Analysis of Toxic Metals in Commercial Moist Snuff and Alaskan *Iqmik*

R.S. Pappas*, S.B. Stanfill, C.H. Watson, and **D.L. Ashley**

Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia 30341

Abstract

The extent to which smokeless tobacco endangers human health is an ongoing subject of debate. Studies have shown that smokeless tobacco products contain high levels of biologically available nicotine and tobacco-specific nitrosamines. Toxic metals in smokeless tobacco products have been less extensively studied. In this study, concentrations of arsenic, barium, beryllium, cadmium, chromium, cobalt, lead, and nickel were measured in snuff products and *iqmik* tobacco, a product popular among some Alaska Natives. The average arsenic, cadmium, lead, and nickel concentrations in 17 commercially available brands were 0.23 ± 0.06 µg/g, 1.40 ± 0.31 µg/g, 0.45 ± 0.13 µg/g, and 2.28 ± 0.36 µg/g, respectively. In 17 *iqmik* tobacco samples, the average arsenic, cadmium, lead, and nickel concentrations were 0.19 ± 0.06 µg/g, 1.41 ± 0.56 µg/g, 0.55 ± 0.19 µg/g, and 2.32 ± 1.63 µg/g, respectively. Using artificial saliva, the extractable levels of beryllium and lead were relatively low and consistent, whereas barium extracted from tobacco samples ranged from 2 to 21%. The group 1 and 2B carcinogens cadmium, cobalt, and nickel were more efficiently extracted by artificial saliva (30–65% of the cobalt, 20–46% of the nickel, and 21–47% of the cadmium).

Introduction

In the United States, tobacco smoking is the leading cause of preventable disease (1). Consequently, most studies concerning the toxicity and health consequences of tobacco use have focused on cigarette smoking. Studies examining the relationship between smokeless tobacco use and health consequences are, however, fewer in number. Thus to cover the full spectrum of modern tobacco use, further studies of tobacco-associated health risks should include the risks associated with repeated oral exposure and absorption of the toxic and carcinogenic substances found in smokeless tobacco products.

In the United States smokeless tobacco is available in a variety of forms, such as dry or moist snuff, currently the most popular form based on revenues (2), and loose or plug chewing tobacco leaves. More than 7.7 million Americans use smokeless tobacco (3). In 1997, smokeless tobacco use in 17 states among U.S. adults over 18 years of age ranged from 1.4% in Arizona to 8.8% in West Virginia, the majority of users being male (4). In 2005, an estimated 2.3% of U.S. adults used smokeless tobacco (5). Certain snuff products have been gaining popularity, especially among adolescent males (6). In 2004, for example, 6.0% of U.S. high school students and 2.9% of middle school students reported using smokeless tobacco products (7).

Among a large number of American Indians, however, smokeless tobacco use reportedly starts at a much earlier age and is prevalent in a larger fraction of the population. Surveys of American Indian schoolchildren before 1988 reported regular smokeless tobacco use ranging from 24% to 64%. Of even greater concern, 21% of kindergarten children who lived on the Rosebud Sioux Reservation reported using smokeless tobacco in 1986. Of the 184 regular users on this reservation in grades 7–12, 37% had oral lesions of the mouth or buccal mucosa. Among Northern Plains American Indian schoolchildren, smokeless tobacco use was similar among boys and girls (8). In the Lumbee Tribe of North Carolina, 35% of women age 44 or younger began using smokeless tobacco before age 6 (9). Among Alaska Natives, 27.5% of girls and 33.7% of boys reported using smokeless tobacco. The majority of these adolescents reported using snuff (10), though *iqmik* use has also been reported as the most popular form of smokeless tobacco among some Alaska Natives, including females (11,12). More recently, 20.2% of the 5654 eligible students surveyed in high schools funded by the Bureau of Indian Affairs reported current use of smokeless tobacco in 2001 (13).

A snuff user typically consumes this smokeless tobacco product by inserting a small "pinch" of loose tobacco or a tobacco-containing pouch in the mouth. To produce *iqmik*, some Alaska Natives chew tobacco leaf together with punk ash (ashes produced from burned *Phellinus igniarius* fungus) or Alaska willow (*Salix alaxensis*) ash (11). The ashes make the saliva alkaline, resulting in a significant increase in nicotine absorption.

Tobacco is known to contain numerous classes of carcinogenic substances, and tobacco-specific nitrosamines are often

* Author to whom correspondence should be addressed: R. Steven Pappas, CDC, 4770 Buford Hwy. NE, MS F-44, Atlanta, GA 30341. E-mail: RPappas@cdc.gov.

regarded as a major factor in smokeless tobacco-related carcinogenesis (14,15). Tobacco-specific nitrosamines have been the focus of several previous studies and are of course one potent class of possible causative agents in carcinogenesis resulting from smokeless tobacco exposure (14–16). Still, carcinogenesis more likely results from the combined exposure to nitrosamines and other classes of organic and inorganic substances, including toxic metals and metalloids.

The International Agency for Research on Cancer (IARC) has identified several toxic metals as carcinogens. Arsenic, beryllium, cadmium, chromium (VI), and nickel are classified as group 1 human carcinogens (17); lead compounds are classified as group 2A probable human carcinogens (18); and cobalt compounds are classified as group 2B possible human carcinogens (19,20). Although not considered a carcinogen, barium is very toxic in soluble form and is known to act in acute exposures as a potassium channel antagonist, resulting in hypokalemia. To date, little solid evidence supports the view that barium in smokeless tobacco might contribute to local oral toxicity or inflammation. That said, however, of the toxic metals that were studied, barium was found at the highest concentrations in tobacco. Thus to expand the science base of information on the possible contents and emissions of concern in smokeless tobacco, barium was included in our analysis.

In this study the concentrations of arsenic, barium, beryllium, cadmium, chromium, cobalt, lead, and nickel were determined in popular snuff products and samples of *iqmik*, a smokeless product popular among some native Alaskans. The extent to which we could use artificial saliva to extract these metals from smokeless tobacco was also tested. This study was undertaken to help better understand the levels of toxic metals and metalloids to which users of smokeless tobacco are exposed, substances that have not been studied as thoroughly as other toxic constituents.

Experimental

Smokeless tobacco samples

In 2004 popular smokeless tobacco products were purchased at various retail outlets in the greater metropolitan area of Atlanta, Georgia, USA. The samples were assigned unique identification numbers and logged into a database. The samples were stored at –70°C until removed for testing. Leaf tobacco, punk ash, and willow ash used to make *iqmik* were obtained with help from sources listed in the Acknowledgments. Only authorized personnel had access to the samples. 1S3 Reference moist smokeless tobacco was obtained from the University of Kentucky Tobacco Research and Development Center (Lexington, KY). Oriental and Virginia tobacco leaf certified reference materials (CRM) CTA-OTL-1 and CTA-VTL-2 were obtained from the Institute of Nuclear Chemistry and Technology (Warsaw, Poland). Between uses, reference materials were stored in a vacuum desiccator.

