokeless tobacco products probably reflect the manufacturer's effort to reduce toxicity of their products and to limit TSNA formation during tobacco processing."

Comparison of nitrite levels observed for U.S. smokeless tobacco products (Table 7) with reference smokeless tobacco products (Table 10) confirms that the 2S3 reference moist snuff, the 1S2 reference dry snuff and the 2S1 reference loose leaf tobacco are each generally consistent with the U.S. smokeless tobacco products tested, with the exception of the moist snuff products that were found to have high nitrite values noted above. There have been few reports of U.S. smokeless tobacco nitrite values, as nitrate is more frequently reported. Nitrite values that have been reported are in agreement with results found in this work (Hoffmann et al., 1995; McNeill et al., 2006; Stepanov et al., 2008). Most values are below 20 μg/g nitrite dry weight, although a few reported moist snuff values range much higher (Hoffmann et al., 1995). Stepanov et al. (2008) have reported that new smokeless tobacco products studied generally contain <10 μg/g nitrite dry weight.

3.3.3. NDMA results

N-Nitrosodimethylamine (NDMA) is a volatile nitrosamine classified by IARC as a probable human carcinogen. Several NDMA formation mechanisms have been reported in drinking water (Mitch et al., 2003). It is generally thought that NDMA in tobacco is formed from the nitrosation of volatile amines, including dimethlyamine specifically. Differences in mean NDMA levels are evident for the different categories of U.S. smokeless tobacco surveyed in this study, with mean concentrations spanning approximately an eightfold range. Mean NDMA levels are rank-ordered as follows: dry snuff > plug ≈ moist snuff > loose leaf ≈ dissolvable (Table 7). Within the moist and dry snuff categories, NDMA levels found for snus tobacco brands are either the lowest, or among the lowest, observed for each category (Appendix E).

Comparison of NDMA levels observed for U.S. smokeless tobacco products (Table 7) with reference smokeless tobacco products (Table 10) suggests that the 2S3 reference moist snuff, the 1S2 reference dry snuff and the 2S1 reference loose leaf tobacco are each generally consistent with U.S. smokeless tobacco products evaluated in this work. Consistent with the category rankings observed in this work, NDMA values for the 1S2 dry snuff > 2S3 moist snuff > 2S1 loose leaf reference smokeless tobacco. Category rankings for Swedish smokeless tobacco products studied in this work (Table 7) and ranges historically reported for U.S. smokeless tobacco (IARC, 2007) are somewhat different. For the Swedish products, moist snuff > dry snuff ≈ twist. IARC (2007) reports moist snuff > -chew > dry snuff.

3.3.4. Chloride results

Chloride is an essential tobacco micronutrient provided by soil and fertilizer. Excess levels of chloride are detrimental to tobacco quality (Flower, 1999). Chloride salts, as much as 7–10% by weight, may be added to smokeless tobacco products to impart specific taste characteristics (Wahlberg and Ringberger, 1999). For example, the smokeless tobacco reference products studied in this work contained ~0.4–7.3% sodium chloride (Table 3). It has been reported that high amounts of sodium chloride may contribute to several disease mechanisms, including inflammation, tumor promotion and co-carcinogenisis (Takahashi et al., 1994; Sugimura, 2000; Boffetta et al., 2008). However, the amounts of sodium chloride taken up when using smokeless tobacco products are relatively low compared to dietary intake sources of sodium chloride.

Differences in mean chloride levels are evident for the different categories of U.S. smokeless tobacco with mean concentrations spanning approximately a two order of magnitude range. Mean chloride levels are rank-ordered as follows: moist snuff > dry snuff > loose leaf ≈ plug > dissolvable (Table 7). Within the moist snuff category, the lowest chloride levels are generally found for snus tobacco brands (Appendix E). However, snus tobacco brands are found at both the lower and upper extremes of the chloride range determined for the dry snuff category. Chloride values found for Swedish moist snuff tobacco products are similar to U.S. values, while Swedish dry snuff and twist chloride values are greater than U.S. values.

Comparison of chloride levels observed for U.S. smokeless tobacco products (Table 7) with reference smokeless tobacco products (Table 10) suggests that the 2S3 reference moist snuff, the 1S2 reference dry snuff and the 2S1 reference loose leaf tobacco are each generally consistent with U.S. smokeless tobacco products evaluated. Stepanov et al. (2008) have reported chloride values for selected new and traditional smokeless tobacco products. Comparison of chloride values from this work with values reported by Stepanov et al. are in agreement.

3.4. Nicotine and calculated "free nicotine" results

Smokeless tobacco products contain nicotine. The amount of nicotine found in a particular product depends upon the types of tobacco and amounts of each tobacco that comprise a particular product. In addition to nicotine content, the amount of nicotine present in the unprotonated form is also generally considered of interest to scientists and regulators as the unprotonated form of the molecule transfers readily across a physiological membrane (Tomar and Henningfield, 1997). The amount of unprotonated nicotine in a smokeless tobacco product is a calculated, rather than a measured, quantity and is often termed "free nicotine" or unionized nicotine. For regulatory reporting purposes, "free nicotine" is calculated based on the Henderson–Hasselbalch equation, an equation which describes the effect of pH on the dissociation of a weak acid in aqueous solution (CDC, 1999). Thus, "free nicotine" is a theoretical, calculated value which provides a basis for comparing products, rather than a quantity that is derived from actual conditions of product use. As such, the physiological relevance of estimated "free nicotine" values has not been established. The scientific limitations associated with currently mandated methods for the estimation of unionized (free) nicotine content of tobacco and the relevance of product design parameters to estimated "free nicotine" values has been recently summarized by Lauterbach et al. (2011).

It has been reported that nicotine absorption from smokeless tobacco is dependent upon not only physical or chemical characteristics of the product, but also a number of behavioral and physiological factors (IARC, 2007; Fant et al., 1999; Ciolino et al., 2001). Product characteristics that have been reported to affect absorption include the form of the tobacco product (i.e., loose tobacco vs. pouch or sachet), tobacco-cuts and particle size, total nicotine content, pH, moisture content and buffering capacity. Behavioral characteristics such as the amount of smokeless tobacco product used, the length of time held in the mouth, movement in the mouth and frequency of use have been reported to affect nicotine absorption. Physiological characteristics such as an individual's saliva pH, saliva buffering capacity, rate of salivation, extent of expectoration and mucosal characteristics have also been reported as factors that may affect nicotine absorption from smokeless tobacco.

Scientific views vary regarding how changes in smokeless tobacco nicotine content may promote tobacco harm reduction. One view is that smokeless tobacco products with lower levels of nicotine or "free nicotine" have "the advantage of being lower in addiction potential" and are thus preferred. However, such products may not provide a viable tobacco alternative to cigarettes for smokers (Hatsukami et al., 2007). It has also been reported that products with low levels of nicotine may appeal to new tobacco users, particularly youths, because they are better tolerated by a new or inexperienced tobacco user compared to products with higher nicotine levels (Alpert et al., 2008). An alternative view is that smokeless tobacco products with higher levels of nicotine and that are associated with more rapid absorption of nicotine are the most likely to successfully promote migration of cigarette smokers to smokeless tobacco use (Hatsukami et al., 2007; Foulds and Furberg, 2008). In keeping with harm reduction principles, it has also been suggested that such products should be as low in tobacco toxicant concentrations as possible (Zeller et al., 2009). Clearly, if migration of smokers to smokeless tobacco products to reduce population-level exposure to tobacco combustion products is the goal, the latter alternative is preferred.

Table 8 summarizes nicotine content and calculated "free nicotine" levels found for U.S. and Swedish products by category (nicotine content and calculated "free nicotine" levels for the individual smokeless tobacco products surveyed in this work are summarized in Appendices G and H). Consistent with regulatory reporting requirements, values in table 8 are reported on a per gram of product (i.e., "as is") basis. Nicotine content for the U.S. smokeless tobacco product categories investigated spanned more than a twofold range with mean nicotine levels rank-ordered as dry snuff > moist snuff > plug ≅ loose leaf ≅ dissolvable. As expected, since "free nicotine" is calculated from pH, rank ordering of pH and calculated "free nicotine" were identical (moist snuff > dissolvable > dry snuff > plug > loose leaf), but differed from that of nicotine content.

