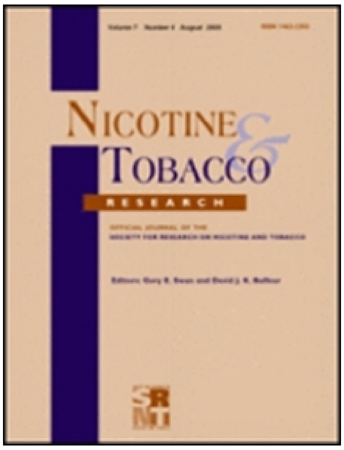

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Surveillance of moist snuff: total nicotine, moisture, pH, un-ionized nicotine, and tobacco-specific nitrosamines

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In 2005, approximately 2.3% of U.S. adults used smokeless tobacco. Moist snuff leads all types of smokeless tobacco in revenues and marketing expenditures. The U.S. Surgeon General has concluded that smokeless tobacco use can lead to nicotine addiction. The National Toxicology Program of the National Institutes of Health has classified smokeless tobacco as a human carcinogen. Tobacco-specific nitrosamines (TSNAs) are potent carcinogens in smokeless tobacco products, and the pH of the product influences the content of un-ionized nicotine which is the form of nicotine most rapidly absorbed in the mouth. The Centers for Disease Control and Prevention analyzed 40 top-selling brands of moist snuff to measure nicotine, moisture, pH, un-ionized nicotine, and TSNAs, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). The study findings indicate that moist snuff brands varied widely in content of rapidly absorbed, addictive un-ionized nicotine (500-fold range) and of carcinogenic TSNAs (18-fold range). Product characteristics such as packaging and moisture content appeared to be correlated with concentrations of un-ionized nicotine, and flavor characteristics of low-priced brands may correlate with TSNA concentrations. These findings warrant further study in light of (a) the marketing of smokeless tobacco for use in places where smoking is prohibited, (b) the promotion of smokeless tobacco as a harm-reduction product, and (c) the ever-expanding number of highly flavored smokeless varieties brought to the market.

Introduction

In 2005, approximately 2.3% of U.S. adults used smokeless tobacco (Centers for Disease Control and Prevention [CDC], 2006). Smokeless tobacco use is much higher among U.S. adults with low levels of education and for some racial and ethnic groups such as American Indians and Alaska Natives (Nelson, Mowery, Tomar, Marcus, Giovino, & Zhao, 2006). Among the smokeless tobacco products, moist snuff receives the greatest advertising and marketing support and leads in revenues and marketing expenditures. In 2005, expenditures on advertising and promoting moist snuff totaled $210.43 million compared with $16.75 million for loose leaf tobacco and chewing tobacco, $72,000 for plug tobacco and twist tobacco, and $103,000 for Scotch snuff and dry snuff (Federal Trade Commission [FTC], 2007). Dollar sales for moist snuff ($2.23 billion) represented 85.2% of total sales of smokeless tobacco in 2005, and more pounds of moist snuff were sold in 2005 (75.7 million) than all other smokeless tobacco products combined (FTC, 2007). From 1986 to 2003, use of moist snuff increased more than 80-fold while large decreases in sales of chewing tobacco and dry snuff products fueled an overall decline in sales volumes for smokeless tobacco (Nelson et al., 2006).

In 1986, the U.S. Surgeon General concluded that smokeless ("spit") tobacco is not a safe substitute for smoking cigarettes or cigars and that smokeless

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tobacco products cause various cancers, noncancerous oral conditions, and nicotine addiction (U.S. Department of Health and Human Services [USDHHS], 1986). The National Toxicology Program of the National Institutes of Health determined that smokeless tobacco is a human carcinogen (National Toxicology Program [NTP], 2005). Smokeless tobacco causes pancreatic cancer (International Agency for Research on Cancer [IARC], 2007) and has also been linked to cardiovascular disease, dental diseases, and adverse pregnancy outcomes (Critchley & Unal, 2003). *N′*-Nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are quantitatively the most prevalent strong carcinogens in smokeless tobacco. (4-[Methylnitrosamino]-1-[3-pyridyl]-1-butanol) (NNAL) is also carcinogenic while NAB (*N′*-nitrosoanabasine) is a moderately potent carcinogen and *N′*-nitrosoanatabine (NAT) is generally considered inactive as a rodent carcinogen (Hecht & Hoffmann 1988). Both NNK and NNN have recently been classified as reasonably anticipated by the IARC as carcinogenic to humans (Group 1) (IARC, 2007). Nicotine is the substance in smokeless tobacco that causes addiction; un-ionized (unprotonated or free) nicotine is the form of nicotine most rapidly and easily absorbed in the mouth (Armitage & Turner, 1970; USDHHS, 1986, 1988). The pH of a smokeless tobacco product is a major determinant of the amount of un-ionized nicotine delivered to the user (Tomar & Henningfield, 1997).