Tobacco sample preparation

All tobacco samples were dried in Teflon® pfa containers for a minimum of 4 h at 100°C and tightly sealed until weighed for analysis. *Iqmik* tobacco leaves were rendered more homogeneous to minimize stem to leaf ratio differnces with a Smart Grind model coffee bean grinder (Black and Decker, Towson, MD) after drying. They were only homogenized for 10 seconds to produce particle sizes similar to those of the commercial tobacco samples. Tobacco samples were prepared for digestion according to Environmental Protection Agency (EPA) method 3052. 9.00 mL nitric, 0.500 mL hydrochloric, 0.500 mL hydrofluoric double-distilled acids (GFS, Powell, OH) and 0.500 mL Tamapure AA-10 35% hydrogen peroxide (Moses Lake Industries, Moses Lake, WA) were added to tobacco weighing between 0.100 and 0.170 g. These mixtures were digested with 1000 watts maximum power, 25 bar maximum pressure settings in a Milestone (Shelton, CT) Ethos model microwave system. Because of the safety precautions advised by EPA when addition of hydrogen peroxide is deemed necessary to achieve complete organic digestions, the 5-min ramp time to 180°C in EPA 3052 was doubled to 10 min. To achieve complete organic digestion, the digestion time at 180°C was more than doubled from 9.5 min to 20 min. Digested samples were diluted to 50.0 mL with ultrapure water. Before analysis, aliquots of 0.250 mL were diluted with 1.000 mL internal standard solution (1.25 µg/L Rh and Ir in 2% v/v HNO_3). Blanks were prepared by adding all reagents to the digestion vessels and proceeding through the digestion and analysis procedures without addition of tobacco.

Ash sample preparation

An Alaska willow ash sample and Coal Fly Ash standard reference materials 2690 and 2691 (NIST, National Institute for Standards and Technology, Gaithersburg, MD) were prepared for triplicate digestions with a variation in the acid volumes compared to tobacco, but still according to Environmental Protection Agency (EPA) method 3052. Nine milliliters nitric, 0.500 mL hydrochloric, and 0.250 mL hydrofluoric double distilled acids and 2.00 mL 35% hydrogen peroxide were added to ash weighing between 0.100 and 0.170 g. These mixtures were digested as described. Digested samples were diluted to 50.0 mL with ultrapure water. Blanks were prepared by adding all reagents to the digestion vessels and proceeding through the digestion and analysis procedures without addition of ash.

Using artificial saliva to extract smokeless tobacco samples

Specific samples were selected for extraction to represent different cuts and popular commercial brands together with *iqmik* tobacco samples for which sufficient quantity was available for additional triplicate analyses. *Iqmik* tobacco extractions were performed in parallel, with and without punk or willow ash addition. Blanks were prepared from artificial saliva taken through the extraction and digestion procedure without addition of tobacco.

Extractions were performed with artificial saliva prepared according to calculated amounts based on Chou and Hee (21) with one exception. Salts were obtained from Sigma-Aldrich-Fluka (St. Louis, MO) in the highest purity on metals basis: NaCl (204439), KCl (409316), $CaCl_2$ (499609), $MgCl_2$ (449172), NaH_2PO_4 (229903), urea (51459), and glucose (49139). The so-

lution pH was adjusted to 7.0 with NaOH before addition of proteins, also from Sigma-Aldrich: mucin from bovine submaxillary gland (M3895), α-amylase from human saliva (A0521), lysozyme from chicken egg white (L2879), and acid phosphatase from potato (P1146). The suspension was stirred for at least 2 h with a Teflon bar to homogeneity before aliquotting for artificial saliva blanks and sample extracts. The exception to the Chou and Hee (21) formula was that 2.5 units/mL (2500 units/L) amylase were used instead of 100 units/mL (100,000 units/L). This was because of the significant salt content of the enzyme preparation, the brown color indicating contaminants, and because of the cost.

Approximately 0.5 g of each sample was added to 30.0 mL of artificial saliva in a 50-mL Teflon pfa tube previously cleaned with a Milestone Traceclean system and heated to 37°C in three different digestions. In each of the triplicate digestions and analyses, duplicate *iqmik* tobacco samples were prepared. To one of each pair, 0.500 g punk or willow ash was added, with the exception of one punk ash sample, which had insufficient mass (0.401 g) for triplicate digestions and analyses. In that case, the same digest was analyzed in triplicate. All samples were inverted seven times to wet the tobacco surfaces and incubated for 30 min at 37°C. To sediment solids from the supernatant, samples were then centrifuged for 5 min at 1620 × *g* in an Eppendorf 5804 centrifuge with an A4-44 swinging bucket rotor. 1.000 mL of the extract was withdrawn from each supernatant and placed in a new pfa vessel. The extracts were tightly capped after addition of 0.500 mL of nitric acid and digested for 1 h at 120°C in a digital heatblock (model VI, VWR, West Chester, PA). The digests were cooled and diluted to 10.0 mL with ultrapure water. Aliquots (1.000 mL) were diluted with 0.500 mL internal standard solution (3 µg/L Rh and Ir in 2% nitric acid) for analysis. After analysis, results from extractions to which ash were added were divided by results from digested tobacco (total metals) analyses for determination of percent extractable for comparison with extractions performed without addition of ash.

Standard preparation

Quantification was based on five calibration standards, with subtraction of the solvent blank and the filter blank. Calibration standards were prepared by dilution in the final digestion reagent concentrations: 18% (v/v) nitric acid, 1% (v/v) hydrochloric acid, and 1% (v/v) hydrofluoric acid, before dilution with internal standard solution. Calibration standard solutions were prepared by dilution of NIST molybdenum, beryllium, and lead standard reference materials (SRMs) 3134, 3105a, 981; and NIST-traceable standards cadmium 10008-1 (High Purity Standards, Charleston, SC), barium, chromium, cobalt, nickel, and arsenic standards 140-051-561, 140-051-241, 140-051-271, 140-051-281, and 140-051-331 (SCP Science, Baie D'Urfé, QC, Canada). Calibration ranges for tobacco samples were determined from preliminary experiments and spanned the range of levels found in 50-mL diluted digests of smokeless tobaccos. The following standard concentration ranges (calculated before dilution in internal standard), were used for tobacco samples: Be, 0.010 to 2.000 µg/L, Mo, 0.100 to 20.00 µg/L; Cd, 1.00 to 200.0 µg/L; Ba, 10.00 to 2000 µg/L; total Pb, 0.500 µg/L to 100.0 µg/L; Cr, 0.500 to 100.0 µg/L, Co, 0.100 to 20.00 µg/L, Ni, 0.500 to 100.0 µg/L; and As, 0.100 to 20.00 µg/L. Standards and solvent blanks (0.250 mL) were diluted, as were tobacco digest samples with 1.000 mL internal standard solution containing 1.25 µg/L rhodium (SPEX PLRH3 Spex, Mettuchen, NJ) and 1.25 µg/L iridium (High Purity Standards 100025-3) in 2% v/v nitric acid. Rhodium was assigned as the low mass internal standard for molybdenum, cadmium, barium, chromium, cobalt, and nickel. Iridium was assigned as high mass internal standard for lead, and, because of their high ionization potentials, for beryllium and arsenic as well.

