Exposure to Nicotine and Carcinogens among South Western Alaskan Native Cigarette Smokers and Smokeless Tobacco Users

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*The findings and conclusions of this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention. Use of trade names and commercial sources is for identification only and does not constitute endorsement by the U.S. Department of Health and Human Services or the Centers for Disease Control and Prevention
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Abstract

Background

The prevalence of tobacco use, both cigarette smoking and smokeless, including iqmik (homemade smokeless tobacco prepared with dried tobacco leaves mixed with alkaline ash), and of tobacco-related cancer is high in Alaska Native people (AN). To investigate possible mechanisms of increased cancer risk we studied levels of nicotine and tobacco-specific nitrosamines (TSNA) in tobacco products and biomarkers of tobacco toxicant exposure in South Western AN people.

Methods

Participants included 163 cigarette smokers (CS), 76 commercial smokeless tobacco (ST), 20 iqmik, 31 dual CS and ST (DT) and 110 non-tobacco (NT) users. Tobacco use history, samples of tobacco products used and blood and urine samples were collected.

Results

Nicotine concentrations were highest in cigarette tobacco and TSNAs highest in commercial ST products. AN participants smoked on average 7.8 cigarettes per day (CPD). Nicotine exposure, assessed by several biomarker measures, was highest in iqmik users, and similar in ST and CS. TSNA exposure was highest in ST users, and polycyclic aromatic hydrocarbon exposure highest in CS.
Conclusions

Despite smoking fewer CPD, AN CS had similar daily intake of nicotine compared to the general US population. Nicotine exposure was greatest from iqmik, likely related to its high pH due to preparation with ash suggesting high addiction potential compared to other ST products. TSNA exposure was much higher with ST compared to other product use, possibly contributing to the high rates of oral cancer.

Impact

Our data contribute to an understanding of the high addiction risk of iqmik use and of the cancer-causing potential of various forms of tobacco use among AN people.
Introduction

The prevalence of tobacco use among Alaskan Native (AN) people is much higher than that of Alaskan non-Native people and the U.S. general population. More than 40% of AN people smoke cigarettes and 11% use smokeless tobacco, including both commercial smokeless tobacco and iqnik [1]. Iqnik is a home-made preparation of tobacco leaves or commercial twist of leaf chewing tobacco combined with ash from a fungus (punk ash) or ash from willow bushes or drift wood [2]. The tobacco leaves and ash are mixed and either pre-chewed in the mouth (premasticated) or placed in a bowl with water and stirred with a knife (non-premasticated). After mixing, iqnik is stored in a can for future use. While AN smokers consume fewer cigarettes per day compared to the U.S. population of smokers [3, 4], the incidence of tobacco-related cancers, including lung cancer, oral, gastric and esophageal cancer are higher among AN people. [5]. To better understand possible mechanisms of higher cancer rates, we studied the levels of nicotine and carcinogenic tobacco specific nitrosamines (TSNAs) in the cigarettes and smokeless tobacco products used by AN people and measured biomarkers of tobacco toxicant exposure among AN tobacco users.

Methods

Participants
The details of the study procedures and smoking behaviors of the participants are described in another paper.[6] The participants were 400 residents of the Bristol Bay region of southwest Alaska. Participants had to be an Alaskan Native person, 18 years of age or older, not pregnant, not taking medications that would interfere with nicotine metabolism, and not using marijuana in the last 7 days (to avoid the potential confounding effects of marijuana use as a source of exposure to combustion products). Participants were required to not have used other illicit drugs in the last 30 days, not to be enrolled in a tobacco cessation program or to be taking nicotine medications, and not to have consumed alcohol on the day of enrollment. Tobacco users must have used tobacco in the 24 hours prior to enrollment. For comparison to tobacco users, we also included non-tobacco users who reported not using any tobacco for the past 12 months. AN ethnicity was defined by self-report, with information collected on the ethnic group of the grandparents. 385 of the participants had 3 or 4 grandparents who were AN people; the remaining 15 had 2 grandparents who were AN. 89% of the subjects were Yupik, 5% Aleut, 3% Athabascan, 1% Inupiaq and 2% others.

