Novel Determinants and Measures of Smokeless Tobacco Use

Study 1: Toxicant Exposure Across Brands of Smokeless Tobacco

Principal Investigator:

Dorothy K Hatsukami, PhD Department of Psychiatry

UMN Co-investigators:

Stephen S Hecht, PhD Masonic Cancer Center

Sharon Allen, MD Family Medicine and Community Health

Chap Le, PhD Biostatistics, School of Public Health

Oregon Research Institute Co-investigators:

Herb Severson, Ph.D.

West Virginia University Co-investigators:

Kimberly Horn, Ed.D.

Robert Anderson

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REVISION HISTORY

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PI CONTACT INFORMATION:

Dorothy Hatsukami, PhD
Forster Family Professor in Cancer Prevention
Professor of Psychiatry
University of Minnesota
Minneapolis, MN 55454-1015
Telephone (612) 627-1808
Fax (612) 627-4899
Email: hatsu001@umn.edu

A. Specific Aims

Reducing toxicants in tobacco products and establishing performance standards for tobacco products have been discussed and recommended by the World Health Organization Tobacco Regulation Study Group and by tobacco control scientists and advocates [1, 2]. Although the focus to date has been primarily on cigarettes, reducing tobacco toxicants in smokeless tobacco should also be strongly considered and recommended [3]. The extent of toxicity varies considerably across different brands of smokeless tobacco (ST) products and data suggest that greater toxicity may result in greater health risks. However, little is known about the actual extent of human exposure to toxicants from current ST products and factors that might moderate the extent of this exposure. The overall goals of this study are to determine toxicant exposure across brands of ST that vary in levels of nicotine and TSNAs using a panel of biomarkers of exposure and effect, and to examine factors other than product toxicants that influence the extent of this exposure.

B. Research Design and Methods

B1. Overview

Subjects using the brands listed in Table 2 will be recruited from three different regions in the U.S. They will undergo two assessment sessions. During these sessions, urine and blood samples will be collected. During the time of data collection, ST users will be recording the amount of tobacco products that they use on a daily basis using their diary cards.

B2. Procedures

Subject recruitment. Subjects (N=600) will be recruited from a) Minneapolis/St. Paul, MN, b) Eugene, OR, and c) Morgantown, WV. Each site will recruit 200 subjects over the course of 2 years, with a total of 100 subjects recruited for each brand that is listed in Table 2. We anticipate no problems with recruitment. Surveys conducted in 2007or 2006 estimated Minnesota and Oregon male ST prevalence of 6.0% and 6.5%, respectively. The 2006 West Virginia Adult Tobacco Survey estimated the male ST prevalence to be about 16%. Multiple avenues of recruitment will be pursued to ensure as representative of a sample as possible. These methods include radio, cable, newspapers (want ads and display ads), restroom stall ads, sporting good stores, WIC clinics, internet and press releases. The recruitment advertisements will state that daily ST users are needed for a study that examines the effects from ST use; blood (only at U of MN site), oral cells (only at U of MN site) and urine samples will be obtained. It will further state that subjects will be paid for participation. Potential adult subjects will be asked to call the research clinic number where the study will be explained, brief phone screening will be conducted, and interested and potentially eligible subjects will be asked to attend an orientation meeting. The brands of ST will not be listed on the advertisements to ensure that we are recruiting actual users of the brands rather than subjects who falsely claim using one of the brands of interest in order to obtain money for study participation. Subjects will be informed that the study determines the extent of exposure to toxicants and the effects of these exposures across different brands of ST.

B2a. Subject inclusion/exclusion criteria

Adult ST users (18 years or older) who are interested in the study will telephone the research clinics, be informed about the study, and initially screened in order to determine whether they meet specific inclusion/exclusion criteria.