The purpose of this study was to independently measure nicotine, moisture, pH, and un-ionized nicotine, and TSNAs in 40 top-selling brands of moist snuff. In addition, we examined the relation between select product characteristics (e.g., packaging, cut, flavor) and un-ionized nicotine levels and TSNAs.

Materials and methods

Materials

Forty brand varieties of U.S. moist snuff smokeless tobacco products manufactured by five companies were selected for analysis on the basis of market share. Unit-based market share measured in percent (%) was determined from proprietary sales data (Scan-Trac data, Grocery Channel, 50 Retail Markets; ACNielsen, Schaumburg, Illinois). Collectively, these brand varieties held more than a 97% share of the U.S. market in the year they were purchased. CDC personnel purchased five packs of each of the 40 moist snuff products from Internet retailers or in the metropolitan Atlanta area in 2004. Samples were stored in the original packaging at −70°C until testing. The five packs of each product were pooled, and the pooled samples were used for all analyses.

Methods

Samples were analyzed for moisture, pH, and nicotine content as previously described (Richter & Spierto, 2003) except for the use of mass selective detection instead of flame ionization detection. This method has been shown to obtain equivalent results for nicotine over the range investigated. TSNAs were measured by CDC in the following manner. Samples were ground and approximately 0.25 g was weighed into a stainless steel extraction cell. 100 ng each of $^{13}C_6$-labeled *N′*-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *N′*-nitrosoanabasine (NAB), *N′*-nitrosoanatabine (NAT) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) were added to the vial. Samples were then extracted into ethylacetate at 100°C and 1500 psi for 5 min using a Dionex ASE® 200 Accelerated Solvent Extractor (Dionex, Sunnyvale, California). The extracts were dried under nitrogen and reconstituted in 10 mL of methanol. Then samples were diluted 10-fold in 20 mM ammonium acetate aqueous solution for analysis by high-performance liquid chromatography and electrospray ionization tandem mass spectrometry (LC/MS/MS). Details of the mass spectral detection method were published previously (Wu, Ashley, & Watson, 2003).

Statistical analysis

Statistical analyses were performed by using the Statistical Analysis System software (SAS for Windows, Version 9.1; SAS Institute, Cary, NC). Statistical comparisons were performed by using analysis of variance and Student *t*-test; $p < .05$ was considered statistically significant.

Results

Total nicotine, moisture, pH, and un-ionized nicotine

Levels of nicotine and TSNAs measured in the 40 brands of moist snuff products are expressed as the amount detected per gram of wet tobacco weight. Results are shown with brands ordered by total nicotine content (Table 1). One brand, Oregon Mint Snuff, had no detectable nicotine. Excluding Oregon Mint Snuff, total nicotine concentrations ranged from 4.4 milligrams of nicotine per gram of tobacco (mg/g) (Hawken Rough Wintergreen) to 25.0 mg/g (W.B. Cut Regular) (mean, 11.9 mg/g; median, 12.6 mg/g). Total un-ionized nicotine ranged from 0.01 mg/g (Hawken Rough Wintergreen) to 7.8 mg/g

(Kodiak Ice Long Cut Regular) (mean, 3.8 mg/g; median 4.4 mg/g). Un-ionized nicotine, expressed as a percentage of total nicotine, ranged from 0.3% (Hawken Rough Wintergreen) to 79.9% (Kodiak Ice Long Cut Regular). Moisture content for all 40 brands ranged from 3.2% (Oregon Mint Snuff) to 54.5% (Rooster Long Cut Bold Wintergreen) (mean, 50.4%; median 53.1%).

