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New and traditional smokeless tobacco: Comparison of toxicant and carcinogen levels

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Declining cigarette use and spreading bans on smoking in public places in the United States are encouraging the U.S. cigarette industry to turn to another tobacco category, smokeless tobacco products. Currently, a number of new brands are being test marketed, including Taboka, Marlboro Snus, Camel Snus, and Skoal Dry. We report here levels of tobacco-specific nitrosamines (TSNAs), alkaloids, anions, polycyclic aromatic hydrocarbons (PAH), and volatile aldehydes in these products, and compare them to the most popular traditional moist snuff brands. Total TSNAs averaged 1.97 μg/g dry weight tobacco in Taboka, Marlboro Snus, and Camel Snus, 4.54 μg/g tobacco in Skoal Dry, and 7.42 μg/g tobacco in traditional brands. The amounts of unprotonated nicotine averaged 0.961 mg/g tobacco in Taboka, Marlboro Snus, and Skoal Dry, 7.22 mg/g tobacco in Camel Snus, and 7.57 mg/g tobacco in traditional brands. Levels of minor tobacco alkaloids were relatively high in Taboka, Marlboro Snus, and Skoal Dry, as compared to other products analyzed here. Levels of nitrite and nitrate in new U.S. smokeless tobacco products and the Swedish snus General were lower than those in the other products. Remarkably high levels of chloride and some PAH were observed in the traditional moist snuff. Crotonaldehyde levels were about five times higher in Taboka and Marlboro Snus than in traditional products. The large variation in the levels of some toxicants and carcinogens analyzed here indicates that more effort is required from the U.S. tobacco industry to further reduce their amounts in new and traditional smokeless tobacco products.

Introduction

The tobacco industry is promoting new types of smokeless tobacco products as a substitute for cigarette smoking (Hatsukami, Ebbert, Feuer, Stepanov, & Hecht, 2007). These products are sold as small pouches of tobacco that users place between the cheek and gum. In contrast to traditional moist snuff which generates excessive saliva and requires spitting, these products are spit-free. In 2006, major U.S. cigarette manufacturers introduced for test marketing two new smokeless tobacco products: Taboka (Philip Morris USA) and Camel Snus (Reynolds American). In 2007, Philip Morris USA introduced Marlboro Snus. The major manufacturer of smokeless tobacco, the U.S. Smokeless Tobacco Company (USSTC), developed Skoal Dry, which may be designed as an intermediate between traditional moist snuff and a flavored spit-free tobacco product Revel, which they had introduced earlier. There are different groups of potential consumers of these products. Current smokers who are unwilling to quit but are inconvenienced by the increasing bans on smoking might consider these new products as an occasional substitute. Smokers who are disturbed by the evidence of serious health risks associated with cigarette smoking might consider these products as a "reduced risk alternative." Another group of potential users are young people initiating tobacco use. An estimated 10% of male high school students in the U.S. report use of smokeless tobacco at least once during the past 30 days (Centers for Disease Control and Prevention [CDC], 2005), and the appearance of new flavored spitless products portioned in small packets and packaged in trendy plastic cases has the potential to increase this number in the future.

Chronic use of smokeless tobacco can result in nicotine addiction (Hatsukami, Lemmonds, &

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Tomar, 2004; Hatsukami & Severson, 1999; Public Health Service [PHS], 1988) and cause precancerous oral lesions, oral and pancreatic cancer, and cardiovascular diseases (Hecht et al, 1986; International Agency for Research on Cancer [IARC], 1985; IARC, 2007; Public Health Service [PHS], 1986). A number of toxicants and carcinogens present in smokeless tobacco are believed to be responsible for these negative health effects (Brunnemann & Hoffmann, 1992; Hoffmann & Djordjevic, 1997; National Cancer Institute [NCI], 1992). Among 28 known carcinogens in smokeless tobacco (NCI, 1992), tobacco-specific nitrosamines (TSNAs) are considered to be the most important due to the combination of abundance and strong carcinogenicity (Hecht, 1998; Hecht & Hoffmann, 1988). The two main carcinogenic compounds in this group, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN), are believed to be involved in the induction of oral cancer in smokeless tobacco users (Hecht, 1998). Other carcinogens in smokeless tobacco include *N*–nitrosamino acids, volatile *N*–nitrosamines, polycyclic aromatic hydrocarbons (PAH), volatile aldehydes, hydrazine, metals, and radioactive polonium (Brunnemann & Hoffmann, 1992; Hoffmann & Djordjevic, 1997; NCI, 1992).