Procedures

Participants who volunteered for the study signed a consent form and responded to a detailed questionnaire on health status, history of tobacco use, as well as a dietary history and history of exposure to secondhand smoke.[6] Samples of cigarettes and smokeless tobacco products, including iqmik, were requested from participants for chemical analysis of nicotine and tobacco-specific nitrosamines. Blood was collected from
participants for measurement for plasma nicotine, cotinine and trans-3’hydroxycotinine (3HC). A urine sample was collected for measurement of nicotine and 8 of its metabolites, and urinary biomarkers of carcinogen exposure: total 4-(methylamino)-4-(3-pyridyl)-1-butanol (NNAL), a major metabolite of the TSNA and lung carcinogen 4-(methylamino)-1-(3-pyridyl)-1-butanone (NNK); total N’-nitrosonornicotine (NNN), another carcinogenic TSNA; and polycyclic aromatic hydrocarbons (PAH) metabolites [7]. The study was approved by the ethics committee and full board at the Bristol Bay Area Health Corporation and the institutional review boards of the Alaska Area Institutional Review Board and the University of California San Francisco.

**Analytical chemistry**

Tobacco samples were analyzed in the Tobacco Smoke Analysis laboratory at the Centers for Disease Control and Prevention (CDC). Concentrations of nicotine and tobacco-specific nitrosamines were measured in each tobacco product, in triplicate if a sufficient sample was available. The target analytes included nicotine, N’-nitrosoanabasine (NAB), N’-nitrosoanatabine (NAT), NNN, NNK, and NNAL. A measured amount of the finely ground tobacco product was spiked with a solution containing isotopically-labelled TSNA's and nicotine as internal standards, and then solvent extracted using 20-mM aqueous ammonium acetate (AA) solution. The extracts were filtered and diluted ten-fold for high-performance tandem mass spectrometric (HPLC/MS/MS) analysis for TSNA analysis and further diluted one thousand-fold for nicotine analysis. The
HPLC/MS/MS analytical run time for each sample was 8 min at a flow rate of 1 ml/min using a Waters Xterra C18 MS column (50mm x 4.6mm i.d. x 5um) at 60°C. Ionization of all analytes was achieved by using electrospray ionization in positive ion mode. Mass spectral data on precursor and product ions were collected in multiple reaction monitoring (MRM) mode on an Applied Biosystems API 4000 mass spectrometer. All chromatographic data were processed using Analyst® 1.4 software from Applied Biosystems.

Plasma cotinine and trans-3’ hydroxycotinine (3HC), and the urine total NNAL and PAH metabolites were measured by the Clinical Pharmacology Laboratory at San Francisco General Hospital using published liquid chromatography – tandem mass spectrometry assays. [8-10] Urine nicotine and 5 nicotine metabolites (nicotine glucuronide, cotinine, cotinine glucuronide, 3HC, 3HC glucuronide) were measured by the Tobacco Exposure Biomarkers Laboratory at CDC using a liquid chromatography – tandem mass spectrometry method that has been previously described [11]. Urine total NNN was measured at the University of Minnesota using a published liquid chromatography – electrospray ionization – tandem mass spectrometry assay[12].

Data analysis

Summary statistics were computed for the nicotine and nitrosamine content of the various types of tobacco products and included the mean and 95% confidence interval. Comparisons were carried out across products using the Kruskal-Wallis test. The
Wilcoxon rank sum test compared the products pair-wise using a p value of <0.01 for significance to control for multiple comparisons.