Total nicotine was not correlated with un-ionized nicotine (r=.01) or pH (r=−.11) while pH demonstrated a highly significant (p<.0001) positive correlation with both total (r=.92) and percentage of un-ionized nicotine (r=.90). The results demonstrated positive correlation between pH and total un-ionized nicotine (p<.0001) and between pH and percentage of un-ionized nicotine (p<.0001), and these correlations showed strong statistical significance. There was also significant positive correlation between moisture and total un-ionized nicotine (p<.0002), percentage of un-ionized nicotine (p<.0016), and pH (p<.0001). Moist snuff is sold in a variety of tobacco cuts, including fine cut, long cut, and rough cut. In this study there was a significant difference in the pH (p=.0057) between the groups.

Five of the varieties of moist snuff in our sample consisted of tobacco in a portion pouch. The other varieties were packaged as loose tobacco. For loose tobacco varieties, but not portion pouches, we observed statistically significant correlations of moisture content with total un-ionized nicotine (p=.0003), percentage of un-ionized nicotine (p=.0034), and pH (p<.0001). In addition, mean pH (p=.01), mean concentrations of moisture (p=.009), total nicotine (p=.01), total un-ionized nicotine (p=.001), as well as mean percentage of un-ionized nicotine (p=.005), were significantly higher in loose smokeless tobacco varieties than in pouch varieties.

In general, the correlations between pH and un-ionized nicotine and moisture existed regardless of retail price or market share. In general, for brands that sold at more or less than the mean retail price of $2.95 per unit or the median market share of 0.72%, the positive correlations between pH and un-ionized nicotine (total or percentage) and between moisture and un-ionized nicotine and between moisture and pH were statistically significant. Brands that sold for less than the mean retail price were exceptions. The results for these brands demonstrated a significant positive correlation of moisture with total un-ionized nicotine (p=.008) but not with percentage of un-ionized nicotine. There was also a significant positive correlation between moisture and total nicotine (p=.0001) and cut and total nicotine (p=.044) for brands with a high market share.

One brand of moist snuff had a market share approximately 3 times higher than that of the brand with the next highest market share and more than 100 times higher than the brand with the lowest market share. When this brand was excluded from the analysis, market share was positively correlated with the percentage of un-ionized nicotine (p=.04) and pH (p=.05) in the high market share group, and this correlation was statistically significant.

Tobacco-specific nitrosamines

Because Oregon Mint Snuff had no detectable TSNAs, this brand was excluded from further statistical analysis of TSNAs. Ranges and differences between the lowest and highest concentrations, means, and medians for concentrations measured in nanograms of each TSNA per gram of tobacco (wet weight) were as follows (Table 1):

- NAB—range, 123–4,242 ng/g (34-fold difference); mean, 616 ng/g; median, 395 ng/g;
- NAT—range, 938–31,866 ng/g (34-fold); mean, 6,452 ng/g; median, 4,369 ng/g;
- NNN—range, 2,204–42,554 ng/g (19-fold); mean, 6,883 ng/g; median, 5,271 ng/g;
- NNK—range, 382–9,950 ng/g (26-fold); mean, 1,811 ng/g; median, 1,261 ng/g;
- NNAL—range, 21–1,412 ng/g (67-fold); mean, 169 ng/g; median, 120 ng/g.

The sums of concentrations of all TSNAs ranged from 4.87 µg/g to 90.02 µg/g. (4,874–90,024 ng/g) (18-fold difference) (Table 1). The sums of concentrations of carcinogenic TSNAs (NNN+NNK+NNAL) ranged from 2.99 mµ/g to 53.92 mµ/g (2,986–53,916 ng/g) (18-fold difference). The brand variety with the highest concentrations of TSNAs had total TSNA and carcinogenic TSNA concentrations 3 times higher than the brand variety with the next highest concentration. The ratios of the most potent carcinogenic TSNAs to total TSNAs ([NNN+NNK+NNAL]/[NAB+NAT+NNN+NNK+NNAL]) ranged from .47 (Timber Wolf Fine Cut Regular) to .78 (Hawken Rough Wintergreen). The average ratio of carcinogenic TSNAs to total TSNAs was .55; the median ratio was .56.