Nicotine and "free nicotine" values observed for snus tobacco brands differ from other endpoints evaluated for those products in this study. Snus tobacco brands are generally among the lowest toxicant concentrations in the moist and dry snuff categories for most analytes. However, nicotine and "free nicotine" values observed for snus tobaccos are comparable to other products in each category.

Nicotine and "free nicotine" values found for U.S. snus products differ somewhat from those found for snus tobaccos from the Swedish market. The Swedish moist snuff products evaluated in this study are lower in nicotine content on average than U.S. moist snuff products, but yield similar amounts of calculated "free

Table 8
Nicotine content and calculated "Free Nicotine" levels observed for different tobacco product categories.[a]

Tobacco type	Products tested	Nicotine (mg/g)[c]	Moisture (%)	pH	Calculated "Free Nicotine" (mg/g)[c]	Calculated "Free Nicotine" (% of total nicotine)
Products from U.S. market[b]						
Dissolvable	2	6.0 (3.9–8.2)	3.8 (2.8–4.8)	7.3 (7.2–7.4)	0.9 (0.7–1.2)	16.1 (14.2–18.0)
Loose leaf	7	6.2 (2.9–8.6)	23.9 (21.6–29.0)	5.6 (5.6–6.1)	0.04 (0.01–0.08)	0.5 (0.1–1.2)
Plug	1	6.4	22.7	6.0	0.1	0.9
Moist Snuff	23	12.0 (7.2–14.1)	51.0 (31.9–56.3)	7.7 (7.2–8.4)	4.2 (1.1–7.4)	36.0 (13.2–68.0)
Dry Snuff	10	15.8 (11.1–23.1)	10.5 (3.6–23.4)	6.5 (5.7–7.4)	0.7 (0.1–2.4)	4.8 (0.4–20.1)
Products from Swedish market[b]						
Moist Snuff	5	8.7 (6.9–10.6)	48.9 (47.1–51.9)	7.9 (7.5–8.4)	4.2 (1.6–6.3)	46.4 (23.6–70.1)
Dry Snuff	4	16.1 (15.3–17.9)	17.9 (7.4–22.8)	7.8 (7.2–9.2)	6.4 (2.1–16.8)	37.9 (13.4–93.5)
Twist	2	23.3 (21.1–25.4)	26.2 (20.4–32.0)	5.3 (5.0–5.6)	0.05 (0.02–0.07)	0.2 (0.1–0.4)

[a] Data for individual products are summarized in Appendices G and H.
[b] Mean (minimum–maximum) values found for the products tested in each category. For products that were tested in both 2006 and 2007, the average values for the two years were used in the assignment of category means and ranges.
[c] Values are expressed on an "as is" or "wet" basis.

Table 9
Comparison of observed smokeless reference product analyte levels with established reference values.

Analyte	2S1 Loose leaf		1S2 Dry Snuff		2S3 Moist Snuff	
	Reference value	This study	Reference value	This study	Reference value	This study
Moisture (%)	21.99 (1.11)	21.70 (0.20)	11.75 (0.41)	13.95 (0.10)	54.46 (0.22)	54.80 (0.20)
Nicotine (mg/g)[a]	0.84 (0.06)	0.78 (0.07)	1.32 (0.04)	1.16 (0.11)	1.34 (0.11)	1.39 (0.21)
pH	5.81 (0.05)	5.87 (0.01)	6.29 (0.07)	6.22 (0.01)	7.32 (0.20)	7.45 (0.20)

[a] As is (i.e., "wet") basis.

nicotine." The Swedish dry snuff products contain more nicotine, on average than the U.S. dry snuff products studied and also yield far greater amounts of calculated "free nicotine." The Swedish twist tobacco products contain much greater amounts of nicotine, but have calculated "free nicotine" values similar to the U.S. plug tobacco brand studied.

Comparison of nicotine, pH and "free nicotine" values observed for U.S. smokeless tobacco products (Table 8) with reference smokeless tobacco products (Table 9) suggests that the 2S3 reference moist snuff, the 1S2 reference dry snuff and the 2S1 reference loose leaf tobacco are each generally consistent with the U.S. smokeless tobacco products surveyed in this study. While the individual reference products may be above or below the mean values observed in this survey, each reference was within the range of response observed for the particular type of smokeless tobacco.

Nicotine, pH and "free nicotine" are among the most widely studied and reported endpoints for characterizing smokeless products. Examples of documents that summarize this type of work include the recent IARC monograph that addresses smokeless tobacco (IARC, 2007), a recent report by Stepanov et al. (2008) that compares new and traditional smokeless products, a moist snuff survey conducted by scientists at the Centers for Disease Control (CDC) (Richter et al., 2008) and a recent survey of products manufactured in the U.S. for sale in Canada (Rickert et al., 2009).

The IARC monograph summarizes much of the nicotine related data reported for smokeless tobacco products from ~1994 to 2004. Compiling data from reports throughout that time period underscores the frequent misclassification of some tobacco products. One example is Hawken Wintergreen which is classified as both a moist snuff and a chewing tobacco, the latter of which is consistent with its tax classification. Another example is the HB cut brand which is also characterized as a moist snuff, rather than a chewing tobacco. Data reported to the Commonwealth of Massachusetts by tobacco manufacturers in 2003 comprises the largest nicotine, pH and "free nicotine" data set in the IARC monograph. Moist and dry snuff results from that data set are in agreement with this work (Table 8). The nicotine content reported for chewing tobacco in the IARC monograph (3.41–39.74 mg/g product) is much greater than the range observed for the more limited number of products evaluated in this survey (2.9–8.6 mg/g product); however, the pH and "free nicotine" results found in each study are in agreement.

Nicotine, pH and "free nicotine" values reported more recently in 2008 are also in good agreement with those found in this work. Moist snuff survey results reported by Richter et al. (2008) align well with the results in this study, excluding the Hawken Rough [*sic*] Wintergreen and WB Cut Regular brands for classification reasons. Results reported by Stepanov et al. (2008) for both traditional and new smokeless products are generally consistent with this study. As such, Stepanov et al. also conclude that new U.S. smokeless tobacco products (e.g., Camel Snus) are "similar to those usually observed in moist snuff." Finally, nicotine and pH values for moist snuff, loose leaf and plug tobacco products manufactured in the U.S for sale in Canada agree well with values found in this survey.

3.5. Smokeless tobacco reference products

Smokeless tobacco reference products were first introduced by the Tobacco and Health Research Institute in the late 1980s (IARC, 2007). Since that time, smokeless reference products have been used to evaluate the effects of storage conditions on tobacco chemistry (Andersen et al., 1989, 1993), to characterize new tobacco chemistries (Djordjevic et al., 1989b; Brunnemann et al., 2002), for biological experiments (Furie et al., 2000) and as one element of a market chemistry survey (Stepanov et al., 2005). However, a general review of the smokeless tobacco literature suggests that inclusion of a smokeless tobacco reference product or products within experimental designs has generally been the exception, rather than the rule.

Smokeless tobacco reference products are formulated to represent smokeless tobacco products typically available in the U.S. market (Table 3). Each product is produced as a single large batch with a unique designation. Therefore, a smokeless reference product is not affected by year-to-year tobacco crop variability, long-term manufacturing variability or potential changes in product formulation. Historically, when a reference product was exhausted, a new batch based on the same product formulation was produced. To designate a new production batch, the leading digit in the reference product designation was incremented (e.g., 2S3 is the second reference product produced with the 1S3 product formulation). Differences in tobacco chemistry from one batch to another are possible since the different batches are produced from tobacco crop years available at the time of manufacture.

