HHS Public Access
Author manuscript
Nicotine Tob Res. Author manuscript; available in PMC 2021 January 19.

Published in final edited form as:
Nicotine Tob Res. 2013 July ; 15(7): 1283–1288. doi:10.1093/ntr/nts270.

Chemical Analysis of Alaskan Iq'mik Smokeless Tobacco

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Abstract

Introduction—Iq'mik, a form of smokeless tobacco (ST), is traditionally used by Cup'ik and Yup'ik Eskimo people of western Alaska. Iq'mik is sometimes incorrectly considered to be a healthier alternative to smoking because its ingredients are perceived as "natural." Our chemical characterization of iq'mik shows that iq'mik is not a safe alternative to smoking or other ST use.

Methods—We measured nicotine and pH levels of tobacco and ash used to prepare iq'mik. We also characterized levels of toxins which are known to be present in ST including tobacco-specific nitrosamines (TSNAs) and polycyclic aromatic hydrocarbons (PAHs) using chromatographic separations coupled with isotope dilution mass spectrometry.

Results—Nicotine content in the iq'mik tobacco was very high, ranging from 35 to 43 mg/g, with a mean of 39 mg/g. The pH of the iq'mik tobacco–ash mixture was 11, an extremely high level compared with most ST products. High levels of PAHs were seen in the fire-cured tobacco samples with a benzo[a]pyrene level of 87 ng/g. Average TSNA levels in the tobacco were 34, 2,700, and 340 ng/g for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), N′-nitrosonornicotine (NNN), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), respectively.

Conclusions—Iq'mik contains high levels of the more easily absorbed unionized nicotine as well as known carcinogenic TSNAs and PAHs. The perception that iq'mik is less hazardous than other tobacco products due to the use of "natural" ingredients is not warranted. This chemical

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Declaration of Interests
None declared.

characterization of iq'mik gives a better understanding of the risk of possible adverse health effects of its use.

Introduction

Oral use of smokeless tobacco (ST) use causes significant health risks, including cancer and a number of noncancerous oral conditions. Some evidence suggests that nicotine may contribute to coronary artery and peripheral vascular disease, hypertension, peptic ulcers, and adverse pregnancy outcomes (Surgeon General Report, 2010). At least 28 carcinogens have been identified in ST (Brunnemann, 1992), with 7 of those being International Agency for Research on Cancer (IARC, 2007) Group 1 carcinogens. Among these known human carcinogens are the tobacco-specific nitrosamines (TSNAs), N′-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and the polycyclic aromatic hydrocarbon (PAH) benzo[a]pyrene (BAP). Smokeless products vary greatly in content of toxic components (Stanfill et al., 2011). Therefore, caution should be exercised before drawing conclusions about all ST products based on data from one or a few of the many forms.

In addition to using conventional forms of commercial ST, Alaska Native (AN) people combine tobacco with fungus or plant ash to make a homemade ST mixture known as iq'mik (Renner et al., 2004). Iq'mik, also known as "blackbull" or "dediguss," is traditionally used by Cup'ik and Yup'ik Eskimo people of western Alaska. A moist dollop of iq'mik is prepared from light and powdery fungus or plant ashes mixed with tobacco in various proportions based on the user's preference (Renner et al., 2005). Fungus ash, also called punk ash, is prepared by burning the basidiocarps of *Phellinus igniarius*, a local available fungus that grows on birch trees. If punk ash is unavailable, ashes from littletree willow wood (*Salix arbusculoides*), alder bushes, or driftwood are sometimes used. The high alkalinity of punk or willow ash facilitates the conversion of the nicotine to the unionized form. This unionized form of nicotine is more rapidly absorbed through oral membranes (Benowitz, 1992; Djordjevic, Hoffmann, Glynn, & Connolly, 1995; Tomar & Henningfield, 1997), resulting in an increased rate of nicotine delivery to the user. The uncut air- or fire-cured full leaf or twist tobacco used in iq'mik is a commercial variety of "chew" tobacco and is available in local stores (Blanchette, Renner, Held, Enoch, & Angstman, 2002; Renner et al., 2004).