We observed no correlations between concentrations of carcinogenic TSNAs or total TSNAs with total nicotine, un-ionized nicotine, moisture, or retail price or market share in the overall sample, and none were observed in evaluations of low versus high retail price or market share groups. There was a statistically significant positive correlation between flavor in moist snuff and total TSNAs (p=.02) and carcinogenic TSNAs (p=.02) among brands in the low retail price group (below mean retail price). Flavored varieties are those that have a flavor descriptor in their name (e.g., Skoal Long Cut Berry Blend). For the high market share varieties (above the median

Table 1. Total nicotine, moisture, pH, un-ionized nicotine, and tobacco-specific nitrosamines in top-selling brands of moist snuff[a].

Brand variety	Manufacturer	Total nicotine (mean mg/g ± *SD*)	Total moisture (mean % ± *SD*)	pH (mean ± *SD*)	Un-ionized nicotine (%)	Un-ionized nicotine (mg/g)	NAB (mean ng/g ± *SD*)	NAT (mean ng/g ± *SD*)	NNN (mean ng/g ± *SD*)	NNK (mean ng/g ± *SD*)	NNAL (mean ng/g ± *SD*)	Sum of total TSNAs (ng/g)	Sum of NNN, NNK, NNAL (ng/g)
W.B. Cut Regular	United States Tobacco	25.03 ± 0.10	33.0 ± 0.30	5.71 ± 0.01	0.5	0.12	142 ± 9.0	3652 ± 220	4184 ± 200	446 ± 62.0	23 ± 12.0	8447	4653
Red Seal Fine Cut Wintergreen	United States Tobacco	14.19 ± 0.08	53.3 ± 0.20	7.55 ± 0.00	25.3	3.59	123 ± 12.0	1907 ± 141	2599 ± 217	583 ± 33.0	60 ± 22.0	5272	3242
Timber Wolf Long Cut, Wintergreen	Swedish Match	14.01 ± 0.03	54.3 ± 0.40	7.62 ± 0.02	28.7	4.01	169 ± 9.5	2543 ± 346	2432 ± 263	902 ± 183	50 ± 13.6	6096	3384
Timber Wolf Fine Cut Regular	Swedish Match	13.91 ± 0.02	50.2 ± 0.30	7.41 ± 0.01	19.8	2.75	1196 ± 270	12056 ± 1100	9556 ± 1571	2287 ± 642	123 ± 17.3	25218	11966
Timber Wolf Straight Regular	Swedish Match	13.88 ± 0.08	54.0 ± 0.40	7.75 ± 0.01	34.9	4.86	193 ± 54.1	2874 ± 682	2877 ± 1127	919 ± 179	37 ± 24.8	6901	3833
Skoal Long Cut Berry Blend	United States Tobacco	13.56 ± 0.04	53.5 ± 0.20	7.52 ± 0.02	24.0	3.25	649 ± 142	6964 ± 346	7630 ± 1576	1739 ± 600	207 ± 26.8	17190	9576
Skoal Straight Cut Regular	United States Tobacco	13.35 ± 0.13	54.2 ± 0.10	7.72 ± 0.01	33.9	4.47	836 ± 83.2	9252 ± 275	8903 ± 1285	3383 ± 949	151 ± 27.6	22527	12438