Inclusion criteria include:

a) use of a consistent brand and daily amount of ST for the past 6 months;
b) in good physical health (no unstable medical condition and no kidney or liver disease);
c) stable, good mental health (e.g., no recent unstable or untreated psychiatric diagnosis, including substance abuse, as determined by the DSM-IV criteria).
d) use of one of the following smokeless tobacco products:

(UPDATED BRANDS BY GROUP): 1) Camel Snus, Marlboro Snus; 2) Skoal Long Cut Mint, Skoal Fine Cut Original, Copenhagen Long Cut Original, Kodiak Wintergreen, Skoal Bandits Wintergreen, Skoal Pouches Straight, Skoal Pouches Wintergreen; 3) Grizzly Long Cut Straight, Skoal Long Cut Straight, Grizzly Long Cut Mint, Grizzly Long Cut Wintergreen, Grizzly Pouches Wintergreen, Skoal Pouches Mint, Kodiak Pouches Wintergreen; 4) Grizzly Fine Cut Regular; 8) Copenhagen Fine Cut Original, Copenhagen Long Cut Straight, Grizzly Pouches Straight, Skoal Long Cut Classic, Skoal Long Cut Wintergreen, and Dual Users of Camel and Marlboro Snus and cigarettes.

Exclusion criteria include:

a) currently using other tobacco (>1 episode of smoking per week on average; no combustible tobacco use in the last 21 days and CO >8 ppm) or nicotine products;
b) female subjects pregnant or nursing;
c) use of alcohol within the last 24 hours of testing and no more than 21 drinks per week at phone screening;
d) use of any inhalable drugs in the last 14 days and regular use >1 times per week;
e) current infection or use of antibiotics;
f) active liver or kidney disease, or UTI which would interfere with biomarker measures.

Subjects meeting these criteria will then be asked to come into the research clinic for an orientation visit, to provide informed consent, and to engage in more thorough screening. Subjects will also be required to complete a tobacco use questionnaire, medical history form (which will be reviewed by a physician), and baseline measurements (see below).

B2b. Measures and Schedule of Administration

The following provides the measures that will be administered. Table 1 describes the measures to be obtained and the timing of the measurement. The majority of these measures have been used in our prior studies and have been found to be dose-sensitive to change in amount of smoking or ST use, or sensitive to switching products (e.g., conventional cigarettes to modified cigarettes, cigarettes to ST products).

Table 1. Schedule of visits and measures Study 1

	Visit	
	Orientation	1
Consent	X	
Baseline and Moderator Measures		
Demographics	X	
Medical History and Concomitant meds	X	X
Tobacco Use History and Exposure,	X (brief)	
Severson ST Dependency Scale	X	
Perceived Health Risk	X	X
Stages of Change		X
CES-D		X
PANAS		X
Perceived Stress Scale		X
Michigan Alcohol Screening Test, NIAAA Alcohol Use Questionnaire		X
Exposure and Lifestyle Measures		
Occupational Health History		X
ETS and Social Influences		X
Dietary – consumption of meats		X
IPAQ		X
Medical status (e.g., infections)		X

Daily Tobacco Use Diary	X (start at screen)	X
Vitals (Blood pressure and heart rate)		X
Weight		X
Concomitant Medications		X
Carcinogen Exposure Urinary total NNAL Total NNN Nitrosoamino acids NPRO and MNPA Phenanthrene tetraol 1-hydroxypyrene (1HOP)		X
Cardiovascular Measures (UMN only) Lipoproteins C-reactive protein Fibrinogen WBC		 X X X X
[1]Buccal assay		X
[2]Nicotine and metabolites	X	X
[3]Product Constituent Testing		X
[4]Weight per dip	X	X
Carbon Monoxide		X

[1]Buccal assays to be placed in biorepository for future analyses (UMN only)
[2]Urinary total nicotine equivalents and nicotine metabolite ratio (3-HC:cotinine)
[3]Product constituent testing will conducted on a random sample of 30 tins for each brand of ST (10 brands from each site)
[4]Estimated weight per dip will be calculated by averaging the weight of 3 typical dips

B2bi. Subjective Measures

1) Demographics such as age and gender, socioeconomic status and if possible, self-reported race or ethnicity.