For artificial saliva samples, calibration standards were prepared by dilution of the same standards as used for tobacco analysis in the final digestion reagent concentrations: 5% v/v nitric acid before dilution with internal standard solution. Calibration ranges were, however, lower: Be, 0.001 to 0.100 µg/L, Mo, 0.050 to 5.000 µg/L; Cd, 0.100 to 10.00 µg/L; Ba, 2.500 to 250 µg/L; total Pb, 0.250 µg/L to 25.00 µg/L; Cr, 0.100 to 10.00 µg/L, Co, 0.250 to 25.00 µg/L, Ni, 0.500 to 50.00 µg/L; and As, 0.100 to 10.00 µg/L. Standards and solvent blanks (1.000 mL) were diluted with 0.500 mL internal standard.

Calibration standards for ash analysis were prepared from the same standards with the following concentration ranges (before dilution in internal standard solution): Be, 5.00 to 100.0 µg/L, Cd, 1.00 to 20.00 µg/L; Ba, 1000 to 20,000 µg/L; total Pb, 10.00 µg/L to 200.0 µg/L; Cr, 20.00 to 400.0 µg/L, Co, 5.00 to 100.0 µg/L, and Ni, 20.00 to 400.0 µg/L. Before analysis, aliquots of 0.100 mL were diluted with 0.900 mL internal standard solution described.

Quality control procedures

Recovery quality control was evaluated by including in every digestion CRMs CTA-OTL-1, CTA-OTL-2, and 1S3, together with samples. Because none of these materials had certified values available for beryllium, a vessel was used for an additional digestion of 1S3. Before addition of nitric acid for digestion, the additional 1S3 tobacco was spiked with 20.0 µL 1000.0 µg/L beryllium in 2% (v/v) nitric acid prepared from National Institute for Standards and Technology beryllium standard 3105a for a calculated concentration of 400.0 ng/L after dilution to 50.0 mL. Recovery results were graphically tracked for acceptability according to Westgard rules with SAS® version 9.1 (SAS Institute, Cary, NC) and, when available, compared to target values for accuracy. For beryllium, the recovery was calculated by comparing beryllium concentrations determined in spiked versus unspiked preparations using the following formula:

Spike Concentration – [(1S3 mass spiked/1S3 mass unspiked) × Unspiked Concentration]

Artificial saliva extraction recovery

To determine the extraction recovery in artificial saliva, 0.500 mL of a solution containing 10.00 µg/L Be, 500.0 µg/L Mo, 1000 µg/L Cd, 25,000 µg/L Ba, 2500 µg/L Pb, 1000 µg/L Cr, 2500 µg/L Co, 5000 µg/L Ni, and 1000 µg/L As was spiked onto 0.500 g 1S3 tobacco before triplicate extraction and digestion analyses. The levels which were determined in triplicate in the unspiked 1S3 extract were subtracted from the

spiked results. Recoveries were expressed as the percentages relative to the spike target value.

Analysis

Analyses were performed with an Element 2 magnetic sector inductively coupled plasma-mass spectrometer (ThermoElectron, Bremen, Germany) with standard high performance aluminum sampler and skimmer cones (Spectron, Ventura, CA), APEX HF model desolvating introduction system with nitrogen addition, high performance torch, o-ring free sapphire injector, pfa 100 µL/min self-aspirating nebulizer (Elemental Scientific, Omaha, NE), and ASX-100 autosampler (Cetac, Omaha, NE).

Be, Mo, Cd, Ba, and Pb were analyzed in low resolution, and Cr, Co, and Ni were analyzed in medium resolution in a combined method at 1250 watts forward power, 15 L/min plasma gas flow, "Both" counting, analog mode, and signal averaging over 3 runs of 10 passes of the scan. Other gas flows and lens settings were optimized using 1 µg/L Mo in diluted digestion acid solution for maximum Mo signal while maintaining less than 1% $^{98}Mo^{16}O$ formation. Mass calibration was maintained with "auto-lock mass," and mass offsets determined for each isotope with the exception of ^{9}Be. All low resolution elements and internal standards except Be were assigned 100% mass windows, 50% search windows, 80% integration windows, 10 ms per sample, and 25 samples per peak. Be was assigned 80% mass, 0% search, 20% integration windows, 100 samples per peak, 10 ms per sample, and a manually set mass offset of +0.0500 mass units to avoid counting the partially resolved H_2O^{2+} peak shoulders in blanks. Medium resolution elements and internal standard had 120% mass, 120% search, and 80% integration windows, 10 ms per sample, and 40 samples per peak except for Co. Co was assigned a 40% search window to avoid locking on the neighboring $^{40}Ar^{19}F^{+}$ peak in blanks. Arsenic (As) was analyzed in high resolution in a separate method at 1450 watts forward power, with parameters the same as the low and medium resolution method except for optimization with 10 µg/L As, 150% mass, 150% search, and 80% integration windows and 40 samples per peak for the Ir internal standard. In addition, 80% mass, 25% search, and 10% integration windows, and 100 samples per peak were used for As to avoid locking onto the $^{40}Ar^{35}Cl^{+}$ peak in blanks.

Limits of detection (LOD) and lowest reportable limits (LRL)

The LODs for smokeless tobacco digest analyses were based on 3 times the standard deviation for analyses of digestion blanks analyzed as samples over 20 runs. For each analysis, this quantity was divided by the mass of tobacco that was used in order to determine the limits of detection per gram of tobacco. The most conservative value (i.e., the highest calculated from all analyses) was reported. The LRLs for smokeless tobacco extracts were based on 3 times the standard deviation of the 10 separate analyses of artificial saliva digestion blanks. The lowest reportable limit per gram of tobacco was calculated as described for tobacco digests. Because the background from artificial saliva for some elements such as chromium and arsenic was above the solvent blank background before subtracting the digest blank, LRL was used for extracts.