Both commercial smokeless products and homemade chew such as iq'mik are used throughout Alaska, but the homemade version is most common in the southwest region of Alaska (Blanchette et al., 2002; Nelson & Powell, 1899; Renner et al., 2005). Iq'mik use is common among select Alaskan Native people with prevalence approaching 16%–22% among adults.

Iq'mik or spit tobacco use during pregnancy is more common among women who live in Southwest Alaska (~56%) than for other Department of Labor regions (0%–7.7%; Perham-Hester, 2007). Iq'mik may be considered a healthier alternative to smoking by some people in the Yukon-Kuskokwim region because its ingredients are perceived as "natural," leading some women to switch to iq'mik from other tobacco products during pregnancy (Renner et al., 2004). A recent study found that one in six AN women in southwest Alaska who used both cigarettes and ST before pregnancy quit smoking but continued to use ST during pregnancy. This suggests that ST may be perceived by these people as a less hazardous product to be used as a substitute for smoking during pregnancy (Kim, England, Deitz, Morrow, & Perham-Hester, 2010; Patten et al., 2008). Because of its widespread use in Alaska, particularly among youth, there is an urgent need to better understand the potential adverse health effects of iq'mik.

Renner et al. (2005) reported preliminary nicotine and pH levels of iq'mik tobacco and ash, which are important to help assess the addictive properties of iq'mik. This study performs a more comprehensive look at these levels as well as reporting levels of PAHs and TSNAs in several variants of tobacco used specifically to make iq'mik. This work thus helps provide a better understanding of the possible adverse health effects from the use of iq'mik.

Methods

Samples of punk ash, willow ash, both air-cured and fire-cured twist and leaf tobacco samples were purchased from local retailers in Bethel and Dillingham, Alaska. They were placed in sealed plastic bags and shipped through commercial carriers to the laboratory. A 3:2 mixture of tobacco to ash by weight was estimated to be an approximate "typical" ratio of ingredients based on anecdotal information from study investigators. The average weight from several individual sized portions was 0.43 g/serving; so, assuming a 3:2 ratio of ingredients, we calculated a 0.26 g tobacco/serving and a 0.17 g ash/serving. This "serving size," however, will inevitably vary greatly from serving to serving as well as from person to person because of individual variations in the manual preparation; so, overall exposure to nicotine and other toxins would vary accordingly.

Nicotine Determination

Nicotine levels in the tobacco used to prepare iq'mik were determined using a previously described procedure (Stanfill, Jia, Watson, & Ashley, 2009). One gram of each tobacco sample was placed in a vial and 50 ml of methyl-t-butyl ether containing quinoline internal standard and 5.0 ml of a 2 N sodium hydroxide solution were added. The sample vial was shaken for 2 hr at 160 rpm. An aliquot of the solvent extract was analyzed by gas chromatography/mass spectrometry (GC/MS) in selected ion monitoring (SIM) mode with a 3.7-min run time. The unionized nicotine content for a typical iq'mik serving was calculated by multiplying the percentage of unionized nicotine (calculated from the pH and pKa using the Henderson–Hasselbalch equation) by the total nicotine content in an estimated serving size. The equations for calculating unionized nicotine are published in the Federal Register (1999).

pH Determination

Tobacco and ash mixtures containing 0.6 g of tobacco and 0.4 g ash (punk and willow) were added to 10 ml of water. After initial stirring, an Orion pH electrode was used to measure the pH levels at 5, 15, 30, and 60 min with constant stirring in accordance with the Federal Register (1999) protocol for determination of pH in smokeless products. The pH measurements at different times ensure the values are stable over time. For comparison, pH measurements on the punk and willow ash samples in water were also made.