Exposure of AN people to nicotine was examined using 3 biomarkers; plasma cotinine, the sum of plasma cotinine plus 3HC and the molar sum of nicotine and 5 metabolites in urine, normalized for creatinine. Cotinine is the proximate metabolite of nicotine. Plasma cotinine is widely used as a marker for nicotine exposure in populations; however the relationship between plasma cotinine and nicotine intake is influenced by individual differences in the rate of metabolism of nicotine to cotinine and the rate of cotinine clearance.[13] The sum of 3HC plus cotinine appears to be superior to cotinine alone as a biomarker of nicotine exposure based on a recent empirical study [14]. The best biomarker of nicotine exposure is the sum of nicotine and its metabolites in urine, the molar sum of which is termed urine nicotine equivalents [14].

Biomarker levels were computed as arithmetic means (nicotine and metabolites) or geometric means (NNAL, NNN and PAH metabolites) with 95% confidence intervals. Values were compared between groups using non-parametric tests (Wilcoxon rank sum test and the Kruskal-Wallis test). The statistical analysis first compared non-users to each of the four tobacco user groups, and then the four tobacco user groups were compared with one another. For both sets of analyses a lower p-value cutoff of p<0.01 was used to account for multiple pair-wise comparisons. Spearman correlations among biomarkers within tobacco use groups were also determined.
Results

Chemical Constituents of Tobacco Products

Constituents of tobacco products were measured in 26 cigarettes, 22 commercial spit tobacco samples and 23 iqmil samples. Table 1 presents nicotine and nitrosamine content of tobacco in the various types of products. The nicotine concentration was highest in cigarette tobacco. Concentrations of NNK, NNN and other tobacco specific nitrosamines in commercial smokeless tobacco products were substantially higher than in cigarettes. Levels of NNK and other tobacco specific nitrosamines in iqmil were considerably lower than that of cigarettes or commercial smokeless tobacco.

Subjects and Tobacco Use Behavior

The participants included 163 smokers (71 men and 92 women, average age 36.0 years, SD 14.4), 76 smokeless tobacco users, (35 men, 41 women, average 39.2 years, SD 12.8), 20 iqmil users (6 men, 14 women, average 42.9 years, SD 17.0), 31 dual cigarette smokers and smokeless tobacco users (16 men, 15 women, average 28.8 years, SD 10.4), and 110 non-tobacco users (52 men, 58 women, average age 38.9, SD 14.7). Three self-reported non-tobacco users were excluded from the analysis because their plasma...
cotinine concentrations were above 14 ng/ml, a level indicating active tobacco use [15].
Additional demographic data on subjects are reported elsewhere [6].

On average the smokers smoked 7.8 cigarettes per day, while dual users smoked 5.7
 cigarettes per day (Table 2). The Fagerstrom test of nicotine dependence score averaged
2.6, SD 2.1; 1.9, SD 1.9 for smokers and dual users, respectively[16]. The smokeless
tobacco users used on average 1.6 tins per week; iqmiq users used 1.2 tins per week; and
dual users used on average either 1.3 tins of smokeless tobacco or 0.5 tins of iqmiq per
week. The Severson smokeless tobacco dependence score averaged 4.0, SD 3.3; 5.5, SD
3.3; and 3.0, SD 4.1 for smokeless, iqmiq and dual users, respectively[17]. Nonsmokers
reported that 97.3% had a smoking ban in their homes and 96.4% had no secondhand
smoke exposure at home or in steam baths.

*Exposure to Nicotine, NNAL, NNN and PAH Metabolites*

Nicotine exposure data in our subjects are provided in Table 2 and Figure 1. Nicotine
intake was estimated using three different measures: plasma cotinine, the molar sum of
plasma cotinine and 3HC, and urine nicotine equivalents. All nicotine intake measures
were twice as high in iqmiq users compared to cigarette smokers. Nicotine intake was
also greater on average in commercial spit tobacco users compared to smokers, and
greater in iqmiq users compared to spit tobacco users. The average plasma cotinine
concentration in tobacco non-users was 0.3 ng/ml.
Concentrations of total NNAL and total NNN in urine are shown in Table 2 and Figure 2. The highest levels of NNAL were seen in commercial smokeless tobacco users followed by dual users and then cigarette smokers. NNAL levels in iqmik users were on average much lower than levels in commercial smokeless tobacco users but not significantly different from those in cigarette smokers. Urine NNN levels were not significantly different comparing the various tobacco user groups, but were significantly lower in non-users.