Table I. Limit of Detection, Lowest Reportable Limit, and Recovery Determinations for Smokeless Tobacco and Artificial Saliva Smokeless Tobacco Extracts

	Be	Cr	Co	Ni	As	Cd	Ba	Pb
LOD* (µg/g)	0.003	0.170	0.031	0.131	0.046	0.315	1.456	0.190
LRL† (µg/g)	0.001	1.019	0.106	0.117	3.327	0.049	0.758	0.13
Recovery (%)	95 ± 10	ND‡	82 ± 1	87 ± 2	ND	74 ± 2	92 ± 1	91 ± 2

* Limit of detection for smokeless tobacco.
† Lowest reportable limit for artificial saliva extracts of smokeless tobacco.
‡ Not determined because of high extraction background.

Table II. Comparison of Experimental Results with Assigned Target Values for Reference Tobacco Samples

	CTA-OTL-1		CTA-VTL-2		
	Our results (µg/g)	Target (µg/g)	Our results (µg/g)	Target (µg/g)	1S3 (µg/g)
Be	0.109 ± 0.004	NA	0.093 ± 0.003	NA	0.039 ± 0.001
Cr	2.70 ± 0.17	2.59 ± 0.32	2.02 ± 0.37	1.87 ± 0.16	3.75 ± 0.21
Co	0.950 ± 0.024*	0.879 ± 0.039	0.409 ± 0.011	0.429 ± 0.026	1.39 ± 0.07
Ni	6.26 ± 0.23	6.32 ± 0.65	2.07 ± 0.14	1.98 ± 0.21	2.73 ± 0.20
As	0.546 ± 0.039	0.539 ± 0.060	0.932 ± 0.058	0.969 ± 0.072	0.317 ± 0.041
Cd	1.22 ± 0.10	1.12 ± 0.12	1.59 ± 0.08	1.52 ± 0.17	1.51 ± 0.11
Ba	94.6 ± 2.3	84.2 ± 11.5	47.8 ± 1.5	42.7 ± 6.6	106 ± 6
Pb	4.26 ± 0.22	4.91 ± 0.80	22.1 ± 1.4	22.1 ± 1.2	1.91 ± 0.10

* All mean values are within one standard deviation of characterized mean values except for the CTA-OTL-1 cobalt, which is within two standard deviations.

Statistical methods

Two-tailed Student *t*-tests were performed with Excel® software (Microsoft, Redmond, WA). Differences were considered statistically significant when $p < 0.05$.

Results

Method performance

The LOD and LRL for all elements are reported in Table I. Concentrations are reported as less than the respective LOD or LRL when one or more of the triplicate analysis results fell below the LOD or LRL. The sensitivity of the instrumentation chosen for the method described was sufficient for all toxic metals examined in tobacco in this study, with the exception of one *iqmik* tobacco arsenic value. Note, however, that because of elevated chromium and arsenic background from the

artificial saliva extraction medium, the LRLs for chromium and arsenic extraction were significantly higher than the tobacco LODs. This prevented the acquisition of reportable extraction data for these two metals. Molybdenum values are not reported here because Mo is not considered an element of significant toxic concern. Still, in all analyses it was monitored to assure negligible interference with cadmium analyses based on optimization conditions to maintain $^{98}Mo^{16}O$ at less than 1% of the ^{98}Mo signal. Beryllium recovery from tobacco digests, calculated as described in section 2.4, was 99.0 ± 5.4% over 20 runs, as determined from the difference in spiked versus unspiked concentrations in 1S3 digests.

Tobacco analysis method accuracy was assessed using a comparison of CDC results with available characterized values for reference materials CTA-OTL-1 and CTA-VTL-2. Results for these and reference tobacco 1S3 are reported in Table II. Results for the two reference materials were within one standard deviation of characterized values, with the exception of the CTA-OTL-1 cobalt mean results. The CTA-OTL-1 cobalt results were within two standard deviations of the characterized value. Results are also shown for the reference materials that have only an information value rather than a characterized value.

Table I shows the percent recovery of trace metals from certified reference materials spiked onto 1S3 smokeless tobacco before digestion and extracted with artificial saliva. The extraction efficiency after centrifugation and digestion of a supernatant aliquot was the lowest for cadmium. All of the other element recoveries were > 82% with exception of Cr and As, which, because of the high background for these elements in the artificial saliva, could not be determined.

Analysis of trace metals in smokeless tobacco

The results of analysis of trace metals in popular moist snuff products are reported in Table III. The Hawken® smokeless tobacco sample was significantly lower in Be ($p \leq 0.01$), Cd ($p \leq 0.03$), Ba ($p \leq 0.008$), Cr ($p \leq 0.02$), and Co ($p \leq 0.005$) than other commercially available brands when compared on individual bases. With the exception of As in Cougar® Regular, Hawken was lower in every metal analyzed than the other commercially available brands, though the differences were not statistically significant in every case. Excluding the Hawken® (Conwood Sales) sample, the levels of trace metals for the remainder of the commercially available brands varied least for nickel, with a range from 19% below to 22% above the mean, and most for lead, with a range from 27% below the mean to 89% above the mean. The two other products of Conwood Sales (Kodiak® and Cougar®) were within the concentration ranges for all metals reported.

Metals concentrations in 17 *iqmik* tobacco samples were beryllium: 0.031 ± 0.015 µg/g, chromium: 1.37 ± 0.48 µg/g, cobalt: 0.98 ± 0.64 µg/g, nickel: 2.32 ± 1.63 µg/g, arsenic: 0.19 ± 0.06 µg/g, cadmium: 1.41 ± 0.56 µg/g, barium: 114.4 ± 40.1 µg/g, and lead: 0.55 ± 0.19 µg/g. Although the metals concentrations varied more widely from sample to sample than for popular commercial brands of moist snuff, the mean chromium concentrations were the only ones that differed significantly between the *iqmik* and snuff tobacco samples ($p = 0.001$). A few individual samples of *iqmik* tobacco showed metals levels higher than the ranges found for moist snuff. These include one sample that had almost twice the concentration of both beryllium and cobalt, and two samples that had more than twice the concentration of nickel found in the highest concentration snuff samples.