Most recent studies involving smokeless tobacco analysis have focused on a limited range of analytes. Since total and unprotonated nicotine content plays an important role in the consumer's acceptance of a particular tobacco product and addiction to it, nicotine levels along with pH and moisture content are commonly measured and reported (Brunnemann, Qi, & Hoffmann, 2002; Chamberlain, Schlotzhauer, & Chortyk, 1988; Hatsukami et al., 2007; Hoffmann & Djordjevic, 1997; Richter & Spierto, 2003). Because of their abundance in some smokeless tobacco products and existing strong evidence supporting their role in causation of oral cancer in smokeless tobacco users, TSNAs are also commonly measured in smokeless tobacco and the results have been reported in the literature periodically (Brunnemann et al., 2002; Chamberlain et al., 1988; Hatsukame et al., 2007; Hoffmann & Djordjevic, 1997; Österdahl, Jansson, & Paccou, 2004; Stepanov, Hecht, Ramakrishnan, & Gupta, 2005; Stepanov, Jensen, Hatsukami, & Hecht, 2006). Nitrite and nitrate content is sometimes reported along with TSNA levels and nicotine (Brunnemann et al., 2002; Stepanov et al., 2005). The only recent comprehensive analysis of smokeless tobacco products was carried out by a group in the UK (McNeill, Bedi, Islam, Alkhatib, & West, 2006). They reported levels of TSNAs, benzo[*a*]pyrene (BaP), *N*-nitrosodimethylamine, Cr, Ni, As, and Pb in a range of smokeless tobacco products available in the UK, and compared them to a few products purchased in other countries. Analysis of a wide range of toxicants and carcinogens in smokeless tobacco products available in the U.S. was last reported two decades ago (Hoffmann et al, 1987).

We present here the results of chemical analyses performed on a range of newly developed smokeless tobacco products and some of the most popular traditional smokeless brands. We analyzed 4 commonly reported TSNAs – NNN, NNK, N'-nitrosoanatabine (NAT), and N'-nitrosoanabasine (NAB). Nitrite, nitrate, total nicotine, and pH were also measured, and unprotonated nicotine was calculated. Among analytes that are not usually reported in the literature are the minor tobacco alkaloids nornicotine, anatabine, and anabasine, anions other than nitrite and nitrate, a range of PAH including acenaphthylene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), and BaP, and 4 aldehydes—formaldehyde, acetaldehyde, acrolein, and crotonaldehyde. The new smokeless tobacco products analyzed here were different varieties of Taboka, Marlboro Snus, Camel Snus, and Skoal Dry. These products were compared to traditional brands: the Swedish snus General, Copenhagen Snuff, Copenhagen Long Cut, Skoal Straight Long Cut, and Kodiak Wintergreen.

Materials and methods

Tobacco samples

Products collected for analysis represent new smokeless spit-free tobacco products and traditional moist snuff. The products were purchased in retail stores between August 2006 and August 2007. Taboka Original and Taboka Green were purchased in Indianapolis, Indiana. Four varieties of Marlboro Snus (Rich, Mild, Spice, and Mint) were purchased in Dallas, Texas, and Camel Snus (Original, Spice, and Frost) and Skoal Dry (Regular, Cinnamon, and Menthol) were procured in Austin, Texas. Swedish snus General was ordered online from Snus Worldwide, Sweden. Conventional smokeless tobacco products were obtained from retailers in Minneapolis. One pack or can of each product was purchased, sealed in a plastic bag, and refrigerated until analysis.

Reagents

Reference NNN, NNK, NAB, 5-methyl-N'-nitrosonornicotine (5-MeNNN), and 5-(methylnitrosamino)-1-(3-pyridyl)-l-pentanone (C5-NNK) were synthesized as previously described (Amin, Desai, Hecht, & Hoffmann, 1996; Carmella, McIntee, Chen, & Hecht, 2000; Stepanov, Carmella, Hecht, & Duca, 2002). NAT was purchased from Toronto Research Chemicals Inc., Toronto, Ontario, Canada. [CD_3]Nicotine was obtained from Sigma, St. Louis, Missouri. [Pyridine-D_4]nornicotine was synthesized as previously described (Munson & Hodgkins, 1977). Deuterium-labeled PAH surrogate cocktail was purchased from Cambridge Isotope Laboratories (Andover, MA). Unlabeled EPA 525 PAH standard mix B and 2,4-dinitrophenylhydrazones of formaldehyde, acetaldehyde, acrolein, and crotonaldehyde were purchased from Sigma-Aldrich (Milwaukee, WI).