Urine PAH metabolite concentrations are shown in Table 2. Levels for two metabolites of PAHs, 2- naphthol and 1-hydroxypyrene, are shown in Figure 3. Urine 2-napthol and fluorene metabolite concentrations were highest on average in cigarette smokers. Average urine hydroxyphenanthrenes and 1-hydroxypyrene levels were similar across all groups, except levels of 1-hydroxypyrene in non-users were significantly lower than cigarette smokers.

*Correlations among Biomarkers and tobacco consumption measures*

Among cigarette smokers there were significant correlations between urine nicotine equivalents and plasma cotinine ($r = 0.73$), urine NNAL ($r = 0.73$), urine NNN ($r = 0.58$), urine 2-napthol ($r = 0.70$), urine 1-hydroxypyrene ($r = 0.53$) and cigarettes per day ($r = 0.49$), with all $p < 0.0001$. Among commercial smokeless tobacco users there were significant correlations between urine nicotine equivalents and plasma cotinine ($r = 0.72$), urine NNAL ($r = 0.87$) and urine NNN ($r = 0.66$), all $p < 0.001$; urine 2-napthol ($r = 0.33$,
p < 0.01) and tins/week (r = 0.26, p < 0.05). There was no significant correlation with 1-hydroxypyrene. For iqmiq users there was a significant correlation between urine nicotine equivalents and plasma cotinine (r = 0.78, p < 0.001) and urine NNN (r = 0.48, p < 0.05); but not with urine NNAL or PAH metabolites or with tins/week. Urine NNAL and urine NNN were significantly correlated in smokers (r = 0.59) and spit tobacco users (r = 0.67), both p < 0.0001.

Discussion

Our study provides several novel findings. To the best of our knowledge, these are the first data on biomarkers of tobacco exposure in a regional population of Alaska Native people. Furthermore we provide novel data on tobacco toxicant exposure in users of the regional smokeless tobacco product iqmiq, as well as dual cigarette and smokeless tobacco users.

Our measurements of nicotine and TSNAs in the tobacco products confirm prior research that nicotine concentrations are higher in general in cigarette tobacco compared to smokeless tobacco [18]. Of note is that nicotine concentrations in iqmiq and commercial smokeless tobacco were similar. However, because of the addition of ash that is quite alkaline, the pH is much higher in iqmiq (average 10.9) than in commercial smokeless tobacco (5.4 to 8.6) [2, 19]. As reported previously, the levels of NNK and other TSNAs are higher in commercial smokeless tobacco compared to cigarette tobacco [18].
contrast to commercial smokeless tobacco, TSNA levels are much lower in iqmik.

Presumably, this is because iqmik is made from dried tobacco leaves that are fire cured and not fermented, resulting in less generation of NNK from nicotine. Our observation agrees with data in the IARC monograph where chewing tobacco (leaf or twist used for iqmik) has substantially lower NNN and NNK levels than the commercial moist snuff varieties sold in the U.S. [18] The lower levels of NNK in iqmik might also be due to decomposition under the alkaline conditions (pH 10.9), via base-induced condensations involving the relatively acidic methylene protons adjacent to the carbonyl group of NNK.

We provide several novel observations related to nicotine exposure of AN smokers and tobacco users. First, the average plasma cotinine level of 170 ng/ml in AN smokers is only slightly lower than the 200 ng/ml average for U.S. smokers in a representative population sample [20]. However, the average number of cigarettes consumed by U.S. smokers overall is about 15 cigarettes per day, while the average in our AN population was 7.8 cigarettes per day. Thus the AN smokers who participated were on average taking in much more nicotine (and presumably other tobacco smoke toxicants) per cigarette than the average U.S. smoker. Similar observations have been made comparing other groups of light smokers [20-22]. Nicotine intake from commercial smokeless tobacco was on average similar to that of cigarette smokers, which has been observed in other U.S. and European populations [23, 24].

