7.5.4-1: INITIAL - CHEMISTRY AND CONSTITUENTS - LITERATURE SUMMARY

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List of Abbreviations

BaA	benz[a]anthracene
BaP	benzo[a]pyrene
CDC	Centers for Disease Control and Prevention
DWB	dry weight basis
GC	gas chromatography
GC-FID	gas chromatography flame ionization detector
GC-MS	gas chromatography-mass spectrometry
HPHC	harmful and potentially harmful constituent
Iso-NNAC	4-(methylnitrosamino)-4-(3-pyridyl)butyric acid
LOD	limit of detection
MNBA	4-(methylnitrosamino)butyric acid
MNPA	3-(methylnitrosamino)propionic acid
MRTP	Modified Risk Tobacco Product
MRTPA	Modified Risk Tobacco Product Application
NAAs	N-nitrosamines
NAB	N'-nitrosoanabasine
NAT	N'-nitrosoanatabine
NDELA	N-nitrosodiethanolamine
NDMA	N-nitrosodimethylamine
NMOR	N-nitrosomorpholine
NNA	4-(N-methyl-N-nitrosamino)-4-(3-pyridyl)butanal
NNAC	4-(methyl-nitrosamino)-4-(3-pyridyl)butyric acid
NNAL	4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol
NNK	4'-(nitrosomethylamino)-1-(3-pyridyl)-1-butanone
NNN	N’-nitrosonornicotine
NO_2	nitrite
NO_3	nitrate
NPRO	N-nitrosoproline
NPYR	N-nitrosopyrrolidine;
NSAR	N-nitrososarcosine
OV	oven volatiles
PAH	polyaromatic hydrocarbon
S-NNN	sinister-N'-nitrosonornicotine
ST	smokeless tobacoo
TSNA	tobacco specific nitrosamines
US	United States
USSTC	United States Smokeless Tobacco Company LLC
VNA	volatile nitrosamines

7.5.4-1. CHEMISTRY AND CONSTITUENTS LITERATURE REVIEW

This section addresses the Food and Drug Administration's (FDA's) Modified Risk Tobacco Product (MRTP) Applications Draft Guidance recommendations for data and information on product analyses to assess users' and nonusers' potential exposure to harmful substances.

The FDA's Modified Risk Tobacco Product Applications Draft Guidance recommends:

- "product analyses regarding the chemistry"
- "product analyses will facilitate FDA's understanding of the product, the potential for exposure to harmful or potentially harmful constituents from use of the product, and provide context for evaluating other data submitted in an MRTPA"

Product chemistry also informs the Health Effects of smokeless tobacco (ST) (Section 6.1) and the relevance/ applicability of existing epidemiologic data to the ST products that are the subject of this application. This literature review section provides a product chemistry overview of the entire relevant ST category since publications containing product specific data are scarce. Specific product chemistry and batch-to-batch information are found in Section 3 Chemistry, Manufacturing, and Controls.

7.5.4-1.1.Literature Search and Review Process

Altria Client Services LLC conducted a comprehensive literature search to identify published information relevant to chemistry and constituents of ST products. A description of our literature search and review process is presented in Section 7.5.1 of this MRTPA. This review is limited to studies of ST products used in the United States that were published between through December 2014. From this search, a total of 6,742 publications were identified, and, after a comprehensive and in depth critical review, 537 were determined to be in scope. These publications were further reviewed to assess which specific category(ies) in the MRTPA Draft Guidance each article addressed. Reports published shortly after the date of our last search were included in this review when deemed to be significant contributions to this body of research. Sixty-seven studies of the chemistry and constituents of ST products were identified. These 67 studies fell into two broad categories. One category of studies contains those that were designed specifically to assess product chemistry and presented data on a relatively wide range of (i.e., 15 or more) ST products and analytes. The second category of studies contains those that were not designed to be product chemistry studies per se, but did contain some product chemistry characterization data, typically collected prior to exposure or clinical studies. We relied primarily on findings from the first category, since they were more comprehensive studies that would address the questions in the MRTP guidance noted above. It was found that findings in the second category of studies were typically redundant with findings from the more comprehensive studies in the first category.

Characteristics of the 67 studies identified in our literature search are presented in Table 7.5.4-1-2. Of the 67 studies, 17 provided data relevant to the candidate product of this application, Copenhagen® Snuff Fine Cut.

An updated literature review was conducted to bridge the original review to February 2017, and updated findings informing health risk of ST are presented in Section 7.5.4-2.

7.5.4-1.2.Chemistry of Smokeless Tobacco Products

Many compounds and classes of compounds have been measured in ST products. Of these, oven volatiles (OV), pH, nicotine, tobacco specific nitrosamines (TSNAs), and polyaromatic hydrocarbons (PAHs) have been the most frequently studied.

The FDA has established a list of 37 harmful and potentially harmful constituents (HPHCs) in ST by the following classes: biological (1), carbonyls (3), metals (7), nitrosamines (8), other (7), PAHs (8), and radioactive (3). Currently, tobacco product manufacturers are required to report to FDA the levels of an abbreviated list of 9 HPHCs in ST.

For decades, scientists and public health researchers have measured levels of chemical constituents to compare products (Brunnemann, Genoble, & Hoffmann, 1985; Hecht, Chen, Hirota, et al., 1978; Hecht, Chen, Ornaf, Hoffmann, & Tso, 1978; Hoffmann, Hecht, Ornaf, & Wynder, 1974; Hoffmann, Hecht, Ornaf, Wynder, & Tso, 1976). An issue identified by Oldham et al. (2014) in a recent comprehensive study, was that measurements of constituents of ST are not consistent as a result of assay, inter-laboratory, and temporal variability. In this study, commercial product from the same manufacturing batch was measured for HPHC regulatory reporting using established regimens. Statistically significant differences for some of these HPHCs were seen when the same lot of the product was put in cold storage and measured 4-6 months later by the same analysts, using the same methods in the same laboratories. The authors conclude that until standardized assays with established reproducibility and methods are used, resulting HPHC data will be unreliable for product comparisons.

Many earlier published studies lacked clear product identification, misclassified product categories by including chewing tobacco and dry snuff incorrectly, used various different analytical methods without quantifying specificity or selectivity, and lacked sophisticated separation technologies and analytical instrumentation. In more recent studies, the inclusion of a certified reference standard, such as Smokeless Tobacco Research Product 2S3 (Moist Snuff) or CORESTA Reference Product CRP2, provides an additional comparator and quality check. CRP2 (American-style loose moist snuff) has been extensively studied annually since its 2009 manufacture via an inter-laboratory study. Sixteen laboratories participated in the 2015 study; reporting levels of nicotine, pH, moisture (OV), and TSNAs using CORESTA Recommended Methods (Wagner, 2015). Statistical analysis provided an assessment of inter-laboratory variability to demonstrate the suitability as a reference product for future studies. The more recent market surveys conducted have identified the commercial products by brand and included a reference product (Borgerding, Bodnar, Curtin, & Swauger, 2012; Oldham et al., 2014; Richter & Spierto, 2003).