Skoal Key	United States Tobacco	13.19 ± 0.10	53.3 ± 0.10	7.90 ± 0.01	43.1	5.68	4242 ± 1174	31866 ± 1288	42554 ± 16049	9950 ± 392	1412 ± 222	90024	53916
Grizzly Fine Cut Regular	Conwood	13.17 ± 0.07	53.8 ± 0.20	7.81 ± 0.00	38.1	5.03	862 ± 111	10908 ± 2968	8690 ± 878	1692 ± 149.2	281 ± 9.0	22434	10664
Skoal Fine Cut Wintergreen	United States Tobacco	13.13 ± 0.17	53.4 ± 0.20	7.66 ± 0.01	30.9	3.99	650 ± 180	7642 ± 1894	8395 ± 1765	2031 ± 575	103 ± 22.5	18821	10529
Red Seal Fine Cut Regular	United States Tobacco	13.09 ± 0.03	54.3 ± 0.60	8.00 ± 0.02	49.7	6.37	1676 ± 531	14544 ± 2552	14080 ± 4472	3878 ± 910	247 ± 35.7	34424	18205
Copenhagen Long Cut Regular	United States Tobacco	12.99 ± 0.02	53.7 ± 0.30	7.91 ± 0.01	44.0	5.67	181 ± 48.2	1793 ± 313	2204 ± 577	911 ± 880	21 ± 14.2	5110	3136
Timberwolf Fine Cut Wintergreen	Swedish Match	12.98 ± 0.50	52.8 ± 0.50	7.22 ± 0.01	13.7	1.76	205 ± 25.0	2738 ± 174	2874 ± 489	1061 ± 37.3	43 ± 13.5	6921	3978
Red Seal Long Cut Wintergreen	United States Tobacco	12.97 ± 0.03	53.6 ± 0.70	7.67 ± 0.01	31.0	4.03	139 ± 9.6	1749 ± 268	2570 ± 132	382 ± 89.6	35 ± 3.3	4874	2986
Copenhagen Regular	United States Tobacco	12.94 ± 0.29	52.6 ± 0.10	7.73 ± 0.01	34.2	4.36	365 ± 42.4	3879 ± 738	3987 ± 302	960 ± 218	62 ± 1.7	9253	5009
Skoal Long Cut Regular	United States Tobacco	12.83 ± 0.15	53.3 ± 0.50	7.77 ± 0.00	36.0	4.59	1046 ± 15.0	16054 ± 1228	14031 ± 614	3428 ± 38.0	269 ± 8.0	34828	17728
Timber Wolf Cool Wintergreen	Swedish Match	12.77 ± 0.04	54.4 ± 0.60	7.84 ± 0.00	39.5	5.04	208 ± 119	2684 ± 1063	2557 ± 905	653 ± 400	26 ± 10.5	6128	3237
Skoal Long Cut Wintergreen	United States Tobacco	12.71 ± 0.29	53.5 ± 0.30	7.75 ± 0.01	35.5	4.41	501 ± 116	5101 ± 823	6455 ± 535	1309 ± 786	85 ± 13.8	13452	7849
Skoal Long Cut Classic	United States Tobacco	12.70 ± 0.21	54.0 ± 0.40	7.83 ± 0.02	39.3	4.99	730 ± 96.5	8305 ± 1179	8186 ± 411	2217 ± 411	178 ± 24.9	19616	10581
Skoal Long Cut Cherry	United States Tobacco	12.64 ± 0.22	52.6 ± 0.20	7.49 ± 0.02	23.4	2.89	530 ± 107	6271 ± 795	6237 ± 1368	1579 ± 957	65 ± 8.6	14682	7881
Renegades Wintergreen	Swedish Match	12.41 ± 0.08	53.0 ± 0.60	6.70 ± 0.01	4.5	0.56	177 ± 17.4	3092 ± 485	2651 ± 245	761 ± 86.9	23 ± 5.7	6705	3436
Copenhagen Black Bourbon	United States Tobacco	12.32 ± 0.05	54.4 ± 0.20	7.82 ± 0.01	38.9	4.79	406 ± 54.5	3366 ± 361	4407 ± 468	2264 ± 2171	56 ± 4.9	10498	6726