Nicotine intake from iqmik was strikingly higher than that from commercial smokeless tobacco. Since the nicotine content of iqmik, the number of dips used per day and
duration of use was similar to commercial smokeless tobacco products, the greater systemic dose is most likely due to greater oropharyngeal absorption of nicotine from the iqmik product related to the addition of alkaline ash. Nicotine is a weak base, and in an alkaline environment more nicotine is in the free or unionized state compared to the ionized state. Alkaline iqmik (pH 10.9) results in 99.9% unionized nicotine, which is expected to be absorbed more rapidly through the buccal mucosa compared to nicotine from commercial smokeless tobacco which has a pH of 5.4 to 8.6 [2, 19]. Anecdotal reports provided by our participants indicated that iqmik frequently results in very intense symptoms of nicotine effects and toxicity. Since the dose of nicotine and the rapidity of absorption of nicotine are thought to influence the risk of addiction, one would predict that iqmik would be more highly addictive than commercial smokeless tobacco products. Not only is it likely that iqmik use would be harder to quit, but iqmik addiction may also serve as a gateway to cigarette addiction [25]. Our findings are consistent with those of Hurt et al., who found that among pregnant women plasma nicotine and cotinine levels were higher in iqmik users compared to users of other forms of tobacco [26].

Exposure to NNK, as indicated by urine levels of NNAL, was higher in smokeless tobacco users compared to smokers, which has been reported by other researchers in different regions of the U.S [23]. The difference reflects the higher level of NNK in commercial smokeless tobacco compared to cigarette tobacco, presumably related to the smokeless tobacco curing process. NNK exposure in iqmik users was much lower than in commercial smokeless tobacco, consistent with lower levels in the product. This is most likely because iqmik is made with leaf or twist chewing tobacco with relatively low
moisture content, unlike popular snuff products that contain much higher water content and are often fermented which may increase the nitrosation reactions leading to higher TSNA content. We did not observe significant differences in urinary NNN among tobacco user groups, which may be due, at least in part, to the contribution of endogenous formation of NNN to total NNN[12].

NNK and NNAL are potent pulmonary carcinogens [7]. Smokeless tobacco use is not associated with increase of lung cancer, but is associated with increased risk of pancreatic cancer and for some products an increased risk of oral and esophageal cancer [27]. Presumably these cancers are caused at least in part by exposure to TSNAs. Dual cigarette smokers and smokeless tobacco users had, on average, higher exposure to NNK than cigarette smokers alone, which suggests that a combination of smoking and smokeless tobacco use might be more harmful than smoking alone. Based on the higher TSNA levels in smokeless tobacco compared to iqmik users, one might predict a higher cancer risk from the former. However the possible contribution of components of ash to cancer risk from iqmik also needs to be considered. Thus, it would be premature to conclude that iqmik poses a lower cancer risk than commercial ST.

PAH analysis confirmed prior research that cigarette smokers are exposed on average to higher levels of some PAHs than non-smokers [9]. This is particularly true of the more volatile PAHs such a naphthalene and fluorene, which are most specific to cigarette smoking [9]. Differences in urine 1-hydroxypyrene excretion between smokers and non-smokers were much less prominent, consistent with other environmental sources of
exposure to pyrene. Although smokeless tobacco made with fire-cured tobacco also contains PAHs [28], we did not observe significant differences in PAH biomarker levels comparing users of smokeless tobacco, iqmik users or non-users.