7.5.4-1.3.TSNAs

TSNAs have received significant attention as important potentially carcinogenic compounds. Brunnemann et al. (2002) conducted a study in which they compared chemical profiles of two brands of oral moist snuff (type A and type B) in 2002. The authors found that the TSNA level was 4.6 µg/g in type A and 37.6 µg/g in type B. When the authors had purchased the same brands in 1994, the TSNA level was 4.1 µg/g in type A and 17.2 µg/g in type B. These numbers were lower than the ones of the same products purchased in 1984 (18.4 µg/g in type

A and 80 µg/g in type B). Brunnemann et al. (2002) noted "[t]hese data suggest that the snuff manufacturers have changed production processes in ways that have led to significant decreases of the major group of carcinogens in snuff, the TSNA." In addition, Borgerding et al. (2012) note in a market survey of 2006 and 2007 products that "TSNA concentrations observed for all commercial products were lower than historically reported values…". Djordjevic et al. (1993) performed a study to compare levels of carcinogenic N-nitrosamines in two U.S. brands and three Sweden brands of oral snuff. They found that for U.S. brand A, there was 75.8% reduction in N'-nitrosonornicotine (NNN) levels, 89.0% reduction in 4'-(nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) levels, and 84.1% reduction in N'-nitrosoanatabine (NAT) levels between 1980 and 1992. For U.S. brand B, there were 85.4% reduction in NNN levels, 70.8% reduction in NNK levels, and 91.1% reduction in NAT levels. The authors note that "[d]uring the past decade, a gradual reduction of the levels of carcinogenic N-nitrosamines was observed in the leading snuff brands in the USA…" Fisher et al. (2012) studied the sources of and strategies to abate TSNA formation. The authors compared TSNA levels of three commercial moist ST products between 1997 and 2010. They showed that for all three brands purchased in 1997, TSNA levels at the start of fermentation were at least doubled by the end of shelf life. However, for all three brands purchased in 2010, the TSNA levels at the start of fermentation were essentially equivalent to the TSNA levels in incoming leaf and at the end of shelf life. Therefore, Fisher et al. (2012) concluded that "[a]s demonstrated herein, [moist ST] production practices have been modified so that since 2005, TSNA formation is inhibited during fermentation and subsequent shelf storage. Therefore, in [moist ST] products manufactured under appropriately sanitary conditions, the levels of TSNA in finished product are a direct function of levels in incoming tobacco leaf." Additional details regarding quality manufacturing practices to mitigate TSNA formation can be found in Section 3.1.

7.5.4-1.4.Other Analytes

Findings for the more common measures of ST chemistry are presented in Table 7.5.4-1-1. Of the 67 relevant articles reviewed, 17 provide data relevant to the candidate product of this application. Oldham et al. (2014) published one of the articles providing chemistry data on the candidate product, and this data is also presented in Table 7.5.4-1-1.This data was reported to the FDA as part of HPHC reporting (Altria Client Services LLC on behalf of United States Smokeless Tobacco Co. LLC), and the product values are within the range of market values reported in the literature. The Copenhagen® Snuff fine cut used in the Oldham et al. (2014) study was obtained in 2012 and unlikely the specific formulation represented in the grandfathered product. HPHC data more specific to the candidate product are included in Section 7.1. We anticipate many HPHC differences are related to tobacco crop or analytical method variation. We assume that publications reporting product specific data prior to 2007 would likely include the grandfathered version of the candidate product.

Table 7.5.4-1-1: Common Measures of Moist Smokeless Tobacco Products

Constituent	Value[1]	Sources	Copenhagen® Snuff Fine Cut[2] (Oldham et al., 2014) Value[3,1] [Average[4,1]]
Oven Volatiles (wt %)			
Average	52.36	(Borgerding et al., 2012; Chen, Isabelle, Pickworth, & Pankow, 2010; Lauterbach, Bao, Joza, & Rickert, 2011; Oldham et al., 2014; Richter, Hodge, Stanfill, Zhang, & Watson, 2008; Rickert et al., 2009; Stepanov et al., 2010)	–
Range	25.9-64.9	–	55.3 [54.4]
pH			
Average	7.59	(Borgerding et al., 2012; Lauterbach et al., 2011; Richter et al., 2008; Rickert et al., 2009)	–
Range	5.54-8.62	–	Not Reported [7.68[5]]
Nicotine (mg/g) as-is			
Average	11.82	(Borgerding et al., 2012; Chen et al., 2010; Lauterbach et al., 2011; Oldham et al., 2014; Richter et al., 2008; Rickert et al., 2009)	–
Range	1.55-15.7	–	11.5 [12.5]
NNN (ng/g) DWB			
Average	8409	(Borgerding et al., 2012; Oldham et al., 2014; Richter et al., 2008; Rickert et al., 2009)	–
Range	855-91,122	–	3,803 [3,829]
NNK (ng/g) DWB			
Average	2363	(Borgerding et al., 2012; Oldham et al., 2014; Richter et al., 2008; Rickert et al., 2009)	–
Range	129-21,306	–	1,063 [1,035]
BaP (ng/g) DWB			
Average	66.9	(Borgerding et al., 2012; McAdam, Faizi, Kimpton, Porter, & Rodu, 2013; Oldham et al., 2014; Rickert et al., 2009; Stepanov et al., 2010)	–
Range	13-198	–	120 [117]
BaA (ng/g) DWB			
Average	298.2	(McAdam et al., 2013; Oldham et al., 2014; Stepanov et al., 2010)	–
Range	5.3-957	–	537[6]

[1] Excludes reference and snus products. Unit conversion was necessary for some references. For example, HPHC values (as-is) were converted to DWB using the reported OV (55.3%). For example, 1,700 ng/g NNN x (1 g as is/ 0.447 g dry) = 3,803 ng/g tobacco DWB.

[2] Copenhagen® Fine Cut and variants thereof have been on the market since 1822. Since 2007, USSTC made minor modifications to Copenhagen® Snuff Fine Cut, which are the subject of a separate pending Substantial Equivalence review. The candidate product subject to the MRTPA is the product for which FDA granted grandfathered status (Grandfather Number – GF1200194) on November 1, 2012.
[3] Values represent the average of n=7, unless otherwise noted.
[4] Section 7.1; Table 7.1-14
[5] While Oldham et al. (2014) measured pH, it was not reported in the publication.
[6] This value represents average of n=3.

7.5.4-1.5.Product Stability

In addition to product chemistry per se, several investigators have studied product chemistry over time; i.e., shelf life stability (Andersen, Burton, Fleming, & Hamilton-Kemp, 1989; M.V. Djordjevic, Fan, Bush, Brunnemann, & Hoffann, 1993; Fisher et al., 2012; Hoffmann & Adams, 1981). Fisher (2012) discusses sources of and technical approaches for the abatement of TSNA formation in ST. The researchers show that the implementation of agricultural growing/curing and manufacturing sanitation processes eliminates any increase in TSNAs from incoming leaf through the end of shelf life as indicated by the "Sell By" date. Additional product specific information on product stability and shelf life can be found in Section 3.1 and Section 7.2.