Results of tobacco artificial saliva extraction analyses

The results of extraction of metals from smokeless tobacco into artificial saliva are reported in Table IV without correcting for extraction efficiency. For all commercially available brands, the amounts of beryllium that could be extracted were below the LRL, with the exception of a very low but measurable concentration in Skoal® Long. The amount of beryllium that could be extracted from *iqmik* tobacco samples with artificial

Table III. Smokeless Tobacco Metal Concentrations ± Standard Deviation

	Be (µg/g)	Cr (µg/g)	Co (µg/g)	Ni (µg/g)	As (µg/g)	Cd (µg/g)	Ba (µg/g)	Pb (µg/g)
Hawken WG	0.010 ± 0.001	0.86 ± 0.16	0.26 ± 0.02	1.39 ± 0.11	0.14 ± 0.03	0.66 ± 0.14	37.9 ± 4.3	0.28 ± 0.03
Kodiak Wintergreen	0.030 ± 0.002	1.71 ± 0.07	1.22 ± 0.05	2.70 ± 0.95	0.21 ± 0.04	1.87 ± 0.03	97.4 ± 1.7	0.42 ± 0.08
Cougar Regular	0.025 ± 0.001	1.32 ± 0.12	1.10 ± 0.04	1.85 ± 0.10	0.13 ± 0.01	1.53 ± 0.02	158.1 ± 6.3	0.48 ± 0.03
Copenhagen Regular	0.033 ± 0.004	2.16 ± 0.18	1.08 ± 0.08	2.48 ± 0.21	0.36 ± 0.06	1.33 ± 0.21	110.6 ± 6.0	0.52 ± 0.07
Copenhagen Long Regular	0.025 ± 0.001	1.90 ± 0.18	0.90 ± 0.02	2.49 ± 0.05	0.20 ± 0.01	1.42 ± 0.05	116.2 ± 3.9	0.35 ± 0.06
Skoal Fine WG	0.029 ± 0.001	2.58 ± 0.26	1.00 ± 0.03	2.45 ± 0.10	0.27 ± 0.06	1.25 ± 0.03	108.4 ± 2.1	0.42 ± 0.09
Skoal Straight Regular	0.025 ± 0.000	2.20 ± 0.11	0.95 ± 0.04	2.49 ± 0.13	0.21 ± 0.01	1.44 ± 0.02	108.8 ± 2.6	0.36 ± 0.09
Skoal Long Classic	0.025 ± 0.001	2.04 ± 0.12	0.98 ± 0.02	2.29 ± 0.03	0.25 ± 0.01	1.33 ± 0.08	108.7 ± 6.7	0.40 ± 0.09
Red Seal Fine Regular Nat	0.031 ± 0.002	2.79 ± 0.03	1.02 ± 0.04	2.73 ± 0.06	0.27 ± 0.03	1.40 ± 0.11	109.6 ± 3.4	0.54 ± 0.08
Red Seal Long WG	0.026 ± 0.001	2.16 ± 0.11	0.95 ± 0.02	2.37 ± 0.05	0.23 ± 0.01	1.39 ± 0.13	103.9 ± 4.5	0.46 ± 0.09
Rooster Long WG	0.027 ± 0.002	1.81 ± 0.14	0.88 ± 0.07	2.32 ± 0.11	0.29 ± 0.02	1.22 ± 0.15	93.8 ± 11.4	0.48 ± 0.07
Rooster Long Icy Mint	0.031 ± 0.001	2.13 ± 0.19	1.00 ± 0.03	2.58 ± 0.05	0.26 ± 0.02	1.38 ± 0.03	112.9 ± 6.0	0.49 ± 0.05
Timberwolf Fine Regular	0.018 ± 0.001	1.75 ± 0.09	0.62 ± 0.06	1.87 ± 0.10	0.20 ± 0.02	1.76 ± 0.12	73.5 ± 5.4	0.33 ± 0.08
Timberwolf Straight Regular	0.020 ± 0.002	1.86 ± 0.02	0.68 ± 0.02	1.88 ± 0.03	0.20 ± 0.02	1.73 ± 0.11	84.5 ± 2.2	0.45 ± 0.08
Timberwolf Long WG	0.021 ± 0.001	1.95 ± 0.11	0.68 ± 0.03	2.01 ± 0.12	0.21 ± 0.04	1.88 ± 0.02	88.1 ± 0.5	0.39 ± 0.14
Silver Creek Long WG	0.028 ± 0.002	2.15 ± 0.15	0.79 ± 0.02	2.34 ± 0.05	0.20 ± 0.02	1.04 ± 0.10	89.8 ± 8.4	0.51 ± 0.02
Redwood Regular	0.038 ± 0.003	3.20 ± 0.40	1.03 ± 0.01	2.55 ± 0.11	0.26 ± 0.03	1.22 ± 0.06	107.0 ± 6.6	0.85 ± 0.01

saliva was from 21 to 36%. The 1S3 reference tobacco was the only sample for which the amount of lead that could be extracted with artificial saliva was above the LRL: approximately 8% of the total tobacco lead concentration. Cobalt (30 to 65%), nickel (20 to 46%), and cadmium (21 to 47%) could be extracted from both commercial moist snuff and *iqmik* tobacco with artificial saliva. The amount of barium that could be extracted from tobacco samples with artificial saliva ranged from only 2 to 21%. Arsenic and chromium levels were indeterminable.

Addition of willow or punk ash to *iqmik* tobacco samples did not appreciably change the levels of lead and that could be extracted from *iqmik* tobacco with artificial saliva. Addition of punk ash decreased the apparent amount of beryllium, cadmium, and barium that could be extracted from *iqmik* tobacco with artificial saliva; whereas the amount of beryllium extracted increased slightly, and the amount of cadmium and barium increased significantly with the addition of willow ash. While it is possible that addition of willow ash increased the extractable concentration of beryllium, cadmium, or barium to some degree, the increase in soluble concentrations of some metals to greater than 100% of total tobacco content after addition of willow ash (112% for cadmium, 250% for barium) indicates that these were contributed predominantly from the ash itself. Cobalt showed a small increase in the soluble fraction from *iqmik* tobacco with punk ash, but 340% of total tobacco content with willow ash, which also supports the ash itself as the addition source. Similarly, the amount of nickel in the soluble fraction of artificial saliva was 1370% of the total nickel content in the *iqmik* tobacco when willow ash was added, compared to 282% and 286% of the total tobacco nickel content when punk ash was added. This also supports the ash as the major source of these elevated metal concentrations.

Results of ash analyses

The complete digestions of the coal fly ash SRMs and of willow ash required additional hydrogen peroxide, and a decrease in hydrofluoric acid, still within EPA 3052 limits, to maintain solubility of all mineral material. Cobalt was indeterminable in ash by this method. Though resolution of 4000 is hypothetically sufficient, the massive amounts of aluminosilicate and calcium available to form overwhelmingly large $^{27}Al^{16}O_2$, $^{29}Si^{14}N\ ^{16}O$, and possibly some contribution from $^{43}Ca^{16}O$, $^{40}Ca^{19}F$, and $^{40}Ar^{19}F$ combined interferences resulted in peak tail overlap into the ^{59}Co mass window.