Table 1. Continued.

Brand variety	Manufacturer	Total nicotine (mean mg/g ± *SD*)	Total moisture (mean % ± *SD*)	pH (mean ± *SD*)	Un-ionized nicotine (%)	Un-ionized nicotine (mg/g)	NAB (mean ng/g ± *SD*)	NAT (mean ng/g ± *SD*)	NNN (mean ng/g ± *SD*)	NNK (mean ng/g ± *SD*)	NNAL (mean ng/g ± *SD*)	Sum of total TSNAs (ng/g)	Sum of NNN, NNK, NNAL (ng/g)
Redwood Regular	Swisher	12.17 ± 0.07	52.2 ± 0.50	7.44 ± 0.01	21.0	2.55	1348 ± 61.2	7787 ± 547	7918 ± 1347	2308 ± 275	255 ± 28.7	19616	10481
Skoal Long Cut Mint	United States Tobacco	12.08 ± 0.08	52.2 ± 0.40	7.85 ± 0.01	40.3	4.87	395 ± 137	5418 ± 1365	5541 ± 1072	1451 ± 468	120 ± 41.5	12925	7112
Silver Creek Fine Cut Wintergreen	Swisher	11.96 ± 0.04	52.6 ± 1.20	7.08 ± 0.00	10.3	1.24	637 ± 62.0	6091 ± 289	5946 ± 192	2925 ± 95.0	268 ± 14.0	15867	9139
Rooster Long Cut Icy Mint	United States Tobacco	11.46 ± 0.13	54.4 ± 0.20	7.85 ± 0.01	40.3	4.61	523 ± 73.2	4369 ± 594	5271 ± 583	1092 ± 925	70 ± 18.2	11326	6433
Rooster Long Cut Bold Wintergreen	United States Tobacco	11.06 ± 0.07	54.5 ± 0.10	7.86 ± 0.01	40.6	4.49	1432 ± 110	11713 ± 386	11081 ± 575	2731 ± 143	150 ± 2.7	27107	13962
Silver Creek Long Cut Straight Regular	Swisher	10.26 ± 0.14	50.7 ± 0.10	6.64 ± 0.01	4.0	0.41	1120 ± 108	11711 ± 2919	10540 ± 1264	4321 ± 559	302 ± 48.1	27994	15162
Silver Creek Long Cut Wintergreen	Swisher	10.21 ± 0.09	50.6 ± 0.60	7.08 ± 0.01	10.2	1.04	212 ± 20.6	2297 ± 60	2722 ± 249	944 ± 31.3	97 ± 2.7	6272	3763
Kodiak Straight Cut Regular	Conwood	9.85 ± 0.07	52.4 ± 0.30	8.29 ± 0.01	65.1	6.39	335 ± 50.4	5082 ± 1212	4476 ± 822	1147 ± 563	217 ± 36.5	11257	5840
Kodiak Ice Long Cut Regular	Conwood	9.80 ± 0.08	53.2 ± 0.10	8.62 ± 0.06	79.9	7.81	314 ± 23.0	4255 ± 149	4550 ± 181	1233 ± 97.0	184 ± 11.0	10536	5967
Kodiak Wintergreen	Conwood	9.56 ± 0.07	52.5 ± 0.40	8.49 ± 0.02	75.6	7.14	492 ± 88.2	7380 ± 1474	6923 ± 1674	1744 ± 503	418 ± 100.6	16958	9085
Grizzly Long Cut Wintergreen	Conwood	9.40 ± 0.08	51.9 ± 0.10	8.42 ± 0.03	71.5	6.71	215 ± 6.1	3100 ± 20	3633 ± 250	975 ± 36.9	168 ± 16.7	8091	4776
Cougar Long Cut Natural	Conwood	9.14 ± 0.06	53.0 ± 0.10	8.16 ± 0.03	58.0	5.27	213 ± 21.7	2813 ± 348	4039 ± 375	1261 ± 206	153 ± 10.0	8478	5453
Cougar Long Cut Wintergreen	Conwood	8.72 ± 0.07	52.2 ± 0.80	7.44 ± 0.02	20.8	1.83	708 ± 45.0	9465 ± 518	9352 ± 1072	1660 ± 58.0	306 ± 32.0	21491	11318
Cougar Regular	Conwood	8.48 ± 0.10	53.9 ± 0.20	8.07 ± 0.01	52.9	4.48	238 ± 15.6	2907 ± 99	4567 ± 155	1053 ± 41	132 ± 10.9	8897	5752
Skoal Bandits Mint	United States Tobacco	7.64 ± 0.10	44.5 ± 0.70	6.72 ± 0.00	4.8	0.37	231 ± 24.6	4257 ± 427	6069 ± 764	931 ± 286	52 ± 18.5	11540	7052
Skoal Bandits Wintergreen	United States Tobacco	6.23 ± 0.09	48.0 ± 0.30	6.84 ± 0.00	6.3	0.39	181 ± 10.6	2789 ± 168	4552 ± 109	751 ± 202	31 ± 4.7	8303	5334
Hawken Rough Wintergreen	Conwood	4.42 ± 0.02	27.4 ± 0.20	5.54 ± 0.01	0.3	0.01	202 ± 22.1	938 ± 238	3203 ± 190	757 ± 241	117 ± 21.3	5218	4077
Oregon Mint Snuff	Oregon Mint Snuff	ND	3.23 ± 0.34	6.51 ± 0.03	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note. SD, standard deviation; NAB, N′-nitrosoanabasine; NAT, N′-nitrosoanatabine; NNN, N'-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; TSNAs, tobacco-specific nitrosamines; ND, not detected .[a] Concentrations of nicotine and TSNAs are expressed as amount detected per gram of wet tobacco.

market share), the correlation between cut and total TSNAs ($p=.03$) and carcinogenic TSNAs ($p=.05$) was significant. No correlation was observed between packaging (loose or in portion pouches) and either total TSNAs or carcinogenic TSNAs.