7.5.4-1.6.Summary

Characteristics of the 67 studies identified in our literature search are presented in Table 7.5.4-1-2. The studies are separated into two groups: those that provide general ST chemistry data (n = 50) and those that provide data relevant to the candidate product of this application, Copenhagen® Snuff Fine Cut (n = 17).

Table 7.5.4-1-2: Summary of Findings from Studies of the Chemical Product Analyses of Smokeless Tobacco

Author	Title	Analyte Class	Author's Findings	Comments
Articles Contributing General ST Chemistry Data				
(Song et al., 2016)	Chemical and toxicological characteristics of conventional and low-TSNA moist snuff tobacco products	pH, moisture, nicotine, free nicotine, TSNAs (4), metals (6), humectants (3), ammonia, BaP, formaldehyde, NDMA, NO_3	Multidimensional data analysis showed a clear difference for constituents between conventional and low-TSNA (i.e., snus) moist snuff products.	Strength: Range of U.S. commercial products studied (7). Comments: Not surprising that snus is different from conventional moist snuff products since they have different leaf blends and processing techniques.
(McAdam et al., 2015)	Analysis of hyrdazine in smokeless tobacco products by gas chromatography-mass spectrometry	Hydrazine	Hydrazine is not quantifiable using currently available analytical methodology in ST products (LOD <10 ng/g).	Strength: Range of U.S. commercial products studied (15).
(Lawler, Stanfill, Zhang, Ashley, & Watson, 2013)	Chemical characterization of domestic oral tobacco products: total nicotine, pH, unprotonated nicotine and tobacco-specific N-nitrosamines	pH, moisture, nicotine, TSNAs	All oral tobacco products should not be thought of as a single, homogenous category; there is wide variation in analytical measures.	Limitation: Only 2 U.S. commercial products studied: Skoal Bandits Mint and Wintergreen.
(McAdam et al., 2013)	Polycyclic aromatic hydrocarbons in US and Swedish smokeless tobacco products	PAHs (21)	Good correlations were obtained between BaP and the other PAHs, providing evidence for the first time that it can be used as a good marker for PAHs in smokeless tobacco products.	Strength: Market survey- Range of U.S. commercial products (16) studied; clear method description and validation for matrix. Thorough, rigorous, well-referenced paper.
(Benowitz et al., 2012)	Exposure to nicotine and carcinogens among Southwestern Alaskan Native cigarette smokers and smokeless tobacco users	Nicotine, TSNAs (4), NNAL	• Concentrations of TSNAs were substantially higher in ST than in cigarettes. • Nicotine concentrations are higher in general in cigarette tobacco than in ST.	Strength: Range of U.S. commercial products (22) studied. Limitation: Reported data as average of 22 brands. Product not identified.
(Fisher et al., 2012)	Sources of and technical approaches for the abatement of tobacco specific nitrosamine formation in moist smokeless tobacco products	TSNAs, NO_2, NO_3	• Since 1997, average TSNA levels have declined by 50% in USSTC ST products. • Production practices have changed so that TSNA formation is inhibited during fermentation/shelf storage. • TSNA finished product levels are a direct function of incoming tobacco leaf levels.	Strengths: Comprehensive access to production process, 13 years of product data. Fundamental study of entire process from harvest and curing to fermentation and shelf life. Limitation: 3 U.S. commercial products studied, not identified.

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Chen et al., 2010)	Levels of mint and wintergreen flavorants: smokeless tobacco products vs. confectionery products	Nicotine, water, OV, flavor compounds (7)	Methyl salicylate was found to be present at levels that averaged eight times the level in Wintergreen-type confectionary products.	Strength: Range of U.S. commercial products (25) and 2 reference samples studied. Limitation: Assumes 100% human exposure.
(Stanfill, Jia, Ashley, & Watson, 2009)	Rapid and chemically selective nicotine quantification in smokeless tobacco products using GC-MS	Nicotine	• The CDC GC-FID method was compared with the GC-MS method. • The GC-MS method was more specific for nicotine with highly flavored interferences.	Limitations: 7 products studied, not identified.
(Hecht et al., 2008)	Exposure to nicotine and a tobacco-specific carcinogen increase with duration of use of smokeless tobacco	Nicotine, NNK, pH	NNK levels for the products used here were similar to those measured previously.	Limitations: Product chemistry was secondary to main exposure focus. 3 products studied: Copenhagen® LC, Skoal LC Straight, Kodiak Premium WG
(Rickert, Wright, Trivedi, Momin, & Lauterbach, 2007)	A comparative study of the mutagenicity of various types of tobacco products	Nicotine, menthol, glycerine, moisture	The two ST products studied have nicotine concentrations typical of the 2S3 reference and those reported for commercial products.	Limitations: Product chemistry not the main focus. Not standard analytical methodology. Three products studied, not identified.
(Clarke, Bezabeh, & Howard, 2006)	Determination of carbohydrates in tobacco products by liquid chromatography-mass spectrometry/mass spectrometry: a comparison with ion chromatography and application to product discrimination	pH, moisture, reducing sugars (glucose, fructose, sucrose)	• Sugars can be used to distinguish tobacco types and added casings. • ST had the lowest sugar content of the 4 product categories studied.	Limitation: Only 1 product studied, not identified.
(Brunnemann et al., 2002)	Chemical profile of two types of oral snuff tobacco	Moisture, NO_2, NO_3, nicotine, minor alkaloids (6), TSNAs (4)	Data suggest that the snuff manufacturers have changed the production processes in ways that have led to significant decreases of TSNAs.	Strength: Included Kentucky 1S3 Reference. Limitations: No product identification. Only 2 products studied.
(Rubinstein & Pedersen, 2002)	Bacillus species are present in chewing tobacco sold in the United States and evoke plasma exudation from the oral mucosa	Microbiology	Five *Bacillus* species were isolated from two popular brands of commercially available chewing tobacco.	Limitations: Product chemistry not the main focus. Contamination assertion incorrect; they are endogenous. Confusing use of “chewing tobacco” product classification (assume authors mean ST).