Tobacco analysis

Moisture content and pH. Moisture content was measured via the difference in weight of a tobacco sample before and after it dried for 3 h in a heating block set at 99°C. To measure pH, ~1 g of tobacco was mixed with 10 ml HPLC-grade H_2O, sonicated for 5 min, and allowed to stand at room temperature for an additional 15 min. The pH of the aqueous extract was measured with a pH meter.

TSNAs. Analysis of TSNAs in smokeless tobacco was carried out essentially as previously described (Stepanov et al., 2006).

Nicotine and unprotonated nicotine. Total nicotine was measured as described elsewhere (Stepanov et al., 2005). The amount of unprotonated nicotine was calculated using the Henderson-Hasselbalch equation, based on the measured total nicotine, pH values, and a pK_a value of 8.02 (Richter & Spierto, 2003).

Nornicotine, anatabine, and anabasine. Tobacco was extracted as described for nicotine (Stepanov et al., 2005), and 500 µl of the methanol extract was mixed with 226 ng [pyridine-D_4]nornicotine internal standard. The mixture was concentrated to dryness, and the alkaloids were converted to tertiary amine derivatives via reductive alkylation with propionaldehyde and sodium borohydride as described (Jacob, Yu, Liang, Shulgin, & Benowitz, 1993). The propyl derivatives were analyzed by gas chromatography-tandem mass spectrometry (GC/MS-MS) with a model 6890 gas chromatograph (GC) equipped with an autosampler and interfaced with a model 5973 mass-selective detector (Agilent Technologies, Palo Alto, CA). The GC was equipped with a 15 m × 0.25 mm × 0.25 µm DB-5MS column (Agilent Technologies, Palo Alto, CA) under conditions similar to those previously described (Jacob et al., 1993). Analyses were carried out by monitoring the transitions *m/z* 190→161 (nornicotine derivative), *m/z* 202→173 (anatabine derivative), *m/z* 204→175 (anabasine derivative), and *m/z* 194→165 ([pyridine-D_4]nornicotine derivative).

Nitrite, nitrate, and other anions. These were analyzed essentially as previously described (Stepanov et al., 2005).

PAH. Tobacco samples were extracted and purified by a modification of a method described for analysis of PAH in cigarette smoke (Ding, Ashley, & Watson, 2007). PAH were extracted by shaking 200 mg tobacco with 1 ml cyclohexane at room temperature for 1 h. The tobacco particles were removed by centrifugation and 500 µl of the extract was mixed with deuterium-labeled internal standard mix. The mixture was loaded on 100-mg BondElut Silica cartridges (Varian) pre-equilibrated with 1 ml cyclohexane. The cartridge was washed with 1 ml cyclohexane, and the eluants from both the load and wash were combined and dried. The residue was reconstituted in 20 µl acetonitrile and transferred to glass microinsert vials. Two µl of the sample were analyzed by GC/MS as described elsewhere (Ding, Trommel, Yan, Ashley, & Watson, 2005).

Volatile aldehydes. These were extracted and derivatized by a modification of a previously described method (Hoffmann et al., 1987). Tobacco (200 mg) was shaken with 2 ml of CH_2Cl_2 for 3 h. The extract was separated from the tobacco particles, 1 ml of the extract was mixed with 1 ml of 0.1% 2,4-dinitrophenylhydrazine (DNPH) in 2 N HCl, and the mix was shaken for 1 h. The aqueous layer was discarded, and 500 µl of the CH_2Cl_2 layer was transferred into a clean vial, dried under a stream of N_2, reconstituted in 100 µl acetonitrile, and analyzed by GC/MS as described elsewhere (Saito, Ueta, Ogawa, & Jinno, 2006). The amounts of DNPH-derivatives of the volatile aldehydes in tobacco samples were determined based on a calibration curve obtained upon analysis of four dilutions of a standard mix containing DNPH-derivatives of formaldehyde, acetaldehyde, acrolein, and crotonaldehyde.