Discussion

Under the Comprehensive Smokeless Tobacco Health Education Act of 1986 (15 U.S.C. 4401 et seq., Pub. L. 99–252), smokeless tobacco manufacturers report annually to the Centers for Disease Control and Prevention (CDC) on the total nicotine content, moisture content, pH, and estimated un-ionized nicotine in smokeless tobacco products. This law does not require information on concentrations of other chemicals such as tobacco-specific nitrosamines (TSNAs) in smokeless tobacco products. The data that are reported to CDC under this act are considered ''trade secret'' and cannot be released to the public in accordance with 5 U.S.C. 552(b)(4) and 18 U.S.C. 1905. The purpose of this study was to provide consumers, researchers, and public health officials with information on pH, levels of total nicotine, un-ionized nicotine, moisture and TSNAs in popular smokeless tobacco brands.

Our previous findings demonstrated that brands of moist snuff with the largest amount of un-ionized nicotine are the most frequently sold brands (Richter & Spierto, 2003). In that study, we used data on market share in an aggregated brand family level (e.g., Skoal). In the present study, this limitation was addressed by using data on market share and retail price reported on a brand variety level (e.g., Skoal Long Cut Berry Blend). The investigation was also expanded to include TSNA concentrations. As in the earlier study, pH proved to be a good predictor of un-ionized nicotine content in the moist snuff products. Market share and retail price were not factors in the relationships between pH and un-ionized nicotine among the brand varieties tested. Exclusion of one very high market share brand resulted in a significant positive correlation between market share and pH and market share and the percentage of un-ionized nicotine among brands in the high market share group (above median market share). This finding suggests that the most popular brands of moist snuff continue to deliver the highest amounts of the rapidly absorbed un-ionized nicotine to consumers.

The observation that moisture concentrations in moist snuff positively correlate with both pH and un-ionized nicotine content is intriguing and, to our knowledge, has not been previously reported. Moisture level is a product characteristic controlled by the manufacturer. Among the brand varieties tested in this study, tobacco cut, another product characteristic, was also positively correlated with pH, both overall and in the high retail price varieties. Others have reported that tobacco cut influences oral absorption of nicotine from smokeless tobacco and that binding agents used with larger cuts of tobacco may allow the smokeless tobacco to be formed in a compacted mass that slows nicotine release (Connolly, 1995; Djordjevic, Hoffmann, Glynn, & Connolly, 1995). Although our analysis was limited by the small number of varieties sold in portion pouches, the results suggest that packaging is a factor in product moisture and un-ionized nicotine content, as well as pH level, but not TSNA content.

The nicotine dose received by a smokeless tobacco user is influenced by the amount of nicotine in the product, the tobacco cut size, pH, and to a much lesser extent, behavioral factors such as the rate of expectoration (Tomar and Henningfield, 1997; USDHHS, 1986). An additional factor that influences the oral absorption of nicotine is the buffering capacity of saliva. The buffering capacity of moist snuff products is higher than the buffering capacity of saliva and the saliva pH for moist snuff products is determined by the acid–base buffering capacities of both the saliva and the moist snuff (Ciolino, McCauley, Fraser, & Wolnik, 2001). Consuming acidic foods such as coffee or cola reduces saliva pH and oral nicotine absorption (Henningfield, Radzius, Cooper, & Clayton, 1990). Among the top-selling U.S. smokeless tobacco products in our current study, there was more than a 5-fold difference in the range of total nicotine concentrations and more than a 500-fold difference in the range of total un-ionized nicotine concentrations. Five brands of moist snuff reported on in 2003 were included in this study (Richter & Spierto, 2003). Compared with the earlier samples, two had lower concentrations of total nicotine and lower (more acidic) pH; one had a higher total nicotine and higher pH; and two were mixed: one had lower total nicotine but higher pH; and one had higher total nicotine but lower pH. Increasing the alkalinity of smokeless tobacco promotes the absorption of nicotine and increases its physiological effects (Tomar & Henningfield, 1997). It is not known whether these differences in nicotine and pH were related to changes in product formulation or whether they reflect variations in an agricultural-based product.