2) Tobacco Dependence as assessed by the Severson Smokeless Tobacco Dependency Scale (SSTDS). This scale consists of 8 items and a total score is calculated as the sum of items 2 through 8. The Cronbach coefficient alpha for the SSTDS was 0.69. Candidate items for this scale were drawn from the following sources: a version of the Fagerström scale used in previous studies [101, 102]; a version of the Cigarette Dependence Scale (CDS) with 5 items (CDS-5) [103] adapted for smokeless tobacco users.

3) Tobacco Use History and Exposure Questionnaire is an interview with variables such as age of initiation of ST use, amount of use, brand used, duration of use, number of quit attempts, duration of quit attempts, and current and past other tobacco product use. Tobacco use questions are taken from a national survey (Tobacco Use Supplement to the Current Population Survey) so that comparisons can be made with national samples.

4) Centers for Epidemiological Studies-Depression (CES-D) is a 20-item scale which assesses current symptoms of depression that has been used in the general population [105].

5) Michigan Alcohol Screening Test (MAST) Short form assesses any negative consequences from alcohol use by self report [106] and the NIAAA Alcohol Use Questionnaire examines rate of alcohol use, in the last 3 months

6) Positive Affect Negative Affect Scale (PANAS-X) [108] has been used in studies to assess negative affect prior to and after cessation [e.g., 109]. This scale contains two higher order scales, positive and negative, and 11 specific affects. We will be focusing primarily on the negative affect scale, which measures subjective distress and unpleasant engagements (aversive mood states such as anger, disgust, guilt, fear and nervousness).

7) Perceived Stress Scale is a 14-item scale that measures self-perceived stressful situations in the last month. The items reflect the extent to which the respondents find that their lives are unpredictable, uncontrollable and overloading [110]. Each item is rated on a 0 to 4 scale that ranges from 0=never and 4=very often. The Cronbach's alpha observed in one study was 0.80 (Manning et al., 2005). The scale has been related to smoking status and cessation [e.g., 111, 112, 113].

8) Perceived Health Risk form assesses perceived health risk of the product for different disease states (heart disease, oral cancer, lung cancer, diabetes, etc.). Subjects rate the product on a 0 to 10 rating scale (0=no risk to 10=very high risk). We have used this scale in a number of tobacco evaluation studies [e.g., 98].

9) Stages of Change assessing intention of quitting in the next 30 days and 6 months.

10) Environmental and Social Influences on Tobacco Use consists of a questions regarding: a) tobacco use (ST, cigarettes and other nicotine containing products) among the subject's social network (e.g., significant other, friends, co-workers, siblings and parents); b) extent of social support for or against ST use; c) restrictions or bans on smokeless tobacco (and smoking) at work, school or in the home; and d) cost of smokeless tobacco products (unit price) and income. These are variables found in studies to be associated with uptake and amount of tobacco use.

11) Food frequency questionnaire for cooking practices assesses frequency of eating charred and processed meats within the last week.

12) Modified International Physical Activity Questionnaire (IPAQ) interview assesses physical activity by recall 7 question assessment of moderate, hard and very hard physical activity engaged in week days and week ends modified to cover the last three months average activity. http://www.ipaq.ki.se/.

13) Medical History and Concomitant Medications lists current diagnosis, symptoms, medications and past health problems, and a record of use of any over-the-counter and prescription medications.

14) Environmental and Occupational Health History assesses environmental and occupational toxicant exposures, which might affect our biomarker measures [124].

15) Tobacco Use Status will be assessed using a *Tobacco Use Questionnaire* that asks about current tobacco use status (ST and other tobacco products). This questionnaire will be administered at the clinic visit. The daily diary cards will collect similar information, but will be hand recorded and returned during the clinic visit. The daily diary cards will be used in Study 1 to conserve costs. We have successfully used daily diary cards in the past when examining the relationship between exposure measures and ST topography [41, 42, 44, 76].

B2bii. Biological measures

Physiological Measures

1) Heart rate and blood pressure. After sitting for 5 minutes, the subject's radial pulse rate will be measured. After the heart rate measurements, blood pressure will be measured twice, 2 minutes apart for reliability. Measurements will be taken using a Critikon Dinamap (GE Medical Systems, Tampa, FL). Although acute increases in heart rate and blood pressure have been observed, most studies have shown a lack of sustained increases in heart rate and blood pressure from ST use [126]. On the other hand, a few studies have shown increased hypertension among ST users [127, 128] and one of the studies has implicated elevated blood pressure as the potential causal pathway for increased risk for fatal myocardial infarction observed [129].