NIST does not certify any of the values for SRM 2690 or 2691, but the values obtained compared with NIST values given for information purposes are presented in Table V. With the exception of slightly low results for beryllium compared to NIST information values obtained with a different method, our results were similar to those obtained by NIST.

Though there were insufficient samples available for analysis of the punk ash, the results of analysis of willow ash presented in Table V show that indeed the ash metal concentrations were far in excess of the additional amounts extracted into the artificial saliva supernatants, when tobacco extractions with ash addition were compared to the results of tobacco extractions without ash.

Discussion

Detectable levels of arsenic, barium, beryllium, nickel, cadmium, cobalt, chromium, and lead were measured in the tobacco of commercial smokeless tobacco samples. The levels of barium were significantly higher than those of the other metals examined. The average arsenic, cadmium, chromium, lead, and nickel concentrations in the 17 commercially available brands reported here were 0.23 ± 0.06 (minimum 0.14) µg/g, 1.40 ± 0.31 (minimum 0.66) µg/g, 2.04 ± 0.53 (minimum 0.86) µg/g, 0.45 ± 0.13 (minimum 0.28) µg/g, and 2.28 ± 0.36 (minimum 1.39) µg/g, respectively. In the 17 *iqmik* tobacco samples that were examined, the average arsenic, cadmium, chromium, lead, and nickel concentrations were 0.19 ± 0.06 µg/g, 1.41 ± 0.56 µg/g, 1.37 ± 0.48 µg/g, 0.55 ± 0.19 µg/g, and 2.32 ± 1.63 µg/g, respectively.

Stephens et al. (22) published concentrations of arsenic, cadmium, chromium, lead, and nickel in eight domestic British cigarette tobacco samples. The arsenic and chromium mean concentrations reported by Stephens were not statistically significantly different between the domestic British cigarette tobacco and U.S. commercial snuff tobaccos reported here. The U.S. commercially available snuff mean cadmium concentrations were, however, higher (1.40 versus 0.6 µg/g, $p < 0.001$). Nickel concentrations were also higher (2.28 versus 1.8 µg/g, $p < 0.05$), but lead concentrations were lower (0.45 versus 0.7 µg/g, $p < 0.01$). The degree to which the comparisons between the levels of metals in tobacco samples can be applied globally is not apparent. Because of differences in soil concentrations and conditions, significant variation in metals contents of smokeless tobacco would be expected. More extensive data on tobacco grown over widely dispersed geographical locations would be required.

Trace metal levels in commercial leafy food products provide additional comparisons. The mean concentrations reported by the U.S. Food and Drug Administration for arsenic, cadmium, lead, and nickel in fresh iceberg lettuce (48 samples) were, respectively, 0 ± 0.002 µg/g, 0.049 ± 0.048 µg/g, 0 ± 0.001 µg/g, and 0.110 ± 0.065 µg/g. Concentrations (48 samples) for arsenic, cadmium, lead, and nickel in boiled spinach were, respectively, 0.003 ± 0.008 µg/g, 0.14 ± 0.0098 µg/g, 0.013 ± 0.013 µg/g, and 0.074 ± 0.036 µg/g (23). The tobacco samples had mean concentrations of these IARC group 1 and 2A carcinogens more than an order of magnitude greater than the mean concentrations in these leafy vegetable samples, with the exception of the comparison of lead in tobacco with lettuce (more than 3 times the mean concentration in lettuce). Beryllium, barium, chromium, and cobalt data were not available from the FDA report.

This comparison of exposures in smokeless tobacco and vegetables, however, has its limitations. In addition to the presence of lower mean carcinogen concentrations in these leafy vegetables than is found in even the lowest tobacco concentrations in the cases of arsenic, cadmium and nickel, vegetables are consumed by brief mastication and swallowing, limiting exposure to the available toxic metals through mouth exposure, rather than maintaining them in a localized position between cheek, tongue, or lip and gum. We cannot rule out the possi-

bility that swallowing and digestion of vegetables or of saliva containing compounds and particulate from tobacco may liberate additional metals and cause health risks other than from oral exposure. Although some exposure to these metals in food and water is unavoidable, the least exposure of people to carcinogens is desirable, especially when some are known to bioaccumulate (24,25). Tobacco plants readily accumulate many toxic metals from soils, depending on soil metal content, pH, and other factors (26,27). Thus, from these comparisons, it appears that a consequence of smokeless tobacco products use is exposure to elevated concentrations of carcinogenic metals that have accumulated in the tobacco, in comparison to exposure to those same metals from consumption of leafy vegetables.

From a few smokeless tobacco samples, Maier et al. (28)

Table IV. Extractable Smokeless Tobacco Metals in Artificial Saliva*

	Be (µg/g)	Co (µg/g)	Ni (µg/g)	Cd (µg/g)	Ba (µg/g)	Pb (µg/g)
1S3	< LRL†	0.559 ± 0.025 (40 ± 2%)	0.706 ± 0.047 (26 ± 2%)	0.432 ± 0.026 (29 ± 2%)	2.22 ± 0.22 (2.1 ± 0.2%)	0.153 ± 0.018 (8.0 ± 0.9%)
Hawken® WG	≤ LRL†	0.171 ± 0.013 (65 ± 5%)	0.554 ± 0.045 (40 ± 3%)	0.309 ± 0.051 (47 ± 8%)	7.88 ± 0.34 (21 ± 1%)	≤ LRL†
Copenhagen®	≤ LRL†	0.407 ± 0.078 (38 ± 7%)	0.781 ± 0.096 (31 ± 4%)	0.310 ± 0.015 (23 ± 1%)	3.080 ± 0.56 (3.0 ± 0.5%)	≤ LRL†
Skoal® Fine WG	≤ LRL†	0.382 ± 0.040 (38 ± 4%)	0.812 ± 0.220 (33 ± 9%)	0.302 ± 0.057 (24 ± 5%)	3.63 ± 1.14 (3.3 ± 1.1%)	≤ LRL†
Skoal® Straight Reg	≤ LRL†	0.390 ± 0.051 (41 ± 5%)	1.153 ± 0.170 (46 ± 7%)	0.310 ± 0.027 (21 ± 2%)	4.377 ± 0.30 (4.0 ± 0.3%)	≤ LRL†
Skoal® Long	0.003 ± 0.002 (11 ± 7%)	0.407 ± 0.019 (40 ± 2%)	1.053 ± 0.090 (43 ± 4%)	0.342 ± 0.046 (25 ± 3%)	4.98 ± 0.29 (4.4 ± 0.3%)	≤ LRL†
IM 10A	0.010 ± 0.001 (30 ± 4%)	0.739 ± 0.035 (34 ± 2%)	0.398 ± 0.051 (23 ± 3%)	0.351 ± 0.021 (26 ± 2%)	11.52 ± 1.05 (12 ± 1%)	≤ LRL†
IM 10A +04D Willow	0.003 ± 0.0003 (36 ± 3%)	2.510 ± 0.025 (340 ± 3%)	5.447 ± 0.070 (1370 ± 18%)	0.414 ± 0.029 (112 ± 8%)	30.80 ± 1.28 (250 ± 10%)	≤ LRL†
IM 10B	0.009 ± 0.002 (32 ± 6%)	0.370 ± 0.008 (30 ± 1%)	0.298 ± 0.005 (20 ± 4%)	0.374 ± 0.023 (23 ± 1%)	11.38 ± 1.72 (9.0 ± 1.4%)	≤ LRL†
IM 10B +05E Punk	0.003 ± 0.0003 (12 ± 1%)	0.495 ± 0.026 (40 ± 2%)	4.328 ± 0.267 (286 ± 18%)	0.216 ± 0.016 (13 ± 1%)	5.40 ± 1.25 (4 ± 1%)	≤ LRL†
IM 30D	0.008 ± 0.002 (21 ± 5%)	0.375 ± 0.018 (40 ± 2%)	0.404 ± 0.038 (26 ± 2%)	0.508 ± 0.020 (30 ± 1%)	19.33 ± 1.83 (17 ± 2%)	≤ LRL†
IM 30D +30E Punk	0.004 ± 0.0003 (11 ± 1%)	0.492 ± 0.030 (53 ± 3%)	4.438 ± 0.294 (282 ± 19%)	0.224 ± 0.011 (13 ± 0.6%)	6.03 ± 1.06 (6 ± 1%)	≤ LRL†