Analysis of within-participant correlations between self-reported product use and biomarkers of exposure revealed moderately strong correlations with number of cigarettes smoked per day and weak correlations with tins per week for commercial smokeless tobacco users, but no significant correlations between amount of iqmik use and exposures. The latter may be due to variability in the composition of the homemade product, including variability in pH. Among smokers there were strong correlations between nicotine intake and exposure to NNK and PAHs, as expected from previous studies.[29]. For commercial smokeless tobacco users there was a strong correlation between nicotine intake and NNK exposures, but weaker correlations with PAH exposure. This observation is consistent with the fact that nicotine is a precursor of TSNAs, but that PAH levels in smokeless tobacco are very dependent on the nature of the curing process and the length and condition of storage of the product. Among iqmik users there were no significant correlations between nicotine intake and carcinogen exposure, most likely related to wide variability in product constituents, including pH.

There are several potential limitations of our study. Foremost with respect to generalizability is that we studied AN people in only one region, the Bristol Bay region of southwest Alaska, who volunteered to participate. Our participants were primarily Yupik. Alaska Native people in other areas of Alaska have different ethnic backgrounds.
and different cultural influences which could influence smoking behavior and exposure to tobacco toxicants. Although, the number of subjects using iqmik only was relatively small our paper provides new data on exposure to carcinogens among users of this tobacco product and indicate the potential increased addiction risk among this population. There are no other published data on human exposure to nicotine and carcinogens among iqmik users, so our data are unique.

Our findings have important implications for tobacco regulation under the auspices of the US Food and Drug Administration as well globally under the Framework Convention on Tobacco Control. First, the TSNA levels of commercial smokeless tobacco are high and may be contributing to the high rate of oral cancer in AN people. TSNA levels in smokeless tobacco can be controlled and reduced to substantially lower levels than those observed in the present study[30], which could have a beneficial effect on cancer risk at the population level. Second, the very high levels of unionized (free) nicotine and the resultant high levels of nicotine absorption from iqmik is consistent with a high addiction risk. A high level of addiction is likely to increase the risk of transition to smoked products and of dual use and to impede efforts to achieve and sustain abstinence, further increasing the risk to health. Thus, the pH and levels of unionized nicotine in smokeless tobacco products need to be evaluated and potentially regulated as a way to reduce addiction and disease risk[31, 32]. Finally, the use of iqmik raises challenges to the regulation of products that are prepared by the user or by small vendors. This is of particular importance because many people think that noncommercial products are less harmful than commercial products[2]. Research is needed on the contribution of ash to
the harmful effects of tobacco, and when regulation is not feasible, public health workers and regulators need to educate the public about the risks of additives such as ash.

Acknowledgements

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Figure Legends

Figure 1. Box and whisker plots of biomarkers of nicotine exposure in different tobacco use groups. Panel A is plasma cotinine; panel B urine nicotine equivalents. The solid line inside the box is the median, the diamond is the mean, the top and bottom of the box represent the 25-75% interquartile range, and the vertical line indicates minimum and maximum values.

Figure 2. Box and whisker plot of urine total NNAL in different tobacco use groups.

Figure 3. Box and whisker plots of urine PAH metabolites in different tobacco use groups. Panel A, 2-naphthol; panel B 1-hydroxypyrene.