When conducting a tobacco chemistry market survey, smokeless tobacco reference products enhance the quality of the data produced in a number of ways. First, since some established "reference values" are available, survey results can be compared to available reference values to confirm the general accuracy and precision of the analytical methodology applied (Table 9). Second, smokeless reference products provide a means of assessing intralaboratory variability. For example, Table 10 provides a comparison of results obtained in this work from the analysis of smokeless reference products in 2006 and 2007. Since each smokeless reference product is produced as a single large "batch," differences observed from one year to the next are expected to result primarily from analytical variability, together with natural tobacco blending variability. Third, smokeless reference products provide a means of assessing interlaboratory variability and detecting differences which may occur when products are tested with more than one analytical method when determining a particular tobacco constituent. Discernment of laboratory-driven differences is critically important as comparison of chemistry survey data from different global marketplaces and from multiples sources within a given market becomes more common.

One goal of this study is to assess the relevance of U.S. smokeless tobacco reference products to the commercial U.S. smokeless tobacco products studied. Moist stuff, dry snuff and loose leaf reference tobacco products were included in this study to evaluate the concordance between commercial product and reference product chemical profiles for each product category. The degree

Table 10
Comparison of smokeless tobacco reference product analyte levels determined in 2006 and 2007 (dry weight basis, unless specified).

Analyte	Moist Snuff (2S3)		Dry Snuff (1S2)		Loose leaf (2S1)	
	2006	2007	2006	2007	2006	2007
Moisture (%)	54.9 (0.3)	54.7 (0.1)	14.2 (0.0)	13.7 (0.2)	21.6 (0.0)	21.8 (0.3)
NNN (ng/g)	3168 (130)	3501 (52)	73,559 (2580)	78,567 (1655)	2845 (78)	2976 (69)
NAT (ng/g)	2393 (128)	2348 (31)	32,721 (735)	33,177 (810)	993 (10)	1125 (51)
NNK (ng/g)	832 (59)	912 (10)	94,489 (2928)	98,261 (2520)	575 (47)	564 (34)
NAB (ng/g)	BLQ	184 (32)	10,067 (481)	11,203 (47)	BLQ	81 (23)
TSNAs (ng/g)[a]	6393 (192)	6945 (69)	210,836 (4000)	221,208 (3122)	4413 (92)	4746 (95)
Arsenic (ng/g)	257 (35)	199 (8)	248 (19)	218 (27)	179 (21)	113 (15)
Cadmium (ng/g)	1305 (21)	1561 (29)	1113 (13)	1478 (39)	518 (8)	618 (29)
Chromium (ng/g)	1565 (11)	1313 (136)	3326 (146)	3200 (160)	1743 (87)	1786 (61)
Lead (ng/g)	454 (36)	326 (13)	1408 (97)	1276 (60)	459 (15)	533 (29)
Nickel (ng/g)	2082 (88)	1880 (114)	4450 (34)	4255 (277)	1157 (77)	1120 (58)
B[a]P (ng/g)	61.8 (7.1)	79.0 (3.1)	19.1 (3.4)	25.9 (4.9)	0.8 (0.2)	1.1 (0.2)
Nitrite (μg/g)	BLQ	11.2 (0.7)	BLQ	5.6 (0.3)	BLD	5.5 (0.6)
NDMA (ng/g)	BLQ	12.9 (2.8)	69.6 (8.3)	47.2 (1.2)	BLD	5.5 (2.2)
Chloride (mg/g)	121 (1)	123 (1)	16.5 (1.7)	17.3 (0.1)	16.5 (0.3)	16.5 (0.6)
Nicotine (mg/g)[b]	14.00 (0.09)	13.80 (0.32)	11.39 (0.23)	11.82 (0.02)	7.91 (0.13)	7.70 (0.04)
pH	7.47 (0.01)	7.42 (0.02)	6.21 (0.01)	6.22 (0.01)	5.85 (0.00)	5.88 (0.02)
Calculated "Free Nicotine" (% of total nicotine)[b]	21.99	20.08	1.53	1.56	0.67	0.72
Calc. "Free Nicotine" (mg/g)[b]	3.08	2.77	0.17	0.18	0.05	0.06

[a] TSNAs = NNN + NAT + NNK + NAB.
[b] As is (i.e., "wet") basis.

Table 11
Comparison of analyte levels for smokeless tobacco products tested in both 2006 and 2007 (dry wt basis, unless specified).

Analyte	Grizzly Moist Snuff		Kodiak Moist Snuff		Catch Dry Eucalyptus Snus		Camel Frost Snus	
	2006	2007	2006	2007	2006	2007	2006	2007
Moisture (%)	53.2 (0.1)	53.2 (0.1)	52.5 (0.2)	54.2 (0.1)	23.4 (0.1)	22.8 (0.4)	34.1 (0.2)	32.2 (0.1)
NNN (ng/cig)	4148 (40)	3448 (64)	4764 (54)	5037 (204)	814 (53)	843 (35)	1068 (67)	1009 (38)
NAT (ng/cig)	4549 (61)	4697 (38)	5489 (115)	6322 (292)	570 (70)	587 (30)	745 (26)	874 (13)
NNK (ng/cig)	1085 (41)	646 (62)	1184 (105)	1325 (29)	NQ	319 (33)	NQ	345 (52)
NAB (ng/cig)	355 (22)	222 (21)	472 (38)	412 (32)	NQ	54 (32.6)	NQ	75 (31.7)
TSNAs (ng/g)[a]	10,137	9013	11,909	13,096	1384	1803	1813	2303
Arsenic (ng/g)	271 (32)	174 (23)	325 (36)	261 (20)	149 (19)	105 (7)	188 (22)	136 (8)
Cadmium (ng/g)	1079 (29)	1228 (195)	1260 (51)	1306 (180)	527 (24)	672 (27)	540 (9)	641 (12)
Chromium (ng/g)	1786 (4)	1488 (275)	1922 (112)	1446 (169)	1270 (79)	955 (122)	1900 (170)	1451 (28)
Lead (ng/g)	382 (8)	244 (24)	437 (8)	301 (14)	218 (16)	198 (17)	220 (14)	225 (13)
Nickel (ng/g)	2079 (111)	1933 (289)	2569 (78)	2065 (296)	1920 (59)	1584 (71)	2187 (200)	1498 (86)
B[a]P (ng/g)	94.4 (3.7)	145 (16)	188 (12)	198 (26)	1.33 (0.04)	1.54 (0.2)	1.06 (0.14)	1.87 (0.17)
Nitrite (μg/g)	BDL	BDL	NQ	NQ	BDL	BDL	BDL	BDL
NDMA (ng/g)	NQ	NQ	BDL	15.8 (1.3)	BDL	NQ	BDL	NQ
Chloride (μg/g)	125 (0)	129 (3)	115 (2)	121 (1)	65.5 (1.4)	63.0 (1.0)	34.1 (0.2)	33.0 (0.6)
Nicotine (mg/g)[b]	10.29	11.20	10.93	10.70	15.93	15.63	13.25	14.10
pH	8.27 (0.03)	8.06 (0.04)	8.19 (0.01)	8.53 (0.01)	7.00 (0.04)	7.21 (0.02)	7.76 (0.01)	7.72 (0.02)
Calculated "Free Nicotine" (% of total nicotine)[b]	64.01	52.3	59.66	76.39	8.72	13.41	35.46	33.39
Calculated "Free Nicotine" (mg/g)[b]	6.59	5.86	6.52	8.18	1.39	2.10	4.70	4.71

[a] TSNAs = NNN + NAT + NNK + NAB.
[b] As is (i.e., "wet") basis.

of concordance found has been discussed on an analyte-by-analyte basis in the preceding sections and, with the exception of dry snuff TSNA levels, the results of this study suggest that the 2S3 moist snuff, 1S2 dry snuff and 2S1 loose leaf reference products were generally representative of commercially available products as of 2006 and 2007.

It has been several years since the most recent reference product formulations were established and the corresponding reference smokeless tobaccos produced. During that time, commercial product formulations may have changed. As of 2010, several new smokeless tobacco reference products have been produced under the auspices of a CORESTA working group. These new smokeless tobacco reference products (CRP1–4) include a snus reference product (CRP1). A detailed description of these new smokeless reference products is available on the NC State website <http://www.tobacco.ncsu.edu/strp.html> where product may also be obtained. Chemical characterization of these new reference products has been conducted by nine laboratories under the coordination of the CORESTA Smokeless Tobacco Subgroup (CSTS). The results obtained were recently presented at an FDA-sponsored workshop on tobacco product analysis (FDA, 2012d). A full report of the characterization effort is anticipated later this year. The CORESTA Smokeless Tobacco Subgroup plans to monitor these new reference products annually and replace them every 5 years.