* Concentrations ± standard deviations. Percent extractable shown in parentheses where possible.
† One or more of triplicate values was determined at or below 0.001 µg/g for Be or below 0.130 µg/g for Pb.

Table V. Results for Willow Ash Analysis Compared with Uncertified Information Values for Reference Samples*

	Be (µg/g)	Cr (µg/g)	Ni (µg/g)	Cd (µg/g)	Ba (µg/g)	Pb (µg/g)
NIST SRM 2690						
CDC Result	6.4 ± 0.1	63 ± 2	47 ± 2	1.0 ± 0.05	5813 ± 251	46 ± 4
NIST Information	8	67	46	0.7	5800	39
NIST SRM 2691						
CDC Result	4.3 ± 0.3	67 ± 2	54 ± 0.3	1.2 ± 0.03	6115 ± 54	31 ± 3
NIST Information	8	68	53	0.9	5900	29
Willow Ash	0.5 ± 0.1	11.3 ± 2.3	44 ± 3	20 ± 2	4330 ± 100	3.4 ± 0.4

* The total metal concentrations (± standard deviations) available in willow ash are greater than sufficient to be responsible for the increases in extractable metals for tobacco alone; data presented in Table IV.

used various chelating agents to determine the extractable availability of nickel, copper, zinc, and cadmium. The metals are expected to be among those with the highest concentrations in tobacco. Indeed, nickel and cadmium are among the metals with the highest concentrations determined in our tobacco samples, although cobalt (group 2B possible carcinogen) and chromium, which Maier et al. (28) did not examine, were at similar levels. The chromium species were not determined in this study, but in smokeless tobacco would be presumed to be in predominantly the +3 oxidation state (29).

For the 1S3 reference tobacco, Maier et al. (28) reported from 13.5% of total cadmium extracted with 0.1 M potassium phosphate buffer pH 6.5 to 91.0% of the total with 0.001 M EDTA. With the artificial saliva used in this study 29% of total cadmium was found to be extractable from 1S3 (Table IV). Maier et al. also reported from 2.5% of total nickel extracted from 1S3 tobacco with 0.1 M potassium phosphate buffer pH 6.5 to 64.4% of the total with 0.001 M EDTA. 26% of total nickel was found to be extractable from 1S3 with the artificial saliva in this study (Table IV). For the metals which were analyzed in both studies, the degree of extraction into soluble fractions with artificial saliva was between those of the phosphate buffer and the EDTA chelating agent used by Maier et al. (28).

For those metals not examined by Maier et al. (28) there were differences in extractability with artificial saliva. A measurable extractable lead level was observed only for the 1S3 reference smokeless tobacco. Beryllium was measurably extractable at low levels only from *iqmik* tobacco samples (Table IV). Because beryllium concentrations in the *iqmik* tobacco samples were comparable to those in the neat, commercially available tobacco samples, perhaps differences in the preparation, curing, additives, or storage of the *iqmik* tobacco made the difference in availability for extraction. In support of this explanation, barium extractability percentages were higher for all *iqmik* tobacco samples than for any of the commercially available tobacco samples except for the Hawken® sample (Tables III and IV). *Iqmik* tobacco barium concentrations in the extraction supernatant decreased with addition of the two punk ash samples, possibly due to coprecipitation with calcium phosphate or carbonate. They increased dramatically, however, with addition of the willow ash sample. This suggests that the willow ash contributed additional barium.

Except for arsenic, the remainder of group 1 and 2B carcinogens were measurably extractable from smokeless tobacco in artificial saliva. Cobalt and cadmium appeared to be more highly extractable from Hawken, although before extraction the tobacco levels were also lower than in other tobaccos. Nickel was extractable at higher percentages from commercially available tobaccos than from *iqmik* tobaccos, as well as from the 1S3 reference tobacco.

In order to interpret the product design features and contents that might influence the levels of chemical components delivered to tobacco users, scientists need to develop research methods for mimicking human smokeless tobacco use. Human saliva is difficult to obtain, preserve, and is variable in composition depending on hydration and time (e.g., before, during, or after eating) (30). A number of artificial saliva formulae have been used for various studies. Mandel (30) pointed out that most natural salivas are supersaturated with calcium phosphate. Leung and Darvell (31) summarized most artificial saliva formulations as supersaturated with calcium phosphate but protein-free, whereas by chelation, the proteins and mucin aid in calcium solution. Precipitation is observed even from natural human saliva collected and maintained at room temperature for a few hours. Chou and Hee (21) published a formula including some proteins, mucin, phosphate, and calcium, more closely resembling human saliva than most formulations. Along with Nasr et al. (32), they used this formula for studies of nicotine recovery. Protein-free, calcium-free, and phosphate-free preparations would be more advantageous for metals analysis extractions because of their lower metal-contaminant backgrounds and calcium solubility. Though the protein-free, calcium-free, and phosphate-free preparations would be more advantageous for extractions for metals analyses, a minor modification of the Chou and Hee (21) formulation was chosen for this metals extraction study because of its greater similarity with human saliva and the obvious impact that human saliva characteristics including high calcium, phosphate, protein, and mucin levels would have on extraction of other metals. This choice enabled greater biological relevance in estimation of the availability of toxic and carcinogenic metals in tobacco for oral exposure. This choice also resulted, however, in increased background levels of chromium and arsenic, thus limited the number of metals for which accurate extraction analyses were possible. Chromium and arsenic could not be quantified in the chosen saliva formula because of reagent contaminant background and possibly coprecipitation with phosphates. Although our method is not proven to replicate human use, it is one approach to investigating extraction of chemical components of concern from smokeless tobacco products. Other possible variables that should be addressed include the presence of enzyme in the saliva and physical breakdown of the product from chewing or from contact with the inside of the mouth.