2) Body weight. Subjects will remove their shoes, coats, sweaters, etc. and be weighed to the nearest 0.25 lb on an electronic scale.

Nicotine metabolism

3) Nicotine metabolite ratio. This ratio is an indicator of CYP2A6 enzyme activity and is the ratio between two nicotine metabolites, cotinine and *trans*-3'-hydroxycontinine (3-HC). Because CYP2A6 is the primary enzyme associated with nicotine metabolism and also catalyzes the conversion of cotinine to 3-HC, the 3-HC:cotinine ratio is considered to reflect the enzymatic activity of CYP2A6 and reflect the extent of nicotine clearance rate (higher the ratio, the faster the clearance rate). This metabolite

ratio has been observed to be moderately correlated to CYP2A6 genotype and the rate of nicotine metabolism [20, 21, 130-133].

Biomarkers.

4) Biomarkers for tobacco exposure measures. Urinary total nicotine equivalents--total nicotine (free plus nicotine *N*-glucuronide) and its main metabolites, total cotinine (free plus cotinine *N*-glucuronide), total *trans*-3′-hydroxycotinine (3-HC) (free plus 3-HC *N*- and *O*-glucuronide)--which together account for 85-90% of the nicotine dose and is a useful measure of daily nicotine exposure, will be assessed by the Hecht laboratory at the Masonic Cancer Center, University of Minnesota. Carbon monoxide, which is rapidly absorbed into the blood stream during smoke inhalation and binds with hemoglobin to form carboxyhemoglobin (COHb), will be measured using the Bedfont Micro Smokerlyzer® (Bedfont Scientific Limited, Kent, UK) device to verify abstinence from smoking. A spot urine samples will be collected at the orientation visit and at the clinic visit.

5) Biomarkers for carcinogen exposure and effect. The following urinary biomarkers of carcinogen exposure will be assessed: a) NNAL and NNAL-glucuronides (total NNAL), metabolites of the tobacco-specific carcinogen NNK; b) *N'*-nitrosonornicotine (NNN) and its glucuronides in urine (total NNN), reflecting the uptake of the tobacco-specific carcinogen NNN; c) the nitrosamino acids NPRO, a widely accepted biomarker of endogenous nitrosation, and MNPA, a biomarker of exposure to the carcinogen MNPA found in smokeless tobacco products; d) 1-HOP, a well established biomarker of exposure to PAH; e) phenanthrene tetraol, a marker for PAH. First urine void in the morning will be collected for the urine analyses [64]. The analyses will be carried out in the Hecht laboratory using well established methods described in our previous publications [62, 134-136]. Results will be expressed per mg urinary creatinine.

For the sample collection at the other sites, staff will be trained on how to collect, process and send the urine samples. Urine samples will be collected, frozen and shipped on dry ice to Minnesota for analysis. The names and other identifying information will be stripped from the data before transmission and the file connecting the data with the subject number will be transferred via secure VPN in a separate file to Minnesota project staff. The file connecting the data to the identifying information of the participant will be stored at each site in a locked file cabinet and will be accessible only to approved study staff.