3.6. Year-to-year variation

Year-to-year differences for individual smokeless tobacco product analyte responses are to be expected. For a given tobacco product, analyte concentrations may vary from year-to-year due to analytical method variability, product variability, or both. Analytical method variability may occur due to the inherent accuracy and precision limitations of the analytical procedure, or may reflect procedural changes in the procedure introduced from one year to another. Product variability may occur due to natural tobacco variability, age of the product when sampled, manufacturing variability and specific product design changes. For example, it has been reported that nitrite and TSNA concentrations may vary significantly for a given smokeless tobacco product based upon aging and storage conditions (Djordjevic et al., 1993).

Analysis of smokeless tobacco reference products provides insight into year-to-year analytical variability in this study (Table 10). While reference product responses are generally consistent from 2006 to 2007 (i.e., most differences observed from 2006 to 2007 are 20% or less), year-to-year differences as great as ~37% are observed for some analytes (e.g., the 2007 1S2 cadmium result is 32.8% greater than the 2006 result; and the 2007 2S1 B(a)P result is 37.5% greater than the 2006 result). Since the reference products tested were unchanged from 2006 to 2007, these results indicate that year-to-year analytical variability was generally less than 20% and potentially as great as ~37%. Similarly, examination of results for products purchased and tested in both 2006 and 2007 (Table 11) suggests year-to-year variability for a given product was less than 20% for most analytes, although some year-to-year differences exceed 37% (e.g., the 2007 Grizzly moist snuff B(a)P value was 53.6% greater than the 2006 value), perhaps better reflecting the natural variability of cured tobacco over time.

3.7. Comparison to GothiaTek® quality standard

The GothiaTek® quality standard is a set of guiding principles developed by the Swedish Match company that provides a quality guarantee for that manufacturer's products. GothiaTek® addresses raw materials, manufacturing processes, content of the final product and communication of information to consumers (Swedish Match, 2008a). However, the principle that deals with content of the final product in terms of "maximum permitted levels of suspected harmful products" has received perhaps the broadest interest and application (Swedish Match, 2008b).

The GothiaTek® limits (in units common to the tables throughout this manuscript) are found in Table 12. The origin of the individual limits is not clear. Swedish Match reports that it is based on many years of research and development (Swedish Match, 2008a). One recent report characterizes the GothiaTek® standard and others as "arbitrary" and "without specific merit," further noting that the limits are not enforced by any regulatory agencies (LSRO, 2008). Recently, the relevance of some GothiaTek® analyte limits has been probed via quantitative risk analysis (Wilson et al., 2008).

Comparison of U.S. survey results for individual smokeless products (Appendices A, C and E) to GothiaTek® limits (Table 12) indicates that many of the smokeless tobacco products evaluated in this study are below the suggested limits. For example, the dissolvable, loose leaf and plug products all fall below the GothiaTek® limits, with the exception of the plug product which exceeds the recommended NDMA limit. Similarly, constituent values found for moist and dry snuff products recently introduced into the U.S. market that include tobaccos selected for low toxicant content, i.e., snus tobacco brands, were below the GothiaTek® limits (note that limits of quantitation and detection for nitrite and NDMA were near the limits for samples tested in 2006). Those products include: Camel Frost, Camel Original, Camel Spice, Catch Dry Eucalyptus, Catch Dry Licorice, General Loose, General Original Portion, General White Portion, Taboka and Taboka Green.

Table 12
GothiaTek® limits (dry wt. basis).

Analyte	Limit
Arsenic (ng/g)	500
B[a]P (ng/g)	20
Cadmium (ng/g)	1000
Chromium (ng/g)	3000
NDMA (ng/g)	10
Nickel (ng/g)	4500
Nitrite (μg/g)	7
Lead (ng/g)	2000
Total TSNAs (ng/g)	10,000

Most traditional moist snuff products contain both fermented and fire-cured tobaccos, the latter of which is exposed to B[a]P and other polycyclic aromatic hydrocarbons during curing practices that involve wood smoke. Traditional moist snuff products exceeded one or more of the following GothiaTek® limits: total TSNAs, B[a]P, cadmium, NDMA and nitrite. Traditional dry snuff products, i.e. those containing fermented and fire-cured tobaccos, exceeded one or more of the following GothiaTek® limits: total TSNAs, B[a]P, cadmium, chromium, nickel, NDMA and nitrite.

3.8. Smokeless tobacco and tobacco harm reduction

The use of tobacco products is associated with increased risk for death and disease. This association is most evident for combustible products, as cigarette smoking significantly increases the mortality risk for lung cancer, cardiovascular disease and chronic obstructive pulmonary disease (Thun et al., 2000). Based on this recognized association, tobacco harm reduction has largely focused on a single product category (i.e., cigarettes), as well as strategies primarily intended to reduce exposure to combustion-related toxicants (IOM, 2001).

No tobacco product has been shown to be safe and without risks; however, the increased harm associated with tobacco use would appear to proceed along a pronounced continuum, influenced significantly by the type of tobacco product used and its associated toxicant profile (Zeller et al., 2009). At one end of the continuum, cigarette smoking produces exposure to nicotine along with tobacco and combustion-related toxicants, and is associated with chronic disease. At the other end of the continuum, smokeless (noncombustible) tobacco products eliminate combustion-related toxicants, producing exposure to nicotine along with tobacco toxicants, the subject of this study. The use of smokeless products does not appear to be associated with many of the smoking-related cancers or to pulmonary disease (Weitkunat et al., 2007; Boffetta et al., 2008; Sponsiello-Wang et al., 2008; Lee and Hamling, 2009a,b; Bertuccio et al., 2011), although the mortality risk for some tobacco-related diseases (e.g., cardiovascular disease) may be slightly elevated relative to non-tobacco users (Henley et al., 2005; Boffetta and Straif, 2009; Arefalk et al., 2011; Hansson et al., 2012).

Tobacco harm reduction, defined in the broadest of terms, should consist of all strategies that effectively achieve reductions for tobacco-related death and disease. Moreover, strategies should be focused on reducing exposure at both the individual and population levels. Reflecting a shift from conventional strategies, an increasing number of public health organizations have recognized the potential health benefits for encouraging inveterate smokers to switch to smokeless or nicotine-based products (RCP, 2002; ACSH, 2006; RCP, 2007; AAPHP, 2008; LSRO, 2008; WHO, 2008). An analysis of cohort data from two large US-based prospective studies (American Cancer Society Cancer Prevention Studies, CPS-I and CPS-II) suggests significant reductions for all cause mortality, cancer (lung, oral and pancreatic), pulmonary disease and heart disease for smokeless tobacco users when compared to cigarette smokers, with limited evidence of increased mortality from heart disease and stroke compared to non-tobacco users (Thun et al., 2000; Henley et al., 2005). Moreover, subsequent analysis of the

CPS-II cohort, in terms of smokers either switching to smokeless products or quitting, indicated mortality hazard ratios that were higher for switchers than quitters, but significantly lower than tobacco users who continued to smoke (Thun et al., 2000; Henley et al., 2007).

Potentially more informative as to the potential health benefits associated with migrating inveterate smokers to smokeless products is the use of moist snuff tobacco (snus) within the Swedish population. During the last several decades, the availability of snus has assisted in drastically reducing smoking prevalence among the male population, i.e., decreasing from 40% to less than 15%; for this same period, daily snus use increased such that tobacco consumption remained stable. With regard to individual health, Swedish men demonstrated notable reductions for smoking-related disease, including a significant decline in the lung cancer incidence rate to the lowest of any developed nation, a continued low oral cancer rate, and dramatically improved cardiovascular health (e.g., Foulds et al., 2003). At the population level, available data consistently demonstrate that dual users of snus and cigarettes were more likely to reduce smoking frequency (Gilljam and Galanti, 2003; Caldwell et al., 2010) and more likely to quit smoking (Furberg et al., 2005, 2008a; Ramström and Foulds, 2006; Stenbeck et al., 2009) compared to exclusive smokers. In addition, snus use appears to be associated with decreased, versus increased smoking initiation (Furberg et al., 2005, 2008b; Ramström and Foulds, 2006; Galanti et al., 2008; Stenbeck et al., 2009).