Throughout the world, tobacco is treated with a variety of materials before use. Many of these treatments are intended to alter chemical properties and shift the fraction of nicotine into a more biologically available form. Punk ash is one such treatment and has had varying effects on the delivery of metals in artificial saliva. When punk ash was added to the *iqmik* tobaccos, extractable cadmium decreased. On the other hand, addition of willow ash increased the extractable cadmium concentration, as was observed for barium—again probably due to contribution from the ash. A slight increase in available cobalt was observed with punk ash addition, compared with more than an order of magnitude increase with willow ash. Available nickel increased more than an order of magnitude with the addition of punk ash, and roughly 2 orders of magnitude with willow ash addition. The increased available levels of carcinogenic metals cobalt and nickel when punk ash is added, and the increased cadmium levels when willow ash was added, are certainly undesirable from a health risk standpoint. Moreover, such health considerations are even more urgent given the early ages at which many Alaska Natives initiate *iqmik* use, and given the fact that many native pregnant females continue its addictive use through pregnancy (33). Note, how-

ever, metals were only analyzed in the centrifuge soluble supernatant fraction. Consequently, bioavailability of metals that precipitated as insoluble salts in the centrifuge, but which would remain in contact with tissues in the oral cavity, or might be absorbed to some degree if swallowed cannot be ruled out. It is possible that burning the wood or fungus in metal containers would also add to the metal content of the ashes. These possibilities suggest need for further studies.

Fineness of cut was evaluated as a design feature that could influence the extraction of metals from smokeless tobacco. Fineness of cut and its effect on surface area available for metals extraction had little influence on extraction efficiencies. No appreciable differences appeared between extraction efficiencies for Co, Ni, Cd, or Ba among Skoal Fine, Straight Regular, and Long. Though no apparent trend emerged from the results, the finest cut had the poorest extraction efficiency for three of the four elements. Thus if surface area were to be a significant contributor to metals extractability, it may be that the differences do not become apparent until the tobacco is ground more finely than as currently marketed. Other possible design features still to be investigated are the use of smokeless tobacco pouches and the effect of additives such as ammonia.

The metals included in this investigation are toxic, carcinogenic, or both. The most immediate and obvious health effects resulting from use of smokeless tobacco are oral irritations and inflammations such as mucosal lesions (34,35), leukoedema, and buccal mucosal "snus changes" (36, Snus is a Swedish term for moist snuff). Leukoedema is a common symptom of "irritant and allergen-induced surface changes" in the oral cavity. These and other tobacco-related lesions are included under the general description of irritant contact stomatitis, which results from "chemical and mechanical" oral cavity irritation. This description of irritations includes such named pathologies as nicotine melanosis, nicotine stomatitis, and snuff dipper's patch (37). Grady et al. (34) discussed an overall strong association between leukoplakia and smokeless tobacco use by 1109 professional baseball players in a study controlled for age, race, smoking, alcohol use, and dental hygiene (odds ratio = 60.0, 95% confidence interval = 40.5–88.8). The authors showed convincing relationships between the number and severity of lesions and the type of smokeless tobacco use, the amount of time used per day, the age at which use commenced, and the recency of use.

Furthermore, certain of the metals under study have adverse health effects in addition to their chemical carcinogenic properties. Beryllium, chromium, cobalt, and nickel are known to cause irritations resulting in allergic contact dermatitis inflammations. Linneberg et al. (38) report that sensitization to nickel, as demonstrated by a positive patch test and symptoms such as nickel contact allergy and allergic nickel contact dermatitis, were significantly associated with the frequency of smoking and the length of smoking history. Nickel is a known cause of oral allergic contact sensitization (39), as are chromate and cobalt (40). Allergic metal sensitization resulting from exposure to metals extracted from tobacco or tobacco smoke by saliva might not contribute to observed oral inflammatory lesions, but this is a topic that requires investigation with regard to nonsmokers and smokers who also use smokeless tobacco. Chronic irritation-induced inflammation has long been associated with risk of neoplastic consequences (41,42,43); and Feron et al. (44) and Mueller (45) have specifically described the risk of carcinogenesis resulting from chronic inflammation of various epithelial and mucosal tissues.

Barium is known to accumulate biologically in bones and teeth (46) in addition to its potassium channel antagonist toxicity. The significance of local barium exposure during smokeless tobacco use to oral cavity pathologies is, however, unclear.

Currently, complete cessation of tobacco products use is the only proven way to reduce tobacco-use health consequences. Additionally, changes in tobacco products that increase exposure of the user to toxic chemicals, including metals, are detrimental to efforts to reduce the morbidity and mortality from tobacco use. Yet a review of reports on the health consequences of smokeless tobacco use compared to consequences of smoking shows that the issue is still controversial. For example, Critchley and Unal (47) saw a strong association between chewing betel quid and tobacco and oral cancer risk in India, whereas in Europe and the Americas, Rodu and Jansson (48) identified a low oral cancer risk from smokeless tobacco products. The IARC accumulated sufficient evidence to classify smokeless tobacco as a group 1 carcinogen (49), and Accort et al. (50) saw significant evidence of increased risk for oral cancers with smokeless tobacco use and epidemiological evidence for other chronic diseases. That said, however, several from the latter group also cautioned that evidence for other cancers lacked credibility or was sometimes conflicting (51). These and other authors criticized several of the studies for lacking some of the requisites of a well-controlled study. Thus, without doubt more research should focus on the possible toxic, carcinogenic, and addictive components of smokeless tobacco and its emissions.

Conclusions

A methodology is presented for measuring heavy metals in various types of smokeless tobacco and for assessing the extent to which artificial saliva can extract heavy metals from smokeless tobacco products. Though smokeless tobacco is described as a group 1 carcinogen by the International Agency for Research on Cancer, little is known regarding bioavailabilty, absorption, and toxicological effects of toxic and carcinogenic inorganic substances from smokeless tobacco. It is hoped that publication of our methods and results will encourage further, similar efforts.

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