References


Table 1: Nicotine and nitrosamine content of tobacco products

<table>
<thead>
<tr>
<th>Biomarker Mean (95% Confidence interval)</th>
<th>Cigarette (N=24)</th>
<th>Commercial ST (N=22)</th>
<th>Iqmik non-premasticated (N=19)</th>
<th>Iqmik premasticated (N=4)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine (mg/g)</td>
<td>18.68 &lt;sup&gt;a&lt;/sup&gt; (17.80-19.56)</td>
<td>12.54 &lt;sup&gt;b&lt;/sup&gt; (11.79-13.30)</td>
<td>12.98 &lt;sup&gt;b&lt;/sup&gt; (11.71-14.26)</td>
<td>12.61 (4.48-20.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NAB (ng/g)</td>
<td>79.7 &lt;sup&gt;b&lt;/sup&gt; (69.6-89.9)</td>
<td>181.2 &lt;sup&gt;a&lt;/sup&gt; (139.8-222.5)</td>
<td>62.5 &lt;sup&gt;b&lt;/sup&gt; (48.0-77.0)</td>
<td>104.0† (0-257.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NAT (ng/g)</td>
<td>1421.6 &lt;sup&gt;b&lt;/sup&gt; (1206.2-1637.0)</td>
<td>2453.5 &lt;sup&gt;a&lt;/sup&gt; (1616.6-3290.5)</td>
<td>938.3 &lt;sup&gt;c&lt;/sup&gt; (733.5-1143.2)</td>
<td>1580.8† (0-4025.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NNK (ng/g)</td>
<td>536.9 &lt;sup&gt;b&lt;/sup&gt; (467.7-606.2)</td>
<td>829.0 &lt;sup&gt;a&lt;/sup&gt; (679.6-978.4)</td>
<td>81.8 &lt;sup&gt;c&lt;/sup&gt; (56.5-107.0)</td>
<td>565.3† (0-2108.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NNN (ng/g)</td>
<td>2028.1 &lt;sup&gt;b&lt;/sup&gt; (1707.1-2349.1)</td>
<td>2874.3 &lt;sup&gt;a&lt;/sup&gt; (2207.6-3541.0)</td>
<td>846.8 &lt;sup&gt;c&lt;/sup&gt; (628.7-1065.0)</td>
<td>1065.8† (0-2466.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NNAL (ng/g)</td>
<td>52.4 &lt;sup&gt;b&lt;/sup&gt; (45.5-59.3)</td>
<td>166.5 &lt;sup&gt;a&lt;/sup&gt; (113.3-219.6)</td>
<td>24.7 &lt;sup&gt;c&lt;/sup&gt; (10.9-38.4)</td>
<td>59.9† (0-155.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* The p-value was derived from the non-parametric Kruskal-Wallis test. “Iqmik premasticated” was excluded from the comparisons between groups due to a very small sample size.

† The lower limit of the confidence interval was truncated at zero.

<sup>a, b, c</sup> – values with the same letter are not significantly different; different letters indicate significant differences compared to other products, p<0.01; comparisons only among commercial cigarettes, commercial ST and Iqmik non-masticated.
Table 2 Tobacco Use and Exposure Biomarker Levels

<table>
<thead>
<tr>
<th>Tobacco Use (n=163)</th>
<th>Cigarette Smoke</th>
<th>Spit Tobacco (n=76)</th>
<th>Iqmik (n=20)</th>
<th>Dual Use (n=31)</th>
<th>Non-user (n=107)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarettes/Day</td>
<td>7.8 (5.21)</td>
<td>–</td>
<td>–</td>
<td>5.7 (3.1)</td>
<td>–</td>
</tr>
<tr>
<td>Tins/Week</td>
<td>–</td>
<td>1.6 (1.5)</td>
<td>1.2 (1.3)</td>
<td>1.3(1.0)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.5(0.6)&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
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### Plasma

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Cigarette Smoke</th>
<th>Spit Tobacco</th>
<th>Iqmik</th>
<th>Dual Use</th>
<th>Non-user</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotinine (ng/ml)</td>
<td>170.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>221.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>343.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>187.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>(154.5-186.6)</td>
<td>(190.7-251.2)</td>
<td>(251.6-434.8)</td>
<td>(141.1-233.1)</td>
<td>(0.1-0.5)</td>
<td></td>
</tr>
<tr>
<td>3HC (ng/ml)</td>
<td>75.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>207.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>(65.4-85.5)</td>
<td>(76.6-113.5)</td>
<td>(120.0-294.3)</td>
<td>(54.5-97.7)</td>
<td>(0.05-0.2)</td>
<td></td>
</tr>
<tr>
<td>(Cotinine + 3HC) (nmol/ml)</td>
<td>1.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.46&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.002&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>(1.23-1.49)</td>
<td>(1.50-1.99)</td>
<td>(2.16-3.89)</td>
<td>(1.10 – 1.81)</td>
<td>(0.001-0.003)</td>
<td></td>
</tr>
</tbody>
</table>