Given the inherent differences between populations, the direct relevance of the Swedish data has been questioned (e.g., Zhu et al., 2009). However, data showing that the use of smokeless products is associated with both qualitative and quantitative decreases in risk compared to cigarette smoking clearly highlight the potential public health benefit that may be derived from even a fraction of existing U.S. smokers switching to smokeless or nicotine-based products. Consistent with the data from Sweden, emerging data from the U.S. population indicate that dual users (i.e., smokeless tobacco and cigarettes) are more likely to reduce cigarette consumption (Carpenter and Gray, 2010; Hatsukami et al., 2011; O'Connor et al., 2011) and more likely to quit smoking (Kozlowski et al., 2003; Rodu and Phillips, 2008; Tomar et al., 2010; Carpenter and Gray, 2010) compared to exclusive smokers. And while it has been suggested that smokeless tobacco use may increase cigarette smoking (Tomar 2003, 2010; Severson et al., 2007), data from studies that control for known psychosocial predictors of smoking initiation and product order appear to indicate that smokeless tobacco use is not associated with subsequent cigarette smoking (O'Connor et al., 2003, 2005; Timberlake et al., 2009; Rodu and Cole, 2010).

4. Conclusions

A survey of smokeless tobacco products sold in the U.S. in 2006 and 2007 has been successfully conducted. The survey establishes the toxicant profiles for a wide range of smokeless tobacco products and directly responds to recent calls for providing such information on a brand and subbrand basis. Levels of individual toxicants vary substantially for different categories of smokeless tobacco with recently introduced smokeless products among the lowest measured levels. Toxicant levels found for U.S. snus tobacco brands are consistent with the low levels found in Swedish snus products. The smokeless tobacco reference products studied were generally representative of commercially available products sampled in 2006 and 2007; with the exception of the 1S2 dry snuff TSNA levels. As such, smokeless reference products should be included when conducting studies of smokeless tobacco chemistry, as inclusion will provide direct comparison of data sets produced by different researchers. Misclassification of smokeless tobacco products according to category (e.g., loose leaf vs. moist snuff) is a longstanding issue that has affected some reported smokeless tobacco toxicant ranges. It would be helpful for researchers to include the basis of product classification in future reports.

5. Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Tobacco specific nitrosamine levels for products purchased from the U.S. market.[a]

Tobacco product	NNN (ng/g)	NAT (ng/g)	NAB (ng/g)	NNK (ng/g)
Ariva	BLQ[b]	113 (14)	BLQ	BLQ
Stonewall Natural	139 (14)	236 (12)	BLQ	BLD[c]
Beech-Nut Chewing Tobacco	1771 (32)	718 (36)	BLQ	368 (11)
Hawken Wintergreen	2853 (124)	1308 (33)	149 (6)	898 (60)
Lancaster Premium Chewing Tobacco	718 (49)	580 (9)	BLQ	BLQ
Levi Garrett Chewing Tobacco	2840 (97)	1316 (36)	179 (12)	840 (75)
Red Man Chewing Tobacco	1099 (73)	713 (18)	BLQ	BLQ
Red Man Golden Chewing Tobacco	662 (31)	503 (46)	BLQ	BLQ
Stoker Chew Apple Chewing Tobacco	2645 (38)	1216 (27)	58 (41)	691 (50)
Taylor's Pride	5053 (130)	1702 (80)	353 (19)	1230 (201)
Catch Dry Eucalyptus	814 (53)	570 (70)	BLQ	BLQ
Catch Dry Licorice	855 (25)	579 (19)	BLQ	BLQ
Skoal Dry	1455 (86)	998 (33)	78 (7)	125 (10)
Taboka	870 (40)	728 (56)	BLQ	BLQ
Taboka Green	901 (19)	682 (12)	BLQ	BLQ
Bruton Scotch Snuff	5565 (38)	4604 (40)	386 (13)	1673 (49)
Dental Sweet Snuff	14,424 (109)	16,124 (212)	3023 (249)	7387 (102)
Levi Garrett Snuff	9990 (557)	9755 (477)	764 (20)	5313 (382)
Railroad Mills Plain Scotch Snuff	12,832 (599)	8850 (103)	723 (43)	4833 (175)
Red Seal Sweet Snuff	7642 (345)	5554 (226)	444 (25)	4950 (304)

Appendix (*continued*)

Tobacco product	NNN (ng/g)	NAT (ng/g)	NAB (ng/g)	NNK (ng/g)
Camel Frost (2006)	1068 (67)	745 (26)	BLQ	BLQ
Camel Frost (2007)	1009 (38)	874 (13)	75 (32)	345 (52)
Camel Original (2006)	1123 (32)	807 (43)	BLQ	BLQ
Camel Original (2007)	1082 (24)	964 (50)	100 (22)	322 (22)
Camel Spice (2006)	1079 (30)	735 (21)	BLQ	BLQ
Camel Spice (2007)	984 (5)	824 (19)	68 (31)	250 (19)
Cooper Long Cut Wintergreen	12,770 (701)	13,908 (1200)	2221 (211)	6761 (238)
Copenhagen	4753 (130)	4375 (245)	354 (10)	1281 (75)
Copenhagen Long Cut	4347 (74)	3506 (133)	369 (24)	1476 (72)
Copenhagen Pouches	4667 (146)	3745 (160)	354 (12)	1098 (65)
General Loose	659 (13)	BLQ	BDL	BLQ
General Original Portion	875 (58)	688 (7)	BDL	BLQ
General White Portion	728 (41)	586 (30)	BDL	BLQ
Grizzly Long Cut Wintergreen (2006)	4148 (40)	4549 (61)	355 (22)	1085 (41)
Grizzly Long Cut Wintergreen (2007)	3448 (64)	4697 (38)	222 (21)	646 (62)
Husky Fine Cut Natural	5019 (73)	4306 (68)	386 (4)	1483 (9)
Kayak Long Cut Wintergreen	9133 (339)	10,713 (283)	1354 (63)	4273 (204)
Kodiak Premium Wintergreen (2006)	4764 (54)	5489 (115)	472 (38)	1184 (105)
Kodiak Premium Wintergreen (2007)	5037 (204)	6322 (292)	412 (32)	1325 (29)
Longhorn Long Cut Wintergreen	3094 (23)	3432 (62)	205 (22)	887 (75)
Red Seal Fine Cut Natural	4301 (58)	4058 (159)	342 (15)	1346 (70)
Renegades Wintergreen	3244 (65)	3506 (145)	252 (5)	883 (69)
Skoal Fine Cut Original	4689 (109)	3760 (180)	271 (16)	1239 (99)
Skoal Long Cut Cherry	4281 (84)	3528 (51)	316 (10)	1024 (6)
Skoal Long Cut Mint	4780 (139)	3922 (156)	333 (16)	1465 (20)
Skoal Long Cut Straight	4729 (190)	3890 (112)	335 (37)	1488 (58)
Skoal Long Cut Wintergreen	6105 (200)	5512 (188)	458 (45)	1791 (102)
Timberwolf Long Cut Wintergreen	3282 (82)	3844 (111)	184 (14)	789 (15)

[a] Average (Std. Dev.), all values are expressed on a dry weight basis.
[b] BLQ – below limit of quantitation.
[c] BLD – below limit of detection.