6) Cardiovascular Disease (CVD) Risk Factors. We will collect samples to measure CVD risk factors only at the Minnesota site to save costs. CVD risk factors will be analyzed at the University or Minnesota Medical Center Fairview, Collaborative Studies Clinical Laboratory, Minneapolis, MN. To date, most of the existing studies on CVD risk factors in ST users have been conducted in Sweden. A review of these studies shows that exclusive ST users do not demonstrate higher CVD risk factors (e.g., lipoproteins, biomarkers of oxidative stress and inflammation, endothelial function and platelet activation, carotid intima media thickness) compared to non-users among Swedish ST users and similar results have been observed with healthy young US snuff users [126, 138]. On the other hand, a few studies conducted with ST users in India have shown elevation in lipoproteins and triglycerides [139, 140]. Because of the limited knowledge of US products and a meta-analyses that shows an increased risk in CVD and stroke observed in the US population of smokeless tobacco users (although not the Swedish population [33] with the exception of fatal cardiovascular disease [128, 141]), the following CVD risk factors, which have been predominantly observed to be increased in cigarette smokers, will be assessed in our study: a) Lipoproteins, such as total plasma cholesterol, high and low density lipoproteins (HDL and LDL, respectively), and triglycerides. These markers were found to be dose-sensitive to cigarette exposure in our prior studies. Subjects will fast for 10 hours prior to blood draws. Preferably sampling will be done in early morning, but samples can be done mid-day according to work schedules of subjects as long as the subjects are fasting. b) C-reactive protein is considered to be an inflammatory marker or mediator and its release by the liver is stimulated by pro-inflammatory cytokines, including interleukin 6 (IL-6). Increases in C-reactive protein have been predictive of future vascular events and course of pre-existing vascular disease, and smoking has been found to modulate levels of this protein [e.g., 142, 143]. Several mechanisms by which C-reactive protein contributes to the risks of cardiac disease have been described [143, 144]. c) Fibrinogen is a marker for thrombosis

and is a glycoprotein involved in the coagulation response to vascular and tissue injury. Fibrinogen is also considered to contribute to vascular disease in other ways besides its role in thrombosis such as stimulating smooth muscle cell proliferation, promoting platelet aggregation and increasing blood viscosity [145]. Elevated fibrinogen levels have been found to be strongly predictive of cardiovascular morbidity and mortality [143]. Prior studies have shown a dose-response relationship between fibrinogen levels and number of cigarettes smoked [146]. d) White blood count, also an inflammatory marker, has been found to be higher in smokers than non-smokers [147, 148] and associated with cardiovascular disease [142] and in our studies was observed to be the most dose-related marker for cigarette reduction [84].

7) Buccal cells biomarkers. Although the grant proposal will not be able to bear the cost for analyses of buccal biomarkers, we will be collecting buccal cell samples to be placed in a biorepository with the hopes of obtaining future funding for their analysis. Buccal cells will be obtained through two methods (oral check swab and mouth wash for DNA extraction). These buccal cells will be frozen, stored and labeled in accordance to the procedures used in our NCI contract (N01-PC-64402) and will be made available to investigators associated with the contract or outside of this contract upon review of their proposal and approval from their respective IRBs. All samples will be de-indentified. Examples of potential biomarkers include cellular autoflorescence (a method being tested by Dr. John Pauly at Roswell Park Cancer Institute), mitochondrial mutations in oral cavity cells (an analyses being tested by Dr. Peter Shields at Georgetown University) and nf Kappa B p65 staining (an analyses conducted for NCI contract N01- CN-15000 by Dr. Frank Ondrey at the University of Minnesota).

B2c. Product constituent testing

In collaboration with the Center for Disease Control and Prevention, we will analyze the constituents of the smokeless tobacco products (un-ionized nicotine, tobacco specific nitrosamines and pyrene) used by our participants. This information will be related to the biomarker data. A random sample of tins of unused ST from 10 subjects for each brand of ST at each site will be analyzed. For Study 1, 180 subject samples (10 random subjects x 6 brands x 3 sites) will be analyzed. The tins will be labeled with the subject number, site identification, date of collection, brand of ST and tin lot number. Samples will be stored in the freezer until a batch is shipped to CDC on dry ice. Subjects will be reimbursed for their tin of ST. The major advantage of taking tins from the subjects is that they may more accurately reflect actual levels of toxicants. Prior studies have shown change in TSNAs based on storage conditions. CDC will analyze samples for NNAL, NNN, NAT, NAB, PAHs, Nitrosamino acids (MNPA & NIS will be added to CDC usual analyses).