Results

TSNA levels in the products, along with pH values, nicotine and unprotonated nicotine, and amounts of other tobacco alkaloids are summarized in Table 1. Overall, Taboka, Marlboro Snus, and Camel Snus contained relatively low amounts of NNN, with the exception of Marlboro Snus Mint which had 3.28 µg NNN/g tobacco—an amount comparable to U.S. traditional moist snuff. NNN levels in Skoal Dry were comparable to those found in traditional

Table 1. Tobacco-specific nitrosamines, pH, total and unprotonated nicotine, and minor tobacco alkaloids in smokeless tobacco products.

	TSNAs[a], µg/g dry weight						Alkaloids, mg/g dry weight				
							Nicotine				
Product	NNN[a]	NNK[a]	NAT[a]	NAB[a]	Total	pH	Total	Free	Nornicotine	Anatabine	Anabasine
New products											
Taboka											
Original	1.05	0.077	0.370	ND[b]	1.50	6.64	21.1	0.844	1.04	3.78	0.149
Green	0.948	0.092	0.292	0.002	1.33	6.85	19.9	1.26	1.02	4.03	0.197
Marlboro Snus											
Rich	1.27	0.259	0.455	ND	1.98	6.83	17.8	1.08	0.438	2.60	0.111
Mild	1.52	0.229	0.234	ND	1.98	6.47	12.8	0.350	0.484	1.82	0.072
Spice	1.56	0.257	0.246	ND	2.06	6.85	17.9	1.13	0.411	2.17	0.097
Mint	3.28	0.215	0.221	ND	3.72	6.58	20.0	0.701	0.454	1.97	0.063
Camel Snus											
Original	1.15	0.270	0.297	0.012	1.73	7.46	28.2	6.09	0.353	1.39	0.164
Spice	1.27	0.157	0.305	0.015	1.75	7.75	25.4	9.16	0.314	1.09	0.183
Frost	1.20	0.267	0.204	0.009	1.68	7.59	23.7	6.40	0.313	0.741	0.103
Skoal Dry											
Regular	3.57	0.360	0.478	ND	4.41	7.23	11.3	1.57	0.345	1.41	0.117
Cinnamon	5.30	0.313	0.572	0.002	6.19	6.85	11.9	0.751	0.324	1.02	0.130
Menthol	2.53	0.279	0.203	ND	3.01	7.18	11.9	1.51	0.386	1.37	0.127
Mean for new products	2.05	0.231	0.323	0.008	2.61		18.5	2.57	0.490	1.95	0.126
Traditional products											
General Snus	1.66	0.464	0.969	0.008	3.10	7.95	16.7	7.69	0.223	0.367	0.072
Copenhagen Snuff	5.12	1.40	1.12	0.152	7.79	7.45	23.0	4.88	0.248	1.43	0.150
Copenhagen Long Cut	3.76	1.10	1.35	0.062	6.27	7.53	26.7	7.14	0.157	0.770	0.037
Skoal Long Cut	4.66	1.64	1.59	0.074	7.96	7.51	25.6	6.03	0.233	1.02	0.049
Kodiak Wintergreen	6.86	1.41	3.58	0.179	12.0	8.23	19.6	12.1	0.164	0.438	0.055
Mean for traditional products	4.41	1.20	1.72	0.095	7.42		22.3	7.57	0.205	0.805	0.073

Note. [a]Abbreviations: TSNAs, tobacco-specific *N*-nitrosamines; NNN, *N′*-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT, *N′*-nitrosoanatabine; NAB, *N′*-nitrosoanabasine. [b]ND, not detected.

products. The lowest NNK levels were found in Taboka, and the levels of this carcinogen were generally lower in new smokeless tobacco products as compared to the traditional ones. Total TSNAs—the sum of 4 measured nitrosamines—averaged 1.97 µg/g tobacco for all varieties of Taboka, Marlboro Snus, and Camel Snus, which is lower than 3.10 µg/g total TSNAs found in the Swedish snus General. Average total TSNAs in 3 varieties of Skoal Dry was 4.54 µg/g tobacco—much higher than total TSNAs in the other new products, and lower than total TSNAs/g tobacco found in traditional Skoal, Copenhagen, and Kodiak.

Nicotine levels of the new tobacco products, with the exception of Skoal Dry, were similar to those usually observed in traditional moist snuff. The amounts of free nicotine were quite low in Taboka, Marlboro Snus, and Skoal Dry. Levels of unprotonated nicotine in Camel Snus were comparable to those found in conventional smokeless tobacco. When expressed as % of nicotine in the same product, the levels of minor tobacco alkaloids—nornicotine, anatabine, and anabasine—were relatively high in Taboka, Marlboro Snus, and Skoal Dry. Thus, in traditional moist snuff, nornicotine, anatabine, and anabasine were on average 0.95%, 3.8%, and 0.32%, respectively, of nicotine measured in the same products, while these values in the new smokeless tobacco products (except Camel Snus) were on average 3.3%, 14%, and 0.77%, respectively. The ratio of minor alkaloids to nicotine in Camel Snus was similar to that observed in traditional products.