PAH Determination

Lower molecular weight PAHs were analyzed by GC/MS, whereas higher molecular weight PAHs were analyzed by LC-MS/MS. The levels of 10 PAHs with molecular weights ranging from 128 to 252 amu were measured using a method similar to that developed by Ding et al. (2006). Tobacco and ash samples were weighed, spiked with an isotopically enriched ^{13}C PAH mixture as an internal standard, extracted with methanol followed by solid phase extraction cleanup. The PAHs were subsequently analyzed by SIM GC/MS in triplicate and normalized to tobacco weight and expressed in nanograms of analyte per gram tobacco. Analytes measured included acenaphthene, acenaphthylene, anthracene, benzo[e]pyrene, chrysene (CHR), fluoranthene (FLR), fluorene, naphthalene, phenanthrene (PHE), and pyrene (PYR).

Additionally, 10 carcinogenic PAH compounds with molecular weights of 228–302 amu were also measured using liquid chromatography/tandem mass spectrometry (LC-MS/MS) to facilitate analysis of the less volatile PAH compounds (Ding, Ashley, & Watson, 2007). The PAHs analyzed by the LC-MS/MS method included benz[a]anthracene (BAA), benzo[b]fluoranthene (BBF), benzo[j]fluoranthene (BJF), benzo[k]fluoranthene (BKF), BAP, dibenz[ah]anthracene (DBA), dibenzo[ai]pyrene (DIP), dibenzo[ae]pyrene (DEP), indeno[1,2,3-cd]pyrene (INP), and 5-methylchrysene. In the LC-MS/MS method, iq’mik tobacco samples were spiked with a ^{13}C-enriched PAH internal standard mixture and extracted with cyclohexane. Following solvent concentration, the extract was filtered and injected into the LC-MS/MS system. As with the PAHs analyzed by GC-MS, the 10 carcinogenic PAHs, measured by LC-MS were normalized to tobacco weight and expressed in ng/g tobacco. For comparison, PAH levels were also measured by the LC-MS method for Copenhagen snuff and mainstream smoke in the Kentucky Reference Cigarette 2R4F.

TSNA Determination

Approximately 0.25 g of the iq’mik tobacco was added to a stainless steel extraction cell and spiked with 100 ng of $^{13}C_6$-labeled TSNAs as internal standards. Samples were extracted with ethyl acetate using a Dionex ASE 200 Accelerated Solvent Extractor. Sample workup followed the procedure described by Richter, Hodge, Stanfill, Zhang, and Watson (2008). Subsequent analysis of the TSNAs by LC-MS/MS used the method developed by Wu, Ashley, and Watson (2003). The TSNAs analyzed in this study included NNN, NNK, N′-nitrosoanabasine (NAB), NAT, and NNAL. Ion chromatograms for standards and samples were similar to those shown in the Cooperation Centre for Scientific Research Relative to Tobacco (2011) TSNA method.

Results

Nicotine

Iq’mik can be formulated in multiple permutations using different types of tobacco (air-cured or fire-cured) and different types of ash (punk or willow) in varying ratios in the final preparation. Nicotine content in the tobacco used in our different iq'mik samples ranged from 35 to 43 mg/g (mean, 39 mg/g) (Table 1), with an overall SD of 5 mg/g.

Product pH

The pH of the punk and willow ash as well as the ash/tobacco mixture, when added to water, gave a pH of 11. When mixed with punk or willow ash, virtually all of the nicotine (99.9%) in iq'mik is converted to the readily absorbed unionized (free) form.

PAHs

PAHs detected in this study ranged from 1.4 ng/g of DEP in aircured twist tobacco to 1,620 ng/g of PHE in fire leaf tobacco (Table 2). A majority of the PAH levels fell in the range of 10–100 ng/g in the tobacco samples. Average levels of BAP, the well-studied IARC Group 1 carcinogen, were 13 ng/g with an SD of 2.6 ng/g for the air-cured tobacco samples and 87 ng/g with an SD of 41 ng/g for the fire-cured tobacco samples. The standard deviations are fairly high in some cases due to the significant sample-to-sample variability, reflecting the varied sources of the product. The PAH levels in the ash samples were found to be near or below the limit of detection for the method and are not reported.