B2d. Tobacco Products

For Study 1, usual brand of ST products will be used. We chose products that had substantial market share and showed a range in nicotine level and in NNK and NNN levels [4, 46, 75, 151]. Table 2 shows the brands that were chosen for testing. Free/unprotonated nicotine levels were considered high (H) if they were > 5 mg/g, medium (M) if they were between 2-5 mg/g and low if they were < 2 mg/g [3]. TSNA levels were based on a classification that was developed by Stephen Hecht (Hecht, report for WHO, TobReg). High (H) levels were NNN plus NNK > than 10 µg/g, Medium (M) levels were NNN plus NNK from 2-10 µg/g, and Low (L) levels were NNN plus NNK < 2 µg/g. Products that are in the Low range for both nicotine and TSNAs tend not be manufactured.

Table 2

Group	Product	Test Date	# Tins/ Regions	Nicotine (mg/g wet)	NNK+NNN (µg/g wet)	Nicotine Group	TSNA Group
1	Camel Snus (All Flavors)	Fall 2010		2.65		Low	Low
	Marlboro Snus (All Flavors)						
	Skoal Long Cut Mint	Fall 2011	3 / 2	1.2	1.23		
	Skoal Fine Cut Original	Spring 2011	1 / 1	2.79	1.88		
2	Copenhagen Long Cut Original	Fall 2010	1 / 1	3.51	2.55	Med	Med
	Kodiak Wintergreen	Spring 2010	9 / 3	4.51	2.73		
	Skoal Bandits Wintergreen	Spring 2010	11 / 3	3.05	3.72		
	Skoal Pouches Straight	Spring 2011	1 / 1	4.02	2.58		
	Skoal Pouches Wintergreen	Spring 2011	1 / 1	3.89	2.81		
	Grizzly Long Cut Straight	Spring 2010	5 / 2	4.23	3.84		
	Skoal Long Cut Straight	Spring 2010	9 / 3	3.15	3.11		
3	Grizzly Long Cut Mint	2010 summary		7.58	3.72	High	Med
	Grizzly Long Cut Wintergreen	Spring 2010	4 / 2	5.12	3.05		
	Grizzly Pouches Wintergreen	Spring 2011	1 / 1	6.25	3.71		
	Skoal Pouches Mint	Spring 2011	1 / 1	5.19	2.15		
	Kodiak Pouches Wintergreen	Spring 2011	1 / 1	5.88	3.44		
4	Grizzly Fine Cut Regular	Spring 2011	1 / 1	4.41	15.3	Med	High
6	Kodiak Long Cut Mint	Spring 2011	1 / 1	3.9	1.91	Med	Low
	Kodiak Long Cut Straight	Spring 2011	1 / 1	4.06	1.88		
8	Copenhagen Fine Cut Original	Spring 2010	9 / 3	2.43	3.45	Low	Med
	Copenhagen Long Cut Straight	2010 summary		2.56	2.07		
	Grizzly Pouches Straight	Spring 2011	1 / 1	0.48	4.0		
	Skoal Long Cut Classic	Spring 2011	1 / 1	2.88	2.88		
	Skoal Long Cut Wintergreen	Spring 2011	1 / 1	2.7	2.51		

Nicotine Group	mg/g wet weight
Low	<3
Medium	3-5
High	>5

B2e: Clinic Visits

B2ei. Orientation meeting. Subjects will attend an orientation meeting to be further informed about the study, to obtain consent, and to determine eligibility. Once determined to be eligible, subjects will provide a spot urine sample for nicotine levels, and then they will be scheduled for a clinic visits and will keep a daily diary to monitor their ST intake (time of dip onset and time when dip was expectorated). We have used daily diaries with ST users for up to a period of 8 weeks with good compliance.