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Carmella, McIntee, Chen, & Hecht, 2000)	Enantiomeric composition of N'-nitrosonornicotine and N'-nitrosoanatabine in tobacco	NNN and NAT: S and R enantiomers	Results indicate that nornicotine is the precursor to NNN and anatabine is the precursor to NAT.	Limitations: Only 2 products studied, not identified. No evidence that S and R enantiomers have any different relative risk.
(Chan, Chowdhry, Chang, & Kew, 1999)	Initial characterization of the complement activating compounds in extracts of smokeless tobacco	Large polyphenolic compound with Mw >400 kDA	No information related to product chemistry.	Limitations: Did not identify analyte. No other reports regarding this topic.
(Ciolino et al., 1999)	Reversed phase ion-pair liquid chromatographic determination of nicotine in commercial tobacco products. 1. Moist snuff	Nicotine	A new method for the determination of nicotine in tobacco products was developed.	Strength: The values from the new method agree well with previously published values. Limitation: Kentucky reference 1S3 only product identified.
(Jacob, Yu, Shulgin, & Benowitz, 1999)	Minor tobacco alkaloids as biomarkers for tobacco use: comparison of users of cigarettes, smokeless tobacco, cigars, and pipes	Minor alkaloids	Levels of various tobacco alkaloids in commercial ST products were determined.	Limitation: Product chemistry not the main focus. Only reports averages of 4 products, not identified
(Tilashalski, Rodu, & Mayfield, 1994)	Assessing the nicotine content of smokeless tobacco products	Nicotine, moisture	ST products had the highest nicotine content of the 3 categories studied.	Limitation: Range of U.S. commercial products (6)

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Andersen, Fleming, Hamilton-Kemp, & Hildebrand, 1993)	pH Changes in Smokeless Tobaccos Undergoing Nitrosation during Prolonged Storage: Effects of Moisture, Temperature, and Duration	pH, TSNAs, NO_2	• The pHs of moist snuff (55.5%) and dry snuff that was remoisturized to 51.4% was increased 0.3-2.1 pH units during storage. • The pHs of decreased water content moist snuff (21.9%) and dry snuff (12.3%) was decreased by 0.2-0.4 unit. • Chewing tobacco pHs at high (49.3%) and low (22.3%) moisture levels and at each moisture-temperature treatment was reduced during storage. • "Nitrosated pyridine alkaloids increased only in tobaccos that became more alkaline during storage." • "Interactions between the effects of moisture and temperature occurred. " • "Heating snuffs at 100° for 30 min at zero storage time prevented the increases of pH, nitrosamines, and nitrite observed in non-heat-treated controls during storage."	Limitation: only studied 1S3 reference product
(M. V. Djordjevic et al., 1993)	The need for regulation of carcinogenic N-nitrosamines in oral snuff	water, alkaloids, NO_2, NO_3, pH, TSNAs, N-nitrosamino acids	A 71%-91% reduction in individual TSNAs was observed during the past decade.	Limitations: No product identification. Cites and uses analytical methods from 1961 and 1967.

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Maier, Purser, Bray, & Pories, 1993)	Trace metal characterization of some standard smokeless tobaccos	Metals: Cd, Cu, Ni, Zn	• "[Cadmium] in the snuff tobacco was 2-3 times more soluble in the buffer solution than was in the cadmium in the chewing tobacco." • "In general, more than half of the [copper] contained in the chewing tobacco and snuff was resistant to solubilization regardless of the extractant use." • "Most of the [nickel] was held in a tightly bound form within the tobaccos examined." • "The [zinc] present in all three tobaccos was held relatively tightly since only about 35-70% of the total [zinc] present was extracted by the chelating extractant. The [zinc] solubility in the buffer solution was higher than the solubility of either [cadmium] or [nickel] and just as in the case of [copper], the zinc may exert a counter effect on the already small amount of [cadmium] and [nickel], which become relatively easily soluble."	Limitations: Data reported as % extracted by 3 chelating agents, not absolute quantities. Only sample identified-1S3 Kentucky reference.
(Prokopczyk, Wu, Cox, & Hoffmann, 1992)	Bioavailability of tobacco-specific N-nitrosamines to the snuff dipper	TSNAs (4)	This method extracted higher levels of NNK (2-7 times) than had been determined previously.	Limitation: 1 product studied, not identified.
(Prokopczyk, Hoffmann, Cox, Djordjevic, & Brunnemann, 1992)	Supercritical fluid extraction in the determination of tobacco-specific N-nitrosamines in smokeless tobacco	TSNAs (4)	This method releases up to 7 times more NNK from tobacco than has been determined previously.	Limitations: 3 products, not identified
(Andersen, 1991)	Nitrosated, Acylated, and Oxidized Pyridine Alkaloids during Storage of Smokeless Tobaccos: Effects of Moisture, Temperature, and Their Interactions	TSNAs (3), NO_2, NO_3, minor alkaloids (5), 2,3'dipyridine, other alkaloid derivatives	Nitrosated alkaloids were higher in the higher water content ST at a given temperature for each storage duration.	Limitation: only studied 1S3 reference product
(Brunnemann & Hoffmann, 1991)	Decreased concentrations of N-nitrosodiethanolamine and N-nitrosomorpholine in commercial tobacco products	NDELA and NMOR	A historical decrease in these 2 analytes was observed due to elimination of their precursors from production processes.	Limitation: 1 product studied, not identified

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Chortyk & Chamberlain, 1991)	The application of solid phase extraction to the analysis of tobacco-specific nitrosamines	TSNAs	A new isolation and separation method has been developed for TSNAs.	Limitation: Only 1 reference product studied
(Hoffmann, Djordjevic, & Brunnemann, 1991)	New brands of oral snuff	Moisture, pH, alkaloids (7), VNAs, NAAs, TSNAs	A gradual decrease in TSNAs occurred in the two leading ST brands.	Limitations: Studied 4 products, not identified. Typo in Table 1: MNPA should be NMPA.
(Sharma, Prokopczyk, & Hoffmann, 1991)	Supercritical fluid extraction of moist snuff	Minor alkaloids, flavor compounds, general screening	A wide variety of components was identified, and several were quantified.	Limitations: Only 1 product studied, not identified. Strength: included 1S3 reference
(Andersen et al., 1989)	Effect of storage conditions on nitrosated, acylated, and oxidized pyridine alkaloid derivatives in smokeless tobacco products	NO_2, nitrosated pyridine alkaloids, acylated pyridine alkaloids	Very large concentration increases in NO_2, nitrosated pyridine alkaloids, and NNK occurred during storage at 24ºC for 52 weeks.	Comment: Sanitation and production practices have improved since this publication such that TSNAs do not increase during shelf life.
(M. V. Djordjevic, Brunnemann, & Hoffmann, 1989)	Identification and analysis of a nicotine-derived N-nitrosamino acid and other nitrosamino acids in tobacco	Nicotine, minor alkaloids, N-nitrosamino acids, TSNAs (4)	A new method was developed to measure iso-NNAC and other nitrosamino acids.	Limitations: ST not the main focus, 1S3 Reference only product studied.
(LaVoie, Tucciarone, Kagan, Adams, & Hoffmann, 1989)	Analyses of steam distillates and aqueous extracts of smokeless tobacco	Flavor compounds	The range of flavor additives present in commercial products was quantified relative to the unflavored 1S3 reference.	Limitations: 9 products studied, not identified
(Maier, Bray, & Pories, 1989)	Trace element status of some commercial smokeless tobaccos	Metals: Cd, Cu, Ni, Se, Zn	The largest metal portion remains bound in the ST and resistant to chemical solubilization.	Limitations: Ambiguous sample category identification. Data reported as mean % of total at various pH and enzyme conditions.
(Chamberlain, Schlotzhauer, & Chortyk, 1988)	Chemical composition of nonsmoking tobacco products	TSNAs(4), alkaloids, sugars, chlorogenic acid, solanesol, organic acids	Nitrosamine levels did not correlate with total alkaloids, indicating that their formation depends on the manufacturing process.	Limitations: 6 products studied, not identified. Obsolete analytical instrumentation used.
(Schroeder, Chen, Iaderosa, Glover, & Edmundson, 1988)	Proposed definition of a smokeless tobacco user based on 'potential' nicotine consumption	Nicotine	The ST products were blindly analyzed by GC to determine nicotine content.	Limitation: Product chemistry not the main focus. 5 products studied, not identified
(Brunnemann, Genoble, & Hoffmann, 1987)	Identification and analysis of a new tobacco-specific N-nitrosamine, 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol	TSNAs (4), iso-NNAL, moisture, nicotine	Iso-NNAL in ST was analyzed for the first time.	Limitations: Very low standard recoveries: 48.6%-62%. No product identification. Only 5 products studied.