Levels of nitrite in new smokeless tobacco products and General were lower than those found in the other traditional products (Table 2). Thus, in all new products nitrite averaged 0.003 mg/g product, and this figure for traditional moist snuff was 0.030 mg/g tobacco, or 10 times higher. The average level of nitrate in new smokeless tobacco was ~3 times lower than that in traditional brands. Camel Snus had the highest levels of formate among all products analyzed here, and more chloride than the other new smokeless tobacco products. General and other traditional moist snuff had even higher chloride levels: 75.7 mg/g product in General and up to 155 mg/g product in the other traditional brands. The levels of sulfate were quite consistent across all the products, and phosphates varied in a low-level range, from 0.309 to 1.32 mg/g product.

PAH and volatile aldehydes in new and old smokeless tobacco products are summarized in

Table 2. Nitrite, nitrate, and other anions in smokeless tobacco products.

Product	Anions, mg/g dry weight					
	Nitrite	Nitrate	Formate	Chloride	Sulfate	Phosphate
New products						
Taboka						
Original	0.004	0.827	3.05	1.78	4.56	0.746
Green	0.004	1.09	3.25	2.19	5.92	1.07
Marlboro Snus						
Rich	ND[a]	1.71	1.89	7.92	7.45	1.28
Mild	ND	1.54	1.56	7.28	6.86	1.28
Spice	0.003	1.69	2.12	7.68	7.01	1.32
Mint	0.003	1.58	1.51	7.41	6.63	1.31
Camel Snus						
Original	ND	3.79	12.7	39.8	9.35	0.820
Spice	0.007	3.79	14.7	39.7	8.42	0.725
Frost	0.003	3.20	15.3	32.4	7.62	0.722
Skoal Dry						
Regular	0.001	1.59	5.53	13.9	7.27	0.431
Cinnamon	0.001	1.42	5.05	11.8	6.72	0.488
Menthol	0.0005	1.25	4.30	11.0	5.72	0.309
Mean for new products	0.003	1.96	5.91	15.2	6.96	0.875
Traditional products						
General Snus	0.004	4.62	4.89	75.7	7.55	0.344
Copenhagen Snuff	0.011	6.60	13.5	107	10.8	0.586
Copenhagen Long Cut	0.055	7.93	3.36	150	11.5	0.922
Skoal Long Cut	0.045	7.96	4.51	137	12.3	0.975
Kodiak Wintergreen	0.035	6.97	1.11	155	9.03	0.445
Mean for traditional products	0.030	6.82	5.47	125	10.2	0.654

Note. [a]ND, not detected.

Table 3. Overall, PAH levels in the new products were comparable or slightly lower than in General snus, and substantially lower than those measured in other traditional products. Anthracene was not detected in any new product. Traces of BaP were detected in Marlboro Snus Rich, Mild, and Mint, and also in Camel Snus Original and Skoal Dry Regular and Menthol, averaging 3.12 ng/g tobacco. All traditional products contained BaP, and the average amount was 38.2 ng/g tobacco. Traces of BbF plus BkF were found in Marlboro Snus Rich and Mild, 2.59 and 2.93 ng/g tobacco, respectively. All other new products and General did not contain these carcinogens, while traditional moist snuff had on average 38.3 ng BbF plus BkF per gram product. Levels of acenaphthylene, phenanthrene, anthracene, fluoranthene, and pyrene were remarkably higher in Skoal, Copenhagen, and Kodiak than in the other products.

Formaldehyde, acetaldehyde, and acrolein were generally lower in the new than in the old products, with a few exceptions (Table 3). Crotonaldehyde was relatively high in Taboka and Marlboro Snus. Thus, in traditional moist snuff, crotonaldehyde levels averaged 2.98 μg/g tobacco, which is about five times lower than the levels found in Taboka and Marlboro Snus.