Tobacco-Specific Nitrosamines

We measured the levels of select TSNAs in the iq'mik tobacco samples and observed values ranging from 15 to 4,910 ng/g (Table 2). There were no consistent differences for TSNAs between air-cured and fire-cured iq'mik tobacco samples. NNN and NNK are classified as IARC Group 1 carcinogens (IARC, 2012); we determined average values of 2,700 and 341 ng/g, with SDs of 898 and 163 ng/g, respectively, for all iq'mik tobacco samples combined. The high relative SDs result from variability between the tobacco samples.

Discussion

The average nicotine value in iq'mik of 39 mg/g tobacco is high compared with values of 12, 18, and 26 mg/g tobacco reported in a survey of chewing tobacco, dry snuff, and moist snuff products, respectively, sold in Massachusetts in 2003 (Massachusetts Department of Public Health, 2004). Because the ash itself does not contain measurable levels of nicotine, the chewing tobacco variety that is typically used for iq'mik must be among commercial smokeless products with the highest total nicotine content in tobacco on a per gram basis.

The estimated serving size for iq'mik of 0.26 g from this study was significantly smaller than the traditional moist snuff dip of 1.2 g previously estimated (Hatsukami & Severson, 1999; Severson, Eakin, Lichtenstein, & Stevens, 1990). Normalizing for the serving size would give a total nicotine value in a serving of iq'mik of 10 mg, somewhat lower than 14 mg estimated for moist snuff. However, the high pH of iq'mik, resulting from the alkalinity of the added punk or willow ash, produces unionized (free) nicotine levels that are extremely high on a per dose basis, typically higher than other U.S. smokeless products. The unionized nicotine content for a typical iq'mik serving was calculated to be 9–11 mg, which is over twice the value of 4.5 mg estimated for moist snuff. A recent paper by Stepanov, Jensen, Hatsukami, and Hecht (2008) on ST products reported unionized nicotine values, accounting for single portion weight, from 0.2 mg for Taboka to 8.2 mg for Kodiak Wintergreen. The mean for new ST products was 0.6 mg, and the mean for traditional ST products was 5.0 mg unionized nicotine. Thus, iq'mik provides a high dose of unionized nicotine compared with other popular ST products.

In contrast to the pH of 11.0 for the iq'mik samples, the alkalinity in domestic moist snuff samples generally ranges from pH 5.4 to 8.6 with 0.3%–79.9% of nicotine in the unionized form (Richter et al., 2008). In a global survey of international products, some khaini, gul, toombak, and South African snuff products had alkalinity in the range of pH 9.2–10.0 (Stanfill et al., 2011). But, even these extreme smokeless products do not attain the high pH level caused by the addition of ash to form iq'mik.

In 1998, the sales-weighted average machine delivery of nicotine for U.S. cigarettes was 0.88 mg (Federal Trade Commission, 2000), which means a single serving of iq'mik can provide at least an order of magnitude higher dose of nicotine over smoking a cigarette. These findings help to explain those of Hurt et al. (2005) who found higher cotinine concentrations in mothers who used iq'mik and their neonates compared with mothers who used cigarettes and/or other forms of tobacco.

The ash samples contained very low amounts of PAHs; most of the measured PAH levels in either the punk or willow ash were below our detection limits. All of the tobacco samples, however, did have detectible levels of a number of PAHs. We saw substantial differences among PAH levels comparing the air-cured with the fire-cured tobacco samples. Lower molecular weight PAHs, such as naphthalene, had similar values for air-cured and fire-cured iq'mik tobacco samples. However, the larger high molecular weight PAHs such as BAP were much higher in the fire-cured iq'mik tobacco samples. This is consistent with ST processed with heat and smoke during the "fire-curing" process (IARC, 2007). Using iq'mik rather than smoking cigarettes may actually increase exposure to select PAHs although the exposure route differs significantly which would significantly affect the internal dose resulting from exposure.

In comparison with the BAP levels of 13 ng/g for the aircured iq'mik tobacco and 87 ng/g for the fire-cured iq'mik tobacco, Copenhagen snuff contains 27 ng/g of BAP. BAP levels in traditional smokeless products in a recent study (Stepanov et al., 2008) ranged from 30 to 57 ng/g and averaged 38 ng/g, intermediate values falling between those measured for air-cured and fire-cured iq'mik tobacco in this study.