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Hoffmann, Adams, Lisk, Fisenne, & Brunnemann, 1987)	Toxic and carcinogenic agents in dry and moist snuff	NO_3, alkaloids, polyphenols, carbonyls, BaP, Po-210, VNAs, TSNAs, NDELA	• VNA concentrations were significantly above the permissible limits set for some food products. • TSNA levels exceeded the levels in other consumer products by at least two to three orders of magnitude.	Limitations: Very little analytical detail: no replicates or standard deviations, many methods not validated for ST matrix. Studied 5 products, not identified
(Whong, Stewart, Wang, & Ong, 1987)	Acid-mediated mutagenicity of tobacco snuff: its possible mechanism	NO_2	Because ST contains a considerable amount of NO_3, it seems that (1) reduction of NO_3 to NO_2 by bacteria and (2) nitrosation of certain constituents by NO_2 under acidic conditions to form mutagenic nitroso-compounds are possible mechanisms.	Limitations: Obsolete method (spectrophotometric), 2 products studied, not identified.
(Hoffmann, Harley, Fisenne, Adams, & Brunnemann, 1986)	Carcinogenic agents in snuff	Alkaloids, VNAs, TSNAs, BaP, Polonium-210	TSNA levels in ST products are exceptionally high and exceed by at least two orders of magnitude the occurrence in other consumer products.	Limitation: 5 products studied, not identified
(Palladino, Adams, Brunnemann, Haley, & Hoffmann, 1986)	Snuff-dipping in college students: a clinical profile	TSNAs(4) and VNAs (5)	ST products used were high in TSNAs.	Limitations: Product chemistry not the main focus. 5 products studied, not identified.
(Brunnemann et al., 1985)	N-nitrosamines in chewing tobacco: An international comparison	Water, pH, NO_3, nicotine, NDELA, nitrosoproline, VNAs(4), TSNAs(4)	TSNA levels in ST exceed by two orders of magnitude the levels in other consumer products.	Limitation: 7 products studied, not identified
(Ohshima et al., 1985)	Identification and occurrence of two new N-nitrosamino acids in tobacco products 3-(N-nitroso-N-methylamino)propionic acid and 4-(N-nitroso-N-methylamino) butyric acid	TSNAs (3) and NAAs (4)	Levels of N-nitrosamino acids were highly correlated with TSNA levels.	Limitation: Very laborious wet chemistry extraction procedure. 5 products studied, not identified.
(Atkinson, Han, & Purdie, 1984)	Determination of Nicotine in Tobacco by Circular Dichroism Spectropolarimetry	Nicotine	• This method can be used to measure nicotine. • Results must be reported as total nicotine since the method cannot distinguish between the alkaloids.	Limitation: Obsolete technique, not specific. 4 products studied, identified
(Hoffmann, Brunnemann, Rivenson, & Hecht, 1982)	N-Nitrosomorpholine and other volatile N-nitrosamines in snuff tobacco	VNAs (NMOR, NDELA, NDMA, NPYR); TSNAs (4)	Waxed cardboard packaging contained morpholine, which could diffuse into ST to produce NMOR.	Limitations: Obsolete methodology, extensive handling of reactive sample, poor chromatography-peaks not baseline resolved. 50%-70% recovery of 14C-NMOR.

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Hoffmann, Adams, Brunnemann, Rivenson, & Hecht, 1982)	Tobacco specific N-nitrosamines: Occurrence and Bioassays	Nicotine, Nornicotine, NNN, NNK, NAT	Variation in TSNA levels was seen between various ST brands, but not between the same brand bought in different cities.	Limitations: Reports data ranges for 3 products (not identified). Assume results are "as-is" since no OV. Number of replicates and standard deviation not reported.
(Hoffmann, Brunnemann, et al., 1982)	N-Nitrosodiethanolamine: Analysis, Formation, in tobacco Products and Carcinogenicity in Syrian Golden Hamsters	N-nitrosodiethanolamine (NDELA)	Maleic hydrazide-diethanolamine is the major precursor for NDELA in tobacco.	Comment: Use of this crop protection agent has been eliminated.
(Hoffmann & Adams, 1981)	Carcinogenic tobacco-specific N-nitrosamines in snuff and in the saliva of snuff dippers	NNN, NNK, NAT, minor alkaloids, moisture	TSNA levels in ST products vary widely, and aging in the open air can lead to an increase in TSNA levels.	Limitation: Unrealistic to age product in an open can for 14 days. Three products not identified.
(Hecht, Chen, Ornaf, et al., 1978)	Chemical studies on tobacco smoke. Tobacco specific nitrosamines: origins, carcinogenicity and metabolism	NNN, NNK	NNN and NNK levels were measured.	Limitations: Analytical methods not fully described. Studied 1 product, not identified
(Hecht, Chen, Hirota, et al., 1978)	Tobacco-specific nitrosamines: formation from nicotine in vitro and during tobacco curing and carcinogenicity in strain A mice	NNN, NNK, NNA	Nicotine is a significant precursor for TSNAs that are formed during the curing and processing of tobacco.	Limitations: Early fundamental precursor paper. Product chemistry secondary to mice studies.
(Hoffmann et al., 1976)	Chemical studies on tobacco smoke. XLII. Nitrosonornicotine: presence in tobacco, formation and carcinogenicity	NNN, NAB, NO_3, NO_2, minor alkaloids, pH	• NNN and possibly other unknown nitrosamines are formed during curing. • NO_3 content is an important factor in nitrosamine formation.	Limitations: ST not the main focus. Unable to quantitate NAB using 1,000 g of tobacco. Unclear as to whether 1 product and 5 replicates or vice versa were measured.
(Hoffmann et al., 1974)	N'-nitrosonornicotine in tobacco	NNN	This compound is the first example of a potential organic carcinogen isolated from tobacco.	Limitations: Ambiguous sample identification, c f. “Chewing tobacco C (fine cut)”. Laborious analytical methodology: 50-60 g extracted for 16 hours.