One goal of this study was to survey concentrations of TSNAs in popular brands of moist snuff and look for correlations between concentrations of TSNAs and other chemical and physical properties. We found an almost 20-fold difference in the range of sums for total TSNAs and for carcinogenic TSNAs. The difference in the range of NNAL concentrations was almost 70-fold (Table 1), a much wider range than that for other carcinogenic TSNAs (NNN, 19-fold; NNK, 26-fold). NNAL has been detected at low concentrations in toombak, a Sudanese fermented snuff (Prokopczyk et al., 1995), but no data were available on NNAL in commercial smokeless tobacco products. Our study found no significant correlation between moisture and TSNAs. However, others have shown that TSNA concentrations in moist snuff products stored at room temperature increase more in high-moisture products than in low-moisture products (Andersen, Fleming, Hamilton-Kemp, & Hildebrand, 1993).

Concentrations of NAB, NAT, NNN, and NNK measured by gas chromatography with a thermal-energy analyzer were recently reported for a variety of tobacco products including traditional moist snuff and new oral tobacco products (Stepanov, Jensen, Hatsukami, & Hecht, 2006). Comparison of the results from that study with those from our study showed that although there is good agreement in total concentrations of these TSNAs for some brands (e.g., Copenhagen long cut); differences are notable for other brands. For example, in our study using high-performance liquid chromatography and electrospray ionization tandem mass spectrometry (LC/MS/MS), the sum of these four TSNAs for Skoal Long Cut Straight Regular was 35 μg/g. In the study by Stepanov and associates, it was 9.2 μg/g. The reproducibility and accuracy of the method we used was reported previously (Wu et al., 2003). This difference may be related to differences in extraction efficiency or year-to-year variations in TSNA concentrations. Further investigation is warranted. In the work by Stepanov and associates, TSNA concentrations in new oral tobacco products were approximately threefold lower than those in traditional smokeless tobacco brands, and the overall ratio of carcinogenic TSNAs to total TSNAs ((NNN+NNK)/(NAB+NAT+NNN+NNK)) was .67, which falls within the range of ratios for the same four TSNAs (.47–.78) in our study. (NNAL concentrations were not reported in the study by Stepanov and associates.) Those results suggest that it is possible to produce commercial smokeless tobacco products with lower TSNA concentrations. However, the biological relevance of small reductions in TSNA concentrations in smokeless tobacco, a human carcinogen, cannot be determined from these data.

The results of our study are subject to two limitations. First, the study samples were the 40 top-selling brands of domestic moist snuff, which were selected by using sales-based data on market share. This sampling captured brands ranging in average retail price from $1.36 to $4.13 per unit. Despite the large number of brands, there may be regional, niche, or deeply discounted varieties that are not represented by the brands included in this study. The study design focused on the most widely available popular brands of moist snuff and did not include new or modified smokeless tobacco products that are being sold or are in test market in some regions of the country. The products were purchased from Internet retailers who ship tobacco products to consumers throughout the United States. Thus they are typical of products available to consumers nationwide.

Second, results are based on five samples from the same lot of a brand that were pooled before analyses. Although this approach ensured an adequate quantity of the moist snuff product to complete all analyses with a single pooled sample, it did not allow evaluation of variations within a product or variations between lots or locations. Consequently, we did not draw direct between-brand conclusions from our comparisons.

To conclude, results from this study indicate that top-selling brands of moist snuff varied widely in content of addictive un-ionized nicotine (500-fold) and carcinogenic TSNAs (18-fold). Product characteristics such as packaging and moisture content appeared to be correlated with concentrations of un-ionized nicotine. In addition, the findings indicate that flavor characteristics of low-priced brands may correlate with TSNA concentrations. Such findings warrant further study in light of (a) the marketing of smokeless tobacco for use in places where smoking is prohibited; (b) the promotion of smokeless tobacco as a harm-reduction product (Henningfield, Rose, & Giovino, 2002; Nelson et al., 2006); and (c) the ever-expanding number of highly flavored smokeless varieties brought to the market (e.g., Vanilla Blend Skoal and Kayak Peach Long Cut).

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The authors report no conflicts of interest.

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