Average TSNA levels ranged from 34 ng/g for NNAL to 3,900 ng/g for NAT. No consistent trends between air- and fire-cured tobacco samples were seen. Values of 2,700 and 340 ng/g measured for NNN and NNK, respectively, are lower than averages of 6,270 and 970 ng/g measured for the top five brands of snuff sold in the United States in 1994 (Hoffmann et al., 1995), average values of 17,400 and 7,500 ng/g for the top five U.S. moist snuff brands in 2001 (Connolly, 2001), and average values of 6,880 and 1,810 ng/g for a survey of 40 U.S. moist snuff brands (Richter et al., 2008). Although these TSNA levels are lower than other smokeless products, the relative levels depend on the type of tobacco used, i.e., fluecured tobaccos tend to have lower levels of TSNAs (Hecht, Ornaf, & Hoffmann, 1974). Thus, if other types of tobacco were used to make iq'mik, these levels would change.

The alkaline ash used in iq'mik has extremely high pH levels, resulting in nearly all nicotine being in the unionized (free) form, which is more rapidly absorbed than the protonated form present at lower pH levels (Henningfield, Radzius, & Cone, 1995). High total nicotine at the high pH levels present in iq'mik result in nearly 100% of all nicotine being available as the more bioavailable unionized form. Such high nicotine and unionized nicotine levels are unprecedented compared with other popular domestic smokeless products. This has serious implications regarding nicotine addiction (Renner et al., 2005).

We have also shown iq'mik tobacco to contain high levels of hazardous TSNAs and PAHs. Moreover, our laboratory found levels of arsenic, cadmium, lead, and nickel, IARC Group 1 carcinogens (IARC, 2012) in iq'mik comparable to levels found in commercial snuff products (Pappas, Stanfill, Watson, & Ashley, 2008). The perception that iq'mik is less hazardous than other tobacco products due to the use of "natural" ingredients is not supported and switching from cigarettes to this form of ST during pregnancy is not a wise health decision. Considering the high levels of addictive and toxic compounds in iq'mik, quitting tobacco use altogether is the much preferred outcome with documented health benefits.

Although we have only focused on a limited set of analytes and a small number of iq'mik samples, a more comprehensive screening could possibly identify the presence of other harmful analytes. Based on our current findings and those of other researchers, there is sufficient evidence to conclude that iq'mik does not provide a safe alternative to smoking or other tobacco use.

Acknowledgments

Funding

This work was supported by the Centers for Disease Control and Prevention.

We would like to acknowledge useful comments from Lucinda England and Shin Kim at the Centers for Disease Control and Prevention for their valuable feedback in preparing this manuscript. We appreciate Ms. Shelley Wallace, of the Bristol Bay Area Health Corporation (BBAHC), Ms. Carrie Enoch, and Ms. Caroline Nevak, of the Yukon-Kuskokwim Health Corporation (YKHC), for their assistance in obtaining the iq'mik samples.

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Table 1

Average Values for Nicotine, pH, and Free-Base Nicotine in Various Types of Tobacco Used to Prepare Iq'mik

	Air-cured leaf tobacco (*n* = 3)	**Air-cured twist tobacco (*n* = 3)**	**Fire-cured leaf tobacco (*n* = 3)**	**Fire-cured twist tobacco (*n* = 6)**
Nicotine (mg/g)	38.0 (0.4)	38.5 (8.8)	42.7 (5.6)	35.0 (3.2)
Nicotine (mg) per	9.9 (0.1)	10.0 (2.3)	11.1 (1.5)	9.1 (0.8)
pH 0.26 g serving	11.0	11.0	11.0	11.0
Percent free base nicotine (%)	99.9	99.9	99.9	99.9
Unionized nicotine (mg) per 0.26 g serving	9.9 (0.1)	10.0 (2.3)	11.1 (1.5)	9.1 (0.8)
Unionized nicotine (mg/g)	38.0 (0.4)	38.5 (8.8)	42.7 (5.6)	35.0 (3.2)

Note. Standard deviations are given within parentheses. U.S. moist stuff averages (for comparison): nicotine = 11.9 mg/g; nicotine per 1.2 g serving = 14.3 mg; pH = 7.6; percent free base nicotine = 31.6%; unionized nicotine per 1.2 g serving = 4.5 mg; unionized nicotine = 3.8 mg/g.