Appendix B. Tobacco specific nitrosamine levels for products purchased from the Swedish market.[a]

Tobacco product	NNN (ng/g)	NAT (ng/g)	NAB (ng/g)	NNK (ng/g)
Catch Dry Cassis Menthol	715 (25)	588 (41)	BLQ[b]	BLQ
Catch Dry Eucalyptus	843 (35)	587 (30)	54 (33)	319 (33)
Catch Dry Vanilla Coffee	704 (26)	521 (9)	BLQ	BLQ
Wise Citrus and Menthol	BLQ	BLQ	BLQ	BLQ
General Onyx	701 (61)	649 (50)	BLQ	BLQ
Gustavus Original	808 (31)	656 (66)	BLQ	BLQ
Nick and Johnny Stark	885 (70)	754 (22)	BLQ	BLQ
Rocker Black	684 (27)	448 (42)	BLQ	BLQ
Rocker Silver	601 (34)	422 (41)	BLQ	BLQ
Oliver Twist Original	1318 (82)	1945 (128)	80 (6)	BLQ
Piccanell Original	1140 (93)	622 (49)	BLQ	236 (20)

[a] Average (Std. Dev.), all values are expressed on a dry weight basis.
[b] BLQ – below limit of quantitation.
[c] BLD – below limit of detection.

Appendix C. Selected metals levels for products purchased from the U.S. market.[a]

Tobacco product	Cadmium (ng/g)	Arsenic (ng/g)	Nickel (ng/g)	Chromium (ng/g)	Lead (ng/g)
Ariva	251 (7)	72 (26)	807 (31)	1418 (57)	181 (17)
Stonewall Natural	471 (8)	181 (6)	1928 (68)	2040 (23)	223 (27)
Beech-Nut Chewing Tobacco	530 (8)	122 (4)	1036 (22)	1063 (26)	378 (6)
Hawken Wintergreen	497 (6)	75 (7.4)	1198 (38)	826 (66)	227 (16)
Lancaster Premium Chewing Tobacco	505 (10)	74 (15)	796 (13)	635 (22)	424 (10)
Levi Garrett Chewing Tobacco	636 (28)	157 (15)	1399 (113)	886 (40)	306 (9)
Red Man Chewing Tobacco	469 (5)	124 (16)	1230 (21)	1432 (14)	309 (22)
Red Man Golden Chewing Tobacco	811 (19)	110 (12)	1410 (20)	1196 (30)	415 (24)
Stoker Chew Apple Chewing Tobacco	748 (11)	74 (12)	648 (35)	585 (38)	267 (7)
Taylor's Pride	681 (40)	149 (17)	1331 (37)	1009 (65)	364 (31)
Catch Dry	527 (24)	149	1920	1270 (79)	218

(*continued on next page*)

Appendix (*continued*)

Tobacco product	Cadmium (ng/g)	Arsenic (ng/g)	Nickel (ng/g)	Chromium (ng/g)	Lead (ng/g)
Eucalyptus		(19)	(59)		(16)
Catch Dry Licorice	494 (40)	157 (9)	1946 (133)	1198 (76)	221 (23)
Skoal Dry	741 (41)	72.4 (5.9)	3107 (75)	5740 (245)	262 (18)
Taboka	356 (35)	142 (21)	1223 (94)	1686 (162)	206 (14)
Taboka Green	372 (4)	69.5 (4.1)	1239 (122)	1634 (67)	179 (25)
Bruton Scotch Snuff	1029 (21)	294 (14)	3502 (41)	3480 (234)	447 (25)
Dental Sweet Snuff	727 (28)	233 (21)	2290 (36)	1184 (4)	791 (41)
Levi Garrett Snuff	1306 (60)	199 (18)	2549 (249)	1255 (31)	570 (44)
Railroad Mills Plain Scotch Snuff	1794 (64)	165 (4)	7540 (402)	5538 (144)	582 (65)
Red Seal Sweet Snuff	1443 (203)	312 (9)	5453 (626)	5396 (293)	472 (25)
Camel Snus Frost (2006)	540 (9)	188 (22)	2187 (200)	1900 (170)	220 (14)
Camel Snus Frost (2007)	641 (12)	136 (8)	1498 (86)	1451 (28)	225 (13)
Camel Snus Original (2006)	566 (26)	157 (15)	2084 (184)	1823 (150)	276 (8)
Camel Snus Original (2007)	512 (99)	BLQ[b]	1379 (94)	1209 (91)	221 (9)
Camel Snus Spice (2006)	526 (43)	151 (11)	1734 (90)	1466 (50)	220 (11)
Camel Snus Spice (2007)	740 (147)	108 (14)	1708 (298)	1540 (271)	216 (12)
Cooper Long Cut Wintergreen	1313 (132)	252 (10)	2187 (260)	1746 (149)	389 (1)
Copenhagen	881 (73)	258 (26)	2019 (226)	1583 (120)	380 (53)
Copenhagen Long Cut	1187 (44)	226 (32)	2203 (99)	1448 (39)	370 (10)
Copenhagen Pouches	1009 (20)	311(46)	2128 (63)	1830 (132)	474 (17)
General Loose	437 (10)	153 (22)	1322 (30)	877 (95)	209 (10)
General Original Portion	365 (8)	152 (14)	1471 (115)	1058 (42)	208 (12)
General White Portion	355 (5)	118 (9)	1384 (28)	1178 (82)	180 (17)
Grizzly Long Cut Wintergreen (2006)	1079 (29)	271 (32)	2079 (111)	1786 (4)	382 (8)
Grizzly Long Cut Wintergreen (2007)	1228 (195)	174 (23)	1933 (289)	1488 (275)	244 (24)
Husky Fine Cut Natural	1527 (191)	132 (11)	2144 (73)	1622 (257)	270 (32)
Kayak Long Cut Wintergreen	1442 (217)	312 (44)	2648 (431)	2073 (472)	471 (67)
Kodiak Premium Wintergreen (2006)	1260 (51)	325 (36)	2569 (78)	1922 (112)	437 (8)
Kodiak Premium Wintergreen (2007)	1306 (180)	261 (20)	2065 (296)	1446 (169)	301 (14)
Longhorn Long Cut Wintergreen	1537 (157)	108 (10)	2073 (238)	1835 (116)	222 (13)
Red Seal Fine Cut Natural	1309 (24)	312 (44)	1887 (45)	1430 (17)	406 (21)
Renegades Wintergreen	1395 (206)	144 (14)	2836 (448)	2112 (218)	261 (18)
Skoal Fine Cut Original	1084 (35)	309 (46)	2519 (50)	2285 (23)	430 (37)
Skoal Long Cut Cherry	1054 (43)	249 (5)	1998 (180)	1372 (26)	364 (3)
Skoal Long Cut Mint	1071 (7)	227 (36)	1988 (80)	1462 (155)	311 (17)
Skoal Long Cut Straight	1053 (64)	277 (36)	1963 (29)	1517 (183)	329 (19)
Skoal Long Cut Wintergreen	1115 (51)	245 (36)	2330 (133)	1503 (75)	358 (14)
Timberwolf Long Cut Wintergreen	1871 (297)	214 (12)	2372 (543)	2007 (437)	330 (14)

[a] Average (Std. Dev.), all values are expressed on a dry weight basis.
[b] BLQ – below limit of quantitation.

Appendix D. Selected metals levels for products purchased from the Swedish market.[a]

Tobacco product	Cadmium (ng/g)	Arsenic (ng/g)	Nickel (ng/g)	Chromium (ng/g)	Lead (ng/g)
Catch Dry Cassis Menthol	420 (3)	96 (10)	1545 (26)	986 (46)	240 (15)
Catch Dry Eucalyptus	672 (27)	105 (7)	1584 (71)	955 (122)	198 (17)
Catch Dry Vanilla Coffee	426 (13)	113 (9)	1668 (239)	1035 (20)	254 (19)
Wise Citrus and Menthol	278 (29)	325 (18)	2318 (151)	4452 (361)	737 (31)
General Onyx	615 (97)	84 (7)	2781 (369)	1822 (318)	193 (9)
Gustavus Original	363 (7)	160 (21)	1182 (130)	1334 (160)	244 (4)
Nick and Johnny Stark	564 (6)	120 (9)	2121 (77)	1044 (66)	157 (15)
Rocker Black	430 (22)	111 (6)	1651 (62)	1229 (61)	228 (21)
Rocker Silver	362 (12)	78 (9)	1923 (70)	870 (84)	162 (15)
Oliver Twist Original	1496 (156)	307 (5)	2141 (232)	726 (29)	386 (18)

Appendix (*continued*)

Tobacco product	Cadmium (ng/g)	Arsenic (ng/g)	Nickel (ng/g)	Chromium (ng/g)	Lead (ng/g)
Piccanell Original	436 (93)	115 (23)	770 (96)	956 (164)	501 (33)

[a] Average (Std. Dev.), all values are expressed on a dry weight basis.