Discussion

As public awareness of the dangers associated with smoking grows, cigarette use declines in the United States. This, along with spreading bans on smoking in public places, is encouraging the U.S. cigarette industry to turn to another tobacco category, smokeless tobacco products. Currently, a number of new brands are being test marketed. Considering the addictive nature of smokeless tobacco, its health risks, and the potential of newly-developed products to attract new consumers among young people and to be accepted as a substitute for smoking by some smokers, it is essential to carry out independent comprehensive chemical analysis of these products in order to provide consumers, researchers, and public health officials with this information. We report here the results of our study in which Taboka, Marlboro Snus, Camel Snus, and Skoal Dry were analyzed for TSNAs, tobacco alkaloids, anions, PAH, and volatile aldehydes, and compared to the most popular traditional moist snuff brands.

Because of their abundance in some smokeless tobacco products and existing strong evidence supporting their role in causation of oral and pancreatic cancer in smokeless tobacco users, TSNAs have become a reference group of carcinogens in these products, their levels to some extent defining the degree of risk. The possibility of limiting TSNA formation during tobacco processing, as demonstrated by the relatively low levels of these carcinogens in some tobacco products (Österdahl et al., 2004), has compelled some tobacco companies to make a serious effort to reduce TSNA levels

Table 3. Polycyclic aromatic hydrocarbons and volatile aldehydes in smokeless tobacco products.

	PAH[a], ng/g dry weight							Aldehydes, μg/g dry weight			
Product	Acenaphthylene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	BbF[a]+BkF[a]	BaP[a]	Formaldehyde	Acetaldehyde	Acrolein	Crotonaldehyde
New products											
Taboka											
Original	2.28	15.6	ND[b]	9.56	9.23	ND	ND	3.14	1.83	0.400	19.4
Green	2.04	19.8	ND	11.0	7.52	ND	ND	2.30	1.96	0.520	16.5
Marlboro Snus											
Rich	ND	14.8	ND	5.54	7.24	2.59	1.55	4.66	5.88	0.483	17.1
Mild	ND	9.44	ND	4.42	4.43	2.93	2.06	4.09	3.33	0.591	18.4
Spice	ND	15.9	ND	5.38	6.24	ND	ND	7.04	8.08	0.383	10.6
Mint	3.15	14.6	ND	5.86	5.68	ND	1.02	5.35	10.5	0.726	4.83
Camel Snus											
Original	3.95	41.7	ND	20.5	20.1	ND	10.5	1.51	6.64	0.310	0.552
Spice	4.14	33.7	ND	19.2	16.4	ND	ND	4.11	13.3	4.42	3.37
Frost	4.99	40.7	ND	22.5	20.3	ND	ND	3.02	16.4	3.31	3.56
Skoal Dry											
Regular	1.27	10.7	ND	3.78	5.08	ND	1.48	1.76	2.51	0.269	3.49
Cinnamon	0.849	24.3	ND	8.38	7.37	ND	ND	0.207	0.970	0.619	8.95
Menthol	0.986	12.8	ND	4.25	4.54	ND	2.10	1.58	2.53	ND	2.74
Mean for new products	2.63	21.2	ND	10.0	9.51	2.76	3.12	3.23	6.16	1.09	9.12
Traditional products											
General Snus	1.70	55.3	ND	31.1	29.7	ND	ND	8.49	31.7	1.01	1.05
Copenhagen Snuff	17.3	699	152	300	351	31.5	34.2	6.58	17.1	3.24	6.35
Copenhagen Long Cut	16.7	528	148	277	323	28.6	31.1	9.54	18.8	2.58	3.29
Skoal Long Cut	67.5	2310	370	522	599	36.1	30.1	10.6	38.6	2.65	0.984
Kodiak Wintergreen	54.0	3920	639	872	1060	57.1	57.3	6.93	72.3	7.85	3.23
Mean for traditional products	31.4	1500	327	400	473	38.3	38.2	8.43	35.7	3.47	2.98

Note. [a]Abbreviations: PAH, polycyclic aromatic hydrocarbons; BbF, benzo[*b*]fluoranthene; BkF, benzo[*k*]fluoranthene; BaP, benzo[*a*]pyrene.
[c]ND, not detected.

significantly in their products (Stepanov et al., 2006). Overall, the results of our study demonstrate the partial success of this effort, with the exception of the Skoal Dry brand, which has TSNA levels comparable to those in some traditional commercial brands. NNN and NNK levels are relatively low in Taboka, Marlboro Snus, and Camel Snus. When expressed per dry weight, NNN levels in these products are comparable to those in the Swedish snus General, while NNK was about two times lower (Table 1). The processing of Swedish snus involves pasteurization, which leads to lower levels of TSNAs. Taboka, Camel Snus, and probably Marlboro Snus, also contain pasteurized tobacco. The reduction in carcinogenic TSNA content in the new smokeless tobacco is encouraging. TSNA levels in traditional moist snuff analyzed in this study are similar to those reported earlier (Stepanov et al., 2006). As known human carcinogens, NNN and NNK are not safe at any level, and even the lower amounts found in the new tobacco products are still 100 to 1,000 times higher than nitrosamine levels in other products, such as food and beer (Bartsch & Spiegelhalder, 1996).