### Urine

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Cigarette Smoke</th>
<th>Spit Tobacco</th>
<th>Iqmik</th>
<th>Dual Use</th>
<th>Non-user</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine equivalents (pmol/mg creatinine)</td>
<td>61.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>116.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>(54.8-68.1)</td>
<td>(59.6-86.0)</td>
<td>(96.3-137.2)</td>
<td>(41.4-69.0)</td>
<td>(0-0.001)</td>
<td></td>
</tr>
<tr>
<td>NNAL (pmol/mg creatinine)</td>
<td>0.89&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>4.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(0.76-1.04)</td>
<td>(3.40-5.04)</td>
<td>(0.40-0.86)</td>
<td>(0.67-1.74)</td>
<td>(0.01-0.02)</td>
<td></td>
</tr>
<tr>
<td>NNN&lt;sup&gt;#&lt;/sup&gt; (pmol/mg creatinine)</td>
<td>0.00059&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00070&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00074&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00114&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00008b</td>
</tr>
<tr>
<td>(0.00048-0.000073)</td>
<td>(0.00053-0.00092)</td>
<td>(0.00028-0.00196)</td>
<td>(0.00071-0.00183)</td>
<td>(0.00004-0.00016)</td>
<td></td>
</tr>
<tr>
<td>2-naphthol (pmol/mg creatinine)</td>
<td>103.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>47.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(89.41192)</td>
<td>(36.6-55.9)</td>
<td>(23.1-37.7)</td>
<td>(48.0-82.8)</td>
<td>(37.6-59.6)</td>
<td></td>
</tr>
<tr>
<td>Sum of hydroxyfluorenes (pmol/mg creatinine)</td>
<td>13.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(11.6-15.1)</td>
<td>(5.7-4)</td>
<td>(4.7-7.2)</td>
<td>(4.9-12.4)</td>
<td>(5.1-7.6)</td>
<td></td>
</tr>
<tr>
<td>Sum of hydroxyphenanthrenes (pmol/g creatinine)</td>
<td>2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(2.4-3.2)</td>
<td>(1.8-3.2)</td>
<td>(1.4-2.6)</td>
<td>(1.3-3.6)</td>
<td>(1.9-2.9)</td>
<td></td>
</tr>
<tr>
<td>1-hydroxypyrene (pmol/g creatinine)</td>
<td>1.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.89&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.83&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(1.08-1.44)</td>
<td>(0.75-1.46)</td>
<td>(0.65-1.71)</td>
<td>(0.69-1.14)</td>
<td>(0.64-1.08)</td>
<td></td>
</tr>
</tbody>
</table>

Tobacco use parameters shown as mean (SD); plasma cotinine, 3HC, (cotinine + 3HC) and urine nicotine equivalents shown as arithmetic mean (95% C.I.); urine NNAL, NNN and PAH metabolites shown as geometric mean (95% C.I.).

<sup>1</sup>- smokeless
<sup>2</sup>- Iqmik

a, b, c, d – values with the same letter are not significantly different; different letters indicates significant differences between groups, p < 0.01

# N: cigarette smokers = 140, spit tobacco = 72, iqmi = 20, dual use = 28, non-users = 23
FIGURE 1 – panel A

FIGURE 1 – panel B
Figure 3

Panel A

Panel B
Cancer Epidemiology, Biomarkers & Prevention

Exposure to Nicotine and Carcinogens among South Western Alaskan Native Cigarette Smokers and Smokeless Tobacco User

Neal L. Benowitz, Caroline Renner, Anne Lanier, et al.

Cancer Epidemiol Biomarkers Prev  Published OnlineFirst April 6, 2012.

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