Appendix E. Selected GothiaTek® analytes and chloride levels for products purchased from the U.S. market.[a]

Tobacco product	B[a]P (ng/g)	Nitrite (μg/g)	NDMA (ng/g)	Chloride (mg/g)
Ariva	0.3 (0.0)	6.1 (1.5)	BLQ[b]	2.2 (0.0)
Stonewall Natural	0.4 (0.0)	3.7 (0.2)	BLQ	2.2 (0.1)
Beech-Nut Chewing Tobacco	1.2 (0.0)	BLD[c]	BLD	21.3 (0.2)
Hawken Wintergreen	1.8 (0.5)	BLQ	BLD	21.8 (0.4)
Lancaster Premium Chewing Tobacco	5.1 (1.3)	5.0 (0.3)	BLQ	18.1 (0.9)
Levi Garrett Chewing Tobacco	4.0 (0.8)	BLD	BLD	19.0 (0.9)
Red Man Chewing Tobacco	2.4 (0.4)	BLD	BLD	22.0 (0.6)
Red Man Golden Chewing Tobacco	2.0 (0.2)	BLD	BLD	21.3 (0.1)
Stoker Chew Apple Chewing Tobacco	8.0 (1.1)	4.8 (0.1)	BLQ	21.3 (0.3)
Taylor's Pride	5.4 (1.1)	BLD	BLQ	19.0 (0.2)
Catch Dry Eucalyptus	1.3 (0.0)	BLD	BLD	65.5 (1.4)
Catch Dry Licorice	1.3 (0.1)	BLD	BLD	72.2 (2.5)
Skoal Dry	1.1 (0.1)	4.6 (0.5)	BLQ	11.5 (0.3)
Taboka	0.7 (0.2)	BLQ	BLQ	2.0 (0.1)
Taboka Green	0.8 (0.1)	4.6 (0.9)	BLQ	2.3 (0.1)
Bruton Scotch Snuff	118 (12)	BLD	BLQ	17.7 (0.9)
Dental Sweet Snuff	4.7 (0.5)	43.6 (0.6)	222 (12)	15.7 (0.1)
Levi Garrett Snuff	115 (16)	2.5 (0.1)	22.9 (3.3)	9.3 (0.4)
Railroad Mills Plain Scotch Snuff	26.0 (2.9)	15.5 (0.1)	30.5 (2.0)	19.2 (0.2)
Red Seal Sweet Snuff	36.3 (4.5)	4.6 (0.1)	16.7 (1.2)	15.2 (0.1)
Camel Snus Frost (2006)	1.1 (0.1)	BLD	BLD	30.8 (0.1)
Camel Snus Frost (2007)	1.9 (0.2)	BLD	BLQ	33.0 (0.6)
Camel Snus Original (2006)	1.2 (0.1)	BLQ	BLD	32.7 (0.6)
Camel Snus Original (2007)	1.3 (0.1)	BLQ	BLQ	35.1 (0.6)
Camel Snus Spice (2006)	1.3 (0.1)	BLD	BLD	33.3 (0.6)
Camel Snus Spice (2007)	1.8 (0.1)	4.0 (0.5)	BLQ	33.1 (0.5)
Cooper Long Cut Wintergreen	72.8 (7.0)	1229 (16)	37.0 (1.7)	75.0 (1.8)
Copenhagen	94.2 (4.3)	BLD	BLQ	110 (7.0)
Copenhagen Long Cut	71.7 (6.8)	BLD	BLQ	116 (3.0)
Copenhagen Pouches	91.1 (2.2)	BLD	BLQ	96.4 (2.9)
General Loose	1.1 (0.4)	BLD	BLD	93.2 (0.8)
General Original Portion	1.0 (0.2)	BLD	BLD	69.5 (1.1)
General White Portion	0.6 (0.2)	BLD	BLD	87.4 (0.3)
Grizzly Long Cut Wintergreen (2006)	94.4 (3.7)	BLD	BLQ	125 (1.0)
Grizzly Long Cut Wintergreen (2007)	145 (16)	BLD	BLQ	129 (3.0)
Husky Fine Cut Natural	62.5 (8.1)	13.1 (0.6)	BLD	120 (1.0)
Kayak Long Cut Wintergreen	102 (8)	975 (8)	39.8 (6.9)	121 (2.0)
Kodiak Premium Wintergreen (2006)	188 (12)	BLQ	BLD	115 (2.0)
Kodiak Premium Wintergreen (2007)	198 (26)	BLQ	15.8 (1.3)	121 (1.0)
Longhorn Long Cut Wintergreen	48.9 (7.3)	BLQ	BLQ	113 (1.0)
Red Seal Fine Cut Natural	69.7 (0.9)	BLD	BLQ	107 (1.0)
Renegades Wintergreen	60.3 (5.3)	BLQ	BLQ	95.7 (2.0)
Skoal Fine Cut Original	78.3 (2.8)	BLQ	BLD	110 (3.0)
Skoal Long Cut Cherry	64.3 (3.0)	BLD	BLD	108 (1.0)
Skoal Long Cut Mint	64.7 (11.1)	47.2 (4.8)	BLD	109 (2.0)
Skoal Long Cut Straight	72.9 (7.8)	BLQ	BLQ	108 (2.0)
Skoal Long Cut Wintergreen	55.9 (6.8)	91.2 (1.7)	BLQ	109 (2.0)
Timberwolf Long Cut Wintergreen	88.7 (15.7)	BLQ	BLD	109 (1.0)

[a] Average (Std. Dev.), all values are expressed on a dry weight basis.
[b] BLQ – below limit of quantitation.
[c] BLD – below limit of detection.

Appendix F. Selected GothiaTek® analytes and chloride levels for products purchased from the Swedish market.[a]

Tobacco product	B[a]P (ng/g)	Nitrite (μg/g)	NDMA (ng/g)	Chloride (mg/g)
Catch Dry Cassis Menthol	1.6 (0.3)	BLD[c]	BLD	61.3 (4.8)
Catch Dry Eucalyptus	1.5 (0.2)	BLD	BLQ[b]	63.0 (1.0)
Catch Dry Vanilla Coffee	1.0 (0.1)	BLD	BLD	63.0 (2.7)
Wise Citrus and Menthol	0.8 (0.1)	BLD	15.0 (1.5)	0.6 (0.1)
General Onyx	0.3 (0.1)	BLD	BLQ	79.5 (1.6)
Gustavus Original	4.1 (0.1)	BLD	BLD	75.9 (0.7)
Nick and Johhny	2.1 (0.3)	5.6 (0.2)	BLQ	60.4 (0.9)
Rocker Black	1.3 (0.2)	BLQ	24.5 (5.0)	64.3 (0.3)
Rocker Silver	1.6 (0.0)	BLQ	19.8 (4.1)	58.5 (0.7)
Oliver Twist Original	88.5 (12.6)	BLQ	BLQ	4.3 (0.1)
Piccanell Original	1.8 (0.1)	5.2 (0.1)	BLQ	104 (1.0)

[a] Average (Std. Dev.), all values are expressed on a dry weight basis.
[b] BLQ – below limit of quantitation.
[c] BLD – below limit of detection.