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
Articles Contributing ST Chemistry Data Relevant to Copenhagen® Snuff Fine Cut				
(Oldham et al., 2014)	Insights from analysis for harmful and potentially harmful constituents (HPHCs) in tobacco products	Food and Drug Administration HPHC Full List (35).	Four of the 7 HPHCs tested at different times for ST products were statistically significantly different even though the same lot of product was tested at both time points. Copenhagen® Snuff Fine Cut contained: • Moisture content: 54.6 (0.0756)% • NNK: 475 (7.27) ng/g as is • Acetaldehyde: 620 (16.0) ng/g as is • Benzo[a]pyrene: 53.8 (0.960) ng/g as is • Cadmium: 579 (40.9) ng/g as is • Crotonaldehyde: 1.13 (0.0456) µg/g as is • Formaldehyde: 0.640 (0.0255 µg /g as is • Nicotine: 11.5 (0.787) mg/g as is • NNN: 1700 (40.4) ng/g as is • Ammonia salts: 2.84 (0.0208) mg/g as is • Anabasine: 42.1 (0.500) µg /g as is • Benz[a]anthracene: 240 (3.61) ng/g as is • Benzo[b]fluoranthene: 54.0 (1.07) ng/g as is • Benzo[k]fluoranthene: 27.6 (1.15) ng/g as is • Chromium: 826 (17.1) ng/g as is • Chrysene: 229 (8.50) ng/g as is • Cobalt: 382 (8.50) ng/g as is • Coumarin: 559 (21.1) ng/g as is • Indeno[1,2,3-cd]pyrene: 14.6 (0.289) ng/g as is • Lead: 223 (12.0) ng/g as is • Naphthalene: 61.5 (2.68) ng/g as is • Nickel: 1050 (63.9) ng/g as is • N-Nitrosopyrrolidine: 26.1 (8.33) ng/g as is • Nornicotine: 131 (19.1) µg /g as is • Polonium-210: 5.33 (0.577) mbq/g as is • Selenium: 209 (43.0) ng/g as is • Below limit of detection: aflatoxin B1, beryllium, dibenz[a,h]anthracene, ethyl carbamate, N-Nitrosopiperidine, uranium-235, and uranium-238	Strengths: Market survey - Range of U.S. commercial products (15) and 35 analytes studied. Controlled product lot selection and known production date. Thorough lab and method descriptions, seven replicates with data reported as mean (standard deviation) and limit of quantitation.

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
			• Below limit of quantification: arsenic, formaldehyde, mercury, N-Nitrosodiethanolamine, N-Nitrosodimethylamine, N-Nitrosomorpholine, N-Nitrososarcosine,	
(Stepanov, Yershova, Carmella, Upadhyaya, & Hecht, 2013)	Levels of (S)-N'-nitrosonornicotine in U.S. tobacco products	S-NNN, moisture	Results demonstrate that S-NNN is the predominant NNN entantiomer. Copenhagen® Snuff moisture level was 53%. It contained 2.32 µg/g wet weight of NNN, 65.5% or 1.52 µg/g wet weight of (*S*)-NNN.	Strength: Range of U.S. commercial products (14) studied. Limitation: obscure analyte studied
(Borgerding et al., 2012)	The chemical composition of smokeless tobacco: a survey of products sold in the United States in 2006 and 2007	BaP, TSNAs (4), NDMA, NO_2, cadmium, lead, arsenic, nickel, chromium, chloride, water, pH, nicotine	• TSNA concentrations for all products were lower than historically reported values. • Year-to-year variability was as great as 54% for some analytes, reflecting natural variability of cured tobacco over time. • Copenhagen® Snuff Fine cut (sampled in 2006) contained 4753 (130) ng/g NNN, 4375 (245) ng/g NAT, 354 (10) ng/g NAB, 1281 (75) ng/g NNK, 881 (73) ng/g cadmium, 258 (26) ng/g arsenic, 2019 (226) ng/g chromium, 380 (53) ng/g lead, 94.2 (4.3) ng/g benzo[*a*]pyrene, 110 (70) mg/g chloride, 12.68 (0.19) mg/g nicotine, 54.4 (0.2) percent moisture, pH = 7.55 (0.02), 3.21 mg/g calculated free nicotine, and 25.31% of calculated free nicotine of total nicotine.	Strength: Range of U.S. commercial products (43) studied over 2 years. Suggests including certified reference as comparison and basis for product classification.

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Lauterbach et al., 2011)	Free-base nicotine in tobacco products. Part II. Determination of free-base nicotine in the aqueous extracts of smokeless tobacco products and the relevance of these findings to product design parameters	Nicotine, free-base nicotine, pH, moisture, ammonia	• Results were in line with those reported by others. • The current approach for determining free-base nicotine levels in ST products is technically incorrect. • Copenhagen® Fine Cut contained 12.4 mg/g total nicotine, pH Result = 7.55, 3.15 mg/g free-base nicotine, 0.25 of free-base nicotine:total nicotine ratio, 46.3% of dry matter, and 53.7% of moisture. • Duncan’s multiple range test of the aqueous extract of Copenhagen® Fine Cut to the nicotine contents was 12.4 mg/g on an as-is basis, the free-base nicotine content was 3.15 mg/g on an as-is basis, and the pH data is 7.55.	Strength: Range of U.S. commercial products (19) studied. Definitive discussion of tobacco pH and overinterpretation of free base nicotine.

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Stepanov et al., 2010)	Analysis of 23 polycyclic aromatic hydrocarbons in smokeless tobacco by gas chromatography-mass spectrometry	PAHs (23), %OV	• Among ST products analyzed, Hawken Long Cut Wintergreen is drastically different. • The tobacco processing method used in the manufacturing of Hawken Long Cut Wintergreen does not lead to its contamination with PAHs. • Copenhagen® Snuff contained 54.7% moisture, 1860 ng/g dry weight of naphthalene, 85 ng/g dry weight of acenaphtylene, 112 ng/g dry weight of acenaphthene, 709 ng/g dry weight of fluorene, 4960 ng/g dry weight of phenanthrene, 784 ng/g dry weight of anthracene, 1650 ng/g dry weight of fluoranthene, 1420 ng/g dry weight of pyrene, 220 ng/g dry weight of benz[*a*]anthracene, 269 ng/g dry weight of chrysene, 95 ng/g dry weight of total 1-methylchrysene, 278 ng/g dry weight of benzo[*b*]fluoranthene and benzo[*j*]fluoranthene, 26 ng/g dry weight of benzo[*k*]fluoranthene, 102 ng/g dry weight of benzo[*e*]pyrene, 60 ng/g dry weight of benzo[*a*]pyrene, 27 ng/g dry weight of indenol[1,2,3-*cd*]pyrene, 26 ng/g dry weight of benzo[*g*,*h*,*i*]perylene, and 12700 ng/g dry weight of total polycyclic aromatic hydrocarbons.	Strength: Range of U.S. commercial products (23) studied.
(Rickert et al., 2009)	Chemical and toxicological characterization of commercial smokeless tobacco products available on the Canadian market	Ammonia, propylene glycol, glycerine, dry matter, nicotine, NO_3, pH, metals, BaP, TSNAs (4)	ST samples tested had TSNA and BaP levels somewhat above the GothiaTek® standard. Copenhagen® Fine Cut contained 6981 µg/g ammonia, 46.3% dry matter, 30.8 mg/g nicotine, 28.9 mg/g nitrate, and pH = 7.55. It also contained 1030 ng/g cadmium, 1303 ng/g chromium, 1417 ng/g nickel, 412 ng/g lead, 281 ng/g arsenic, 75.0 ng/g selenium, 83.2 ng/g benzo[*a*]pyrene, 5818 ng/g NNN, 5657 ng/g NAT, 557 ng/g NAB, 1694 ng/g NNK, and 13726 ng/g total TSNA.	Strength: Range of U.S. commercial products (19) studied.