The value for pH is based on a mixture of the various tobaccos with punk ash. Values for U.S. moist snuff (*n* = 39) are included for comparison.

Table 2

Average Values for PAHs and TSNAs Found in the Various Iq'mik Tobaccos

	Air leaf	Air twist	Fire leaf	Fire twist	Copenhagen	2R4F smoke
Polycyclic aromatic hydrocarbons (ng/g)						
Naphthalene (NAP)	363 (15)	354 (7)	361 (14)	353 (4)	360	240
Acenaphthylene (ACL)	97 (4)	97 (12)	176 (91)	104 (11)	131	78
Acenaphthene (ACT)	27 (5)	21 (1)	46 (30)	31 (12)	36	27
Fluorene (FLU)	167 (6)	154 (26)	463 (482)	218 (117)	319	136
Phenanthrene (PHE)	398 (332)	416 (167)	1,620 (1,946)	910 (619)	1,120	161
Anthracene (ANT)	102 (63)	99 (30)	358 (409)	196 (113)	213	60
Fluoranthene (FLR)	127 (124)	139 (60)	882 (915)	649 (285)	377	54
Pyrene (PYR)	56 (101)	72 (26)	601 (574)	392 (156)	375	43
Benz[a]anthracene (BAA)	51a	36 (8)	292 (137)	226 (112)	81	18
Chrysene (CHR)	37 (22)	41 (9)	359 (177)	285 (147)	97	22
Benzo[b]fluoranthene (BBF)	19.4 (2)	20 (2)	68 (19)	56 (20)	31	7.7
Benzo[k]fluoranthene (BKF)	5.9 (3)	5.4 (0.1)	39 (7)	30 (16)	10	1.7
Benzo[e]pyrene (BEP)	15a	9.7a	82 (11)	58 (24)	27	5.3
Benzo[a]pyrene (BAP)	13 (5)	13 (0.4)	104 (22)	78 (48)	27	7.4
5-Methylchrysene (5MC)	7.6a n/da	18 (4)	17 (4)	8.4	2.7	
Benzo[j]fluoranthene (BJF)	18 (4)	19 (2)	63 (34)	48 (26)	30	10
Indeno[1,2,3-cd]pyrene (INP)	35a	30 (1)	77 (13)	57 (15)	36	5.9
Dibenz[ah]anthracene (DBA)	8.7 (3)	5.7 (5)	19 (2)	14 (6)	6.3	4.7
Dibenzo[ae]pyrene (DEP)	1.5 (0.2)	1.4 (0.0)	3.8 (0.6)	2.9 (1.1)	1.6	1.5
Dibenzo[ai]pyrene (DIP)	1.4 (0.0)	1.5 (0.0)	2.2 (0.1)	1.9 (0.4)	1.5	0.7
Tobacco-specific nitrosamines (ng/g)						
NAB	163 (65)	213 (85)	342 (169)	200 (34)	365	12
NAT	3,220 (1,370)	4,350 (2,130)	4,910 (2,640)	3,140 (539)	3,879	129
NNK	188 (84)	230 (117)	541 (370)	404 (155)	960	131
NNN	1,990 (845)	4,000 (1,600)	2,240 (722)	2,560 (390)	3,987	160

	Air leaf	Air twist	Fire leaf	Fire twist	Copenhagen	2R4F smoke
NNAL	80[a]	42 (11)	n/d	22 (11)	62	n/d

Note. Standard deviations are given within parentheses.

NAB = N′-nitrosoanabasine; NAT = N′-nitrosoanatabine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN = N′-nitrosonornicotine; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.

Levels measured in Copenhagen snuff and in mainstream smoke from a 2R4F Kentucky Reference Cigarette (ng/cig) are given for reference.

[a]Standard deviation not relevant (limited number of data points); n/d = not detected or below instrument limit of quantitation.