Appendix G. Nicotine content and calculated "Free Nicotine" levels observed for products purchased from the U.S. market.[a]

Tobacco product	Nicotine (mg/g)	Moisture (%)	pH	Calculated free nicotine (mg/g)	Calculated free nicotine (% of total nicotine)
Ariva	3.90 (0.08)	4.8 (0.1)	7.36 (0.02)	0.70	17.95
Stonewall Natural	8.19 (0.23)	2.8 (0.1)	7.24 (0.02)	1.17	14.23
Beech-Nut Chewing Tobacco	7.07 (0.08)	23.1 (0.1)	5.56 (0.00)	0.02	0.35
Hawken Wintergreen	2.92 (0.16)	29.0 (0.0)	5.37 (0.03)	0.01	0.22
Lancaster Premium Chewing Tobacco	8.01 (0.24)	22.3 (0.2)	5.05 (0.00)	0.01	0.11
Levi Garrett Chewing Tobacco	5.34 (0.15)	21.6 (0.2)	6.10 (0.01)	0.06	1.19
Red Man Chewing Tobacco	8.58 (0.19)	23.9 (0.2)	5.97 (0.01)	0.08	0.88
Red Man Golden Chewing Tobacco	7.70 (0.13)	25.5 (0.1)	5.88 (0.00)	0.06	0.72
Stoker Chew Apple Chewing Tobacco	3.77 (0.02)	21.7 (0.2)	5.37 (0.03)	0.01	0.22

Appendix (*continued*)

Tobacco product	Nicotine (mg/g)	Moisture (%)	pH	Calculated free nicotine (mg/g)	Calculated free nicotine (% of total nicotine)
Taylor's Pride	6.43 (0.17)	22.7 (0.5)	5.98 (0.02)	0.06	0.90
Catch Dry Eucalyptus	15.93 (0.70)	23.4 (0.1)	7.00 (0.04)	1.39	8.72
Catch Dry Licorice	16.70 (0.94)	21.2 (0.2)	6.65 (0.03)	0.68	4.09
Skoal Dry	11.91 (0.86)	6.6 (0.1)	7.42 (0.03)	2.39	20.08
Taboka	16.73 (0.37)	9.8 (0.1)	6.36 (0.00)	0.36	2.14
Taboka Green	13.01 (1.15)	9.8 (0.1)	6.60 (0.02)	0.48	3.66
Bruton Scotch Snuff	17.49 (0.26)	7.2 (0.1)	6.48 (0.03)	0.49	2.80
Dental Sweet Snuff	11.14 (0.33)	9.0 (0.1)	6.10 (0.01)	0.13	1.19
Levi Garrett Snuff	16.60 (1.03)	3.6 (0.1)	5.65 (0.03)	0.07	0.42
Railroad Mills Plain Scotch Snuff	23.13 (1.78)	6.5 (0.2)	6.35 (0.01)	0.48	2.09
Red Seal Sweet Snuff	15.08 (0.72)	7.5 (0.2)	6.45 (0.02)	0.40	2.62
Camel Snus Frost (2006)	13.25 (0.13)	34.1 (0.2)	7.76 (0.01)	4.70	35.46
Camel Snus Frost (2007)	14.10 (0.11)	32.2 (0.1)	7.72 (0.02)	4.71	33.39
Camel Snus Original (2006)	13.87 (0.06)	34.3 (0.4)	7.73 (0.00)	4.70	33.90
Camel Snus Original (2007)	13.49 (0.29)	31.9 (0.6)	7.95 (0.03)	6.20	45.98
Camel Snus Spice (2006)	13.16 (0.62)	32.8 (0.8)	8.03 (0.01)	6.65	50.58
Camel Snus Spice (2007)	13.35 (0.62)	32.2 (0.5)	7.81 (0.02)	5.09	38.14
Cooper Long Cut Wintergreen	7.97 (0.39)	51.1 (0.3)	7.22 (0.01)	1.09	13.68
Copenhagen	12.68 (0.19)	54.4 (0.2)	7.55 (0.02)	3.21	25.31
Copenhagen Long Cut	13.91 (0.19)	55.0 (0.2)	7.82 (0.01)	5.38	38.69
Copenhagen Pouches	11.21 (0.19)	52.5 (0.4)	8.21 (0.01)	6.81	60.77
General Loose	7.15 (0.18)	56.3 (0.2)	7.57 (0.03)	1.87	26.19
General Original Portion	8.46 (0.11)	50.9 (0.1)	8.20 (0.02)	5.10	60.22
General White Portion	7.92 (0.18)	52.3 (0.2)	8.21 (0.02)	4.81	60.77
Grizzly Long Cut Wintergreen (2006)	10.29 (0.15)	53.2 (0.1)	8.27 (0.03)	6.59	64.01
Grizzly Long Cut Wintergreen (2007)	11.20 (0.26)	53.2 (0.1)	8.06 (0.04)	5.86	52.30

Appendix (*continued*)

Tobacco product	Nicotine (mg/g)	Moisture (%)	pH	Calculated free nicotine (mg/g)	Calculated free nicotine (% of total nicotine)
Husky Fine Cut Natural	12.86 (0.14)	55.5 (0.2)	7.79 (0.01)	4.77	37.06
Kayak Long Cut Wintergreen	11.88 (0.36)	53.9 (0.1)	7.39 (0.03)	2.26	18.99
Kodiak Premium Wintergreen (2006)	10.93 (0.24)	52.5 (0.2)	8.19 (0.01)	6.52	59.66
Kodiak Premium Wintergreen (2007)	10.70 (0.51)	54.2 (0.1)	8.53 (0.01)	8.18	76.39
Longhorn Long Cut Wintergreen	13.79 (0.29)	54.7 (0.1)	7.87 (0.02)	5.72	41.45
Red Seal Fine Cut Natural	13.17 (0.15)	54.6 (0.1)	7.51 (0.02)	3.11	23.61
Renegades Wintergreen	13.36 (0.17)	50.8 (0.1)	7.36 (0.02)	2.40	17.95
Skoal Fine Cut Original	13.31 (0.17)	54.8 (0.1)	7.63 (0.00)	3.85	28.95
Skoal Long Cut Cherry	12.70 (0.15)	53.7 (0.1)	7.20 (0.01)	1.67	13.15
Skoal Long Cut Mint	12.93 (0.06)	54.8 (0.1)	7.62 (0.00)	3.68	28.47
Skoal Long Cut Straight	13.37 (0.20)	54.4 (0.0)	7.64 (0.01)	3.94	29.42
Skoal Long Cut Wintergreen	12.84 (0.24)	54.7 (0.2)	7.48 (0.01)	2.87	22.38
Timberwolf Long Cut Wintergreen	14.13 (0.15)	55.4 (0.2)	7.78 (0.02)	5.16	36.53

[a] Average (Std. Dev.), all values are expressed on an "as is" basis.

Appendix H. Nicotine content and calculated "Free Nicotine" levels observed for products purchased from the Swedish market.[a]

Tobacco product	Nicotine (mg/g)	Moisture (%)	pH	Calculated free nicotine (mg/g)	Calculated free nicotine (% of total nicotine)
Catch Dry Cassis Menthol	15.28 (0.32)	21.1 (0.2)	7.51 (0.03)	3.61	23.61
Catch Dry Eucalyptus	15.63 (0.33)	22.8 (0.4)	7.21 (0.02)	2.10	13.41
Catch Dry Vanilla Coffee	15.60 (0.46)	20.1 (0.1)	7.45 (0.04)	3.31	21.21
Wise Citrus and Menthol	17.92 (1.09)	7.4 (0.3)	9.18 (0.02)	16.76	93.53
General Onyx	10.49 (0.57)	51.9 (0.3)	7.90 (0.03)	4.53	43.14
Gustavus Original	7.48 (0.27)	47.3 (0.2)	7.66 (0.07)	2.27	30.39
Nick and Johnny	10.55 (0.27)	49.8 (0.2)	7.98 (0.03)	5.03	47.70
Rocker Black	8.11 (0.18)	48.4 (0.3)	8.39 (0.03)	5.69	70.10
Rocker Silver	6.91 (0.14)	47.1 (0.1)	7.51 (0.04)	1.63	23.61
Oliver Twist Original	25.37 (1.55)	20.4 (0.1)	4.99 (0.01)	0.02	0.09
Piccanell Original	21.14 (0.10)	32.0 (0.2)	5.57 (0.04)	0.07	0.35

[a] Average (Std. Dev.), all values are expressed on an "as is" basis.

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