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Pappas, Stanfill, Watson, & Ashley, 2008)	Analysis of toxic metals in commercial moist snuff and Alaskan iqmik	Metals (8)	The concentrations of arsenic, barium, beryllium, cadmium, chromium, cobalt, lead, and nickel were determined in popular ST products. Copenhagen® Regular contained 0.033 (0.004) µg/g beryllium, 2.16 (0.18) µg/g chromium, 1.08 (0.08) µg/g cobalt, 2.48 (0.21) µg/g nickel, 0.36 ± 0.06 µg/g arsenic, 1.33 ± 0.21 µg/g cadmium, 110.6 ± 6.0 µg/g barium, and 0.52 ± 0.07 µg/g lead. Extractable Copenhagen® metals in artificial saliva: • 0.407 (0.078) µg/g (38 [7]%) cobalt • 0.781 (0.096) µg/g (31 [4]%) nickel • 0.310 (0.015) µg/g (23 [1]%) cadmium • 3.080 (0.56) µg/g (3.0 [0.5]%) barium	Limitations: Product chemistry not the main focus. Range of U.S. commercial products (17) studied.
(Richter et al., 2008)	Surveillance of moist snuff: total nicotine, moisture, pH, un-ionized nicotine, and tobacco-specific nitrosamines	Nicotine, pH, moisture, TSNAs (5)	ST brands varied widely in content of un-ionized nicotine and TSNAs. Copenhagen® Regular contained 12.94 (0.29) mg/g total nicotine, 52.6 (0.10)% total moisture, pH = 7.73 (0.01), 34.2% un-ionized nicotine, 4.36 mg/g un-ionized nicotine, 365 (42.2) ng/g, 3879 (738) ng/g NAT, 3987 (302) ng/g NNN, 960 (218) ng/g NNK, 62 (1.7) ng/g NNAL, 9253 ng/g sum of total TSNAs, and 5009 ng/g sum of NNN, NNK, and NNAL.	Strengths: Market Survey - Range of U.S. commercial products (40) studied. All samples from the same lot.

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Stepanov, Jensen, Hatsukami, & Hecht, 2008)	New and traditional smokeless tobacco: comparison of toxicant and carcinogen levels	TSNAs, alkaloids, anions, PAH, volatile aldehydes	Large variation in the levels analyzed here indicates that more effort is required from the United States tobacco industry to further reduce their amounts. Copenhagen® Snuff contained 5.12 µg/g dry weight of NNN, 1.40 µg/g dry weight of NNK, 1.12 µg/g dry weight of NAT, 0.152 µg/g dry weight of NAB, pH = 7.45, 23.0 mg/g dry weight of total nicotine, 4.88 mg/g dry weight of free nicotine, 0.248 mg/g dry weight of nornicotine, 1.43 mg/g dry weight of anatabine, 0.150 mg/g dry weight of anabasine, 0.011 mg/g dry weight of nitrite, 6.60 mg/g dry weight of nitrate, 13.5 mg/g dry weight of formate, 107 mg/g dry weight of chloride, 10.8 mg/g dry weight of sulfate, 0.586 mg/g dry weight of phosphate, 17.3 ng/g dry weight of acenaphthylene, 699 ng/g dry weight of phenanthrene, 152 ng/g dry weight of anthracene, 300 ng/g dry weight of fluoranthene, 351 ng/g dry weight of phyene, 31.5 ng/g dry weight of benzo[*b*]fluoranthene and benzo[*k*]fluoranthene, 34.2 ng/g dry weight of benzo[*a*]pyrene, 6.58 µg/g dry weight of formaldehyde, 17.1 µg/g dry weight of acetaldehyde, 3.24 µg/g dry weight of acrolein, and 6.35 µg/g dry weight of crotonaldehyde.	Limitations: 4 U.S. commercial products studied: Copenhagen® Snuff, Copenhagen® LC, Skoal LC, Kodiak WG. No standard deviations.
(Stepanov, Jensen, Hatsukami, & Hecht, 2006)	Tobacco-specific nitrosamines in new tobacco products	TSNAs (4)	Conventional products had levels consistent with those reported previously. Copenhagen® Snuff contained 2.2 µg/g product wet weight of NNN, 0.75 µg/g product wet weight of NNK, 1.8 µg/g product wet weight of NAT, 0.12 µg/g product wet weight of NAB, and 4.8 µg/g product wet weight of total TSNA.	Limitation: Range of U.S. commercial products (6) studied

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Renner et al., 2005)	Iqmik: a form of smokeless tobacco used among Alaska natives	pH, nicotine	A sample of commercial ST (Copenhagen®) that is known to have the highest nicotine content and pH of any commercial ST product was analyzed. Copenhagen® Snuff's weight used for pH determination was 2.008 grams and for nicotine determination was 24.2 grams. Copenhagen® Snuff pH was 8.2 and it contained 9.2 mg/g total nicotine.	Limitations: Product chemistry not the main focus. Very few analytical details. One U.S. commercial product studied: Copenhagen® Snuff.
(Richter & Spierto, 2003)	Surveillance of smokeless tobacco nicotine, pH, moisture, and unprotonated nicotine content	Nicotine, pH, moisture	The ST brands with the highest total amounts of unprotonated nicotine have the highest market shares. Copenhagen® Snuff contained 12.71 (0.15) mg/g nicotine, 53.0 (0.1)%total moisture, pH = 7.41 (0.04), and 24.0% unprotonated nicotine or 3.05 mg/g unprotonated nicotine.	Comment: Range of U.S. commercial products (8) studied.
(Ciolino, McCauley, Fraser, & Wolnik, 2001)	The relative buffering capacities of saliva and moist snuff: implications for nicotine absorption	pH, nicotine, moisture, buffering capacity	ST products' buffering capacity was determined to be 10-20 times higher than that of human saliva. Copenhagen® Moist Snuff pH at 37°C was 7.99 and at ambient temperature was 7.98 (based on average measurements from three product tins). Copenhagen® Snuff nicotine and moisture contents were 13.9 mg/g and 54%, respectively (based on measurements from six product tins with nicotine content reported on a "wet weight" basis). The buffering capacities for Copenhagen® Snuff at acidic (to pH 8.02) was 11 µeq/g (range: 0.0-3.6 µeq/g), at basic (to pH 6.02) was 170 µeq/g (range: 110-210 µeq/g), and at total (pH 6.02-8.02) was 180 µeq/g. Copenhagen® Moist Snuff buffering capacity at 37°C (to pH 6.02) was 57 µeq/g (range: 39-67 µeq/g), and 86 µeq/pinch. The resulting saliva pH range for Copenhagen® Moist Snuff was 7.1-8.1.	Strength: Range of U.S. commercial products (8) studied. Limitation: Large variability for replicates of same product (i.e., 10%-50% free base nicotine)