The consumer's acceptance of a smokeless tobacco product and addiction to it depends on the nicotine content and the pH—parameters defining the amount of biologically available unprotonated nicotine. Total nicotine levels expressed per dry weight of product are quite similar across the brands (Table 1). However, due to differences in pH values, there is a large variation in the levels of unprotonated nicotine. Taboka and Marlboro Snus have the lowest pH values and, consequently, the lowest levels of free nicotine. A positive aspect of the low free nicotine content is the lower addictive potential of these products. However, low unprotonated nicotine products could be more easily accepted by young people who initiate tobacco use. Moreover, the low-nicotine products may not provide a good substitute for cigarette smoking, potentially leading to dual use of cigarettes and smokeless tobacco products (Hatsukami et al., 2007).

Camel Snus, slightly higher in total nicotine and pH than Taboka and Marlboro Snus, contains up to 9 mg unprotonated nicotine per gram dry weight—an amount similar to the most popular traditional brands. This high level of biologically-available nicotine has the potential to satisfy those smokers who are looking for a substitute for smoking, and to keep them addicted to this product.

Levels of nornicotine were relatively high in Taboka, and generally elevated in new products as compared to the traditional brands (Table 1). There are some indications that nornicotine, which may accumulate in the brain (Crooks & Dwoskin, 1997; Crooks, Li, & Dwoskin, 1995), contributes to the addiction associated with tobacco use (Bardo, Green, Crooks, & Dwoskin, 1999). Another potential risk is endogenous nitrosation of nornicotine in the stomach, which can lead to formation of NNN (Porubin, Hecht, Li, Gonta, & Stepanov, 2007). The potential health effects of the relatively high levels of anatabine observed in Taboka are unknown.

Nitrite and nitrate content in smokeless tobacco products are important for a number of reasons. Nitrate in saliva is converted to nitrite (Marletta, 1988). The toxic properties of nitrite include methemoglobin formation (Assembly of Life Sciences [ALS], 1988) and conversion to nitrosating agents, which can participate in endogenous synthesis of nitrosamines from tobacco alkaloids and dietary amines (Porubin, et al., 2007; Shepard, Schlatter, & Lutz, 1987). The 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.

Among the other anions analyzed here, the relatively high levels of chloride in Camel Snus and in traditional moist snuff smokeless products are noteworthy. Sodium chloride is a known additive to smokeless tobacco, and is used as a flavor enhancer and antimicrobial agent. High doses of salt can damage the gastric epithelium, providing favorable conditions for the occurrence of mutations (Charnley & Tannenbaum, 1985; Sugimura, 2000). A positive correlation between daily salt intake and gastric cancer incidence has been reported (Hirayama, 1984; Tsugane et al., 1991). Local irritation from salt may increase the absorption of smokeless tobacco carcinogens in the oral cavity, and also may lead to chronic inflammation and tumor promotion.

The low levels of PAH in the new smokeless tobacco is a very positive sign (Table 3). Anthracene, BbF, BkF, and BaP are virtually undetectable in these products, while other PAH are present in trace amounts. However, PAH levels in the most popular brands currently used by millions of consumers are in some cases remarkably elevated. Even though human toxicity data for acenaphthylene, phenanthrene, anthracene, fluoranthene, and pyrene are not available, animal studies suggest a range of negative effects, including pulmonary, endocrine, and liver toxicity, as well as co-carcinogenicity (U.S. Department of Health and Human Services [USDHHS], 2001). BbF and BkF are IARC group 2B carcinogens (possibly carcinogenic to humans) (IARC, 1983), and to our knowledge, this is the first study to report their presence in smokeless tobacco. The sum of these carcinogens is comparable to the amounts of BaP detected in the same products, which, in turn, are similar to those reported in the literature (Hoffmann et al., 1987; McNeill et al.,

Table 4. Average levels of nicotine, chloride, and some carcinogens per portion.