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Centers for Disease Control and Prevention, 1999)	Determination of nicotine, pH, and moisture content of six US commercial moist snuff products--Florida, January-February 1999	Nicotine, pH, moisture	Substantial differences exist in the pH, amount of moisture and nicotine, and the percentage of free nicotine in the products studied. Copenhagen® Snuff purchased in January-February 1999 in Florida overall contained 54.0 (1.0)% total moisture, pH = 8.18 (0.20), 10.59 (0.17) mg/g nicotine content, 2.30 (0.08)% nicotine dry weight, 6.229 (1.178) mg/g free nicotine, and 58.74 (10.56)% free nicotine.,	Strength: Range of U.S. commercial products (6) studied: Copenhagen® Snuff, Skoal Bandits Straight, Skoal Bandits WG, Skoal LC WG, Kodiak WG, Hawken Wintergreen. Limitation: Did not control for product age or storage conditions at retail

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Hoffmann et al., 1995)	Five leading U.S. commercial brands of moist snuff in 1994: assessment of carcinogenic N-nitrosamines	TSNAs, nicotine, pH, NAAs, moisture %, NO_2, NO_3	The three leading ST brands had high levels of pH, nicotine, free nicotine, and TSNAs compared with the 4th and 5th best-selling ST brands. Copenhagen® Moist Snuff contained 58.8 (1.78)% moisture, pH (of suspension) = 8.00 (0.31), 12.0 (0.7) mg/g nicotine, and 49.0 (16.7)% unprotonated nicotine. Additionally, Copenhagen® Moist Snuff purchased in New York, Massachusetts, Kentucky, Colorado, California, and Michigan in 1994 contained 8.73 (1.44) µg/g dry weight of NNN with coefficient of variation of 16.5%, 1.89 (0.62) µg/g dry weight of NNK with coefficient of variation of 32.9%, 6.13 (1.02) µg/g dry weight of NAT with coefficient of variation of 16.7%, 0.50 (0.50) µg/g dry weight of NAB with coefficient of variation of 23.7%, 17.24 (2.97) µg/g dry weight of Total TSNAs with coefficient of variation of 17.3%, 512.5 (120.6) µg/g dry weight of nitrate-nitrogen with coefficient of variation of 23.5%, 672.0 (296.8) µg/g dry weight of nitrite-nitrogen with coefficient of variation of 44.2%, 0.06 (0.01) µg/g dry weight of NSAR with coefficient of variation of 22.9%, 2.62 (0.62) µg/g dry weight of MNPA with coefficient of variation of 23.8%, 0.34 (0.10) µg/g dry weight of MNBA with coefficient of variation of 28.9%, 5.67 (1.29) µg/g dry weight of NPRO with coefficient of variation of 22.7%, 0.31 (0.21) µg/g dry weight of iso-NNAC with coefficient of variation of 37.9%, and 10.47 (2.70) µg/g dry weight of Total NAAs with coefficient of variation of 25.8%,	Limitations: Product identification ambiguous: attribute discrete values to many products in same brand family (i.e. Skoal (Original, Fine Cut, Wintergreen). Assume used moisture from earlier study (not same samples) for DWB calculation.

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Hoffmann et al., 1995)	Five leading U.S. commercial brands of moist snuff in 1994: assessment of carcinogenic N-nitrosamines	TSNAs, nicotine, pH, NAAs, moisture %, NO_2, NO_3	The three leading ST brands had high levels of pH, nicotine, free nicotine, and TSNAs compared with the 4th and 5th best-selling ST brands. Copenhagen® Moist Snuff contained 58.8 (1.78)% moisture, pH (of suspension) = 8.00 (0.31), 12.0 (0.7) mg/g nicotine, and 49.0 (16.7)% unprotonated nicotine. Additionally, Copenhagen® Moist Snuff purchased in New York, Massachusetts, Kentucky, Colorado, California, and Michigan in 1994 contained 8.73 (1.44) µg/g dry weight of NNN with coefficient of variation of 16.5%, 1.89 (0.62) µg/g dry weight of NNK with coefficient of variation of 32.9%, 6.13 (1.02) µg/g dry weight of NAT with coefficient of variation of 16.7%, 0.50 (0.50) µg/g dry weight of NAB with coefficient of variation of 23.7%, 17.24 (2.97) µg/g dry weight of Total TSNAs with coefficient of variation of 17.3%, 512.5 (120.6) µg/g dry weight of nitrate-nitrogen with coefficient of variation of 23.5%, 672.0 (296.8) µg/g dry weight of nitrite-nitrogen with coefficient of variation of 44.2%, 0.06 (0.01) µg/g dry weight of NSAR with coefficient of variation of 22.9%, 2.62 (0.62) µg/g dry weight of MNPA with coefficient of variation of 23.8%, 0.34 (0.10) µg/g dry weight of MNBA with coefficient of variation of 28.9%, 5.67 (1.29) µg/g dry weight of NPRO with coefficient of variation of 22.7%, 0.31 (0.21) µg/g dry weight of iso-NNAC with coefficient of variation of 37.9%, and 10.47 (2.70) µg/g dry weight of Total NAAs with coefficient of variation of 25.8%,	Limitations: Product identification ambiguous: attribute discrete values to many products in same brand family (i.e. Skoal (Original, Fine Cut, Wintergreen). Assume used moisture from earlier study (not same samples) for DWB calculation.
(Dahl, Stolen, & Oilo, 1989)	Abrasives in snuff?	Inorganics (Na, K, Ca)	ST contains measurable inorganic material. The total weight percentage of inorganic material in Copenhagen® Snuff was 12.35 (0.69)%.	Limitations: Only 1 U.S. commercial product studied: Copenhagen® Snuff

Table 7.5.4-1-2. Summary of Findings From Studies of the Chemical Product Analyses of Smokeless Tobacco (continued)

Author	Title	Analyte Class	Author's Findings	Comments
(Hsu, Pollack, Hsu, & Going, 1980)	Sugars present in tobacco extracts	Fructose, glucose, sucrose, maltose, isomaltose	A number of sugars that are potentially cariogenic were identified and quantified. Snuff (Copenhagen®) contained 1.3% fructose, 0.9% glucose, and no detectable amount of sucrose, maltose, or isomaltose.	Comment: 3 U.S. commercial products studied: Copenhagen®, Skoal, Happy Days

7.5.4-1.7.Literature Cited

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