Product	Single portion weight, g[a]	Moisture content, %	Amount per portion									
			Nicotine mg	Free nicotine mg	Chloride mg	NNN µg	NNK µg	BbF+BkF ng	BaP ng	Formaldehyde µg	Acetaldehyde µg	Crotonaldehyde µg
New products												
Taboka Original	0.233	13.3	4.26	0.171	0.360	0.212	0.016	ND[b]	ND	0.634	0.371	3.91
Marlboro Snus Rich	0.222	10.1	3.55	0.215	1.58	0.253	0.052	0.517	0.309	0.930	1.17	3.42
Camel Snus Original	0.322	31.2	6.25	1.35	8.81	0.255	0.060	ND	2.31	0.335	1.47	0.122
Skoal Dry Regular	0.372	10.3	3.76	0.525	4.64	1.19	0.120	ND	0.495	0.588	0.837	1.16
Mean for new products	0.287	16.2	4.46	0.565	3.85	0.478	0.062	0.517	1.04	0.622	0.962	2.15
Traditional products												
General snus	1.27	48.5	10.9	5.03	49.5	1.08	0.304	ND	ND	5.55	20.7	0.685
Copenhagen Snuff	1.50	55.3	15.4	3.27	71.7	3.44	0.936	21.2	23.0	4.41	11.5	4.26
Copenhagen Long Cut	1.50	56.6	17.4	4.64	97.7	2.45	0.719	18.6	20.3	6.21	12.2	2.15
Skoal Long Cut	1.50	55.4	17.1	4.04	92.0	3.12	1.10	24.2	20.1	7.10	25.8	0.659
Kodiak Wintergreen	1.50	54.8	13.3	8.20	105	4.65	0.956	38.7	38.9	4.70	49.1	2.19
Mean for traditional products		54.1	14.8	5.04	83.2	2.95	0.803	25.7	25.6	5.59	23.9	1.99

Note. [a]Average pouch weight for the new smokeless tobacco products and Swedish snus General, and average reported portion weight for other traditional products. [b]ND, not detected.

2006). BaP has recently been classified by IARC as a group 1 carcinogen (carcinogenic to humans) (IARC, 2007).

Volatile aldehydes commonly occur in the human environment (IARC, 1995; USDHHS, 2004). Overall, their levels are relatively low in the products studied here, when compared to other sources of exposure such as the diet and alcoholic beverages (Table 3). A surprising finding was the relatively elevated levels of crotonaldehyde in Taboka and Marlboro Snus. The manufacturer should identify and eliminate the source of contamination of their products with this mutagen (Neudecker, Eder, Deininger, & Henschler, 1989) and carcinogen (Chung, Tanaka, & Hecht, 1986).

Expression of levels of toxic and carcinogenic constituents per dry weight of tobacco does not allow us to compare the actual exposure to these agents per single doses, or portions, of the products. In Table 4, we estimate the levels of the most important agents analyzed in this study per single portion of some new and traditional tobacco products. The moisture content of new smokeless tobacco products ranges from 10.1% to 31.2%, while that of traditional brands averages 54.1%. If portion sizes were similar for both groups of products, the toxicant and carcinogen intake from the new products would be somewhat similar to that from traditional ones. However, the differences in the portion size between the new and traditional smokeless tobacco products lead to even more drastic differences in toxicant and carcinogen amounts per dose. The mean weight of one pouch of a new smokeless tobacco product in our study was 0.287 g, while the weight of one pouch of General snus was 1.27 g, and the mean reported grams per dip of traditional moist snuff is about 1.5 (Hatsukami & Severson, 1999). As a result, one pouch of a new smokeless tobacco product contains on average about 20 times lower amounts of the analyzed agents than an average portion of traditional products (Table 4). The levels of crotonaldehyde, even though relatively high in Taboka and Marlboro Snus when expressed per dry weight of product, become comparable to the levels of this carcinogen in an average portion of traditional moist snuff. It is not clear, however, whether users of these new products will use more pouches to compensate for the smaller amount of tobacco per pouch.

In summary, we report here a large variation in the levels of important toxicants and carcinogens in a range of recently introduced smokeless tobacco products and some of the most popular traditional moist snuff brands. Some of the new smokeless tobacco products contain much lower levels of most of the carcinogens analyzed here, as compared to the traditional brands. More effort is required from the U.S. tobacco industry to further reduce levels of these important chemical agents in both new and conventional smokeless tobacco products.

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