B2eii. Clinic visit. At the clinic visit, subjects will be asked to complete questionnaires on demographic and tobacco use history, on ST dependence, on psychiatric, medical and alcohol use history, and on factors that might moderate the extent of tobacco exposure. Subjects will also be asked to complete a series of questionnaires during each of the orientation and clinic visits. These questionnaires will assess for tobacco use status and any factors that might confound our biomarker measures. These factors include exposure to environmental tobacco smoke, dietary intake, level of physical activity, medical status (e.g., infections), alcohol intake prior to visit and use of any other nicotine containing products. First morning urine sample will be collected to measure carcinogen biomarkers. Two spot urines (one at orientation and one at the clinic visit) total nicotine equivalents and nicotine metabolite ratio. Vital sign measurements and CO will be obtained and at the U of MN site, blood samples taken (23 ml of blood obtained by a nurse). In addition, subjects will provide us with at least 3 samples (usual size dips) of their smokeless tobacco product at each of the two visits. Each of these dips will be weighed to obtain an estimate of the mean weight per dip. Clinic visits will be scheduled on Tuesdays, Wednesdays or Thursdays. At each site, tins of unused ST will be obtained from a random sample of 10 subjects from each of the 6 brands of ST and sent to CDC for toxicant

analysis. At the end of the study, all subjects will be encouraged to quit ST use. Cessation materials and referrals will be provided to all participants at the conclusion of the second visit.

B2f. Subject payments.

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Subjects will be paid $100 for participation, $25 for the Orientation and $75 for the clinic visit.

***B2g. Adherence to protocol procedures and data integrity*.**

Standard operating procedures will be developed. Face-to face training for research personnel will occur prior to the study, where the protocol and procedures will be carefully described. Case report books will be made for the different sites to maximize parallel recording of data. Each visit will have a checklist of all the measures that need to be taken and the order by which these measures are administered.

Weekly conference calls will occur among the research coordinators to trouble shoot and to make sure that all protocol procedures are followed. On a once a month basis, the PI and co-investigators will participate in this conference call. During these conference calls, the number of subjects enrolled, the data collection process, the results from data monitoring and other issues of concern will be discussed. As another measure to ensure data collection integrity, the project manager from the University of Minnesota will make a visit to the various sites after they have enrolled 10 subjects to make sure that the protocol is being followed carefully and all the data is being collected properly. Another on site visit will occur 3 months from the initiation of the study. An additional visit will be made in Year 1 if necessary, and 2 visits in Year 2.

Human subject consent forms, pen and paper and electronic subjective forms, and containers for the urine biosamples and for ST collections will be given bar codes individualized for each subject. Each site will receive a separate box for each subject, packaged at the University of Minnesota, that contains the bar coded paper and pencil subjective forms and collection containers. Most of the subjective measures will be administered via computer in electronic forms on a secured website. Subject numbers, represented on the bar codes, will be entered into the computer prior to subject data entry. Forms will include programming features to ensure valid data (i.e., input masks, validation criteria, skipout logic) and data will be exported to Microsoft Excel files that are de-identified of any personal health identifiers. All other data not captured directly in the website at the other sites (e.g., pencil-and-paper forms) will be sent to the University of Minnesota on a weekly basis (with photocopies of originals maintained at the other sites). A data monitor will be available at the University of Minnesota site and irregularities (e.g., wrong date on the forms, inconsistent data) or missing data will be flagged by the monitor and sent to the site for comment or correction to ensure the integrity of the data.

All de-identified biosamples will be sent to the University of Minnesota on a monthly basis after both biosamples have been collected for each subject. A list of subject number and biosamples sent will be recorded by each site and when the samples are received, the researcher at the University of Minnesota will note each subject number and biosample that was received. The samples-received list will be posted on a secure website so that each site can check it against the samples-sent list.

C. Statistical analyses

C1. Analytic Approach

Primary Aims

1. To determine the toxicant exposure associated with currently marketed oral tobacco products with varying levels of toxicants and nicotine.

Subject characteristics will be examined across sites and ST users at each site will be compared to the characteristics of the respective state population of ST users. The outcomes for this study are the uptake of toxicants, as measured by biomarkers of exposure including NNAL, NNN, nitrosoamino acids,

1-HOP and total nicotine equivalents and biomarkers of effect such as CVD risk factors. The distribution of each biomarker will be examined to determine if a data transformation is necessary. From our previous experience, the distributions of many biomarkers are often skewed with a long right tail and need to be analyzed in the natural log scale. The two biomarker assessments for nicotine from each subject will be used to evaluate the reliability, as measured by the intraclass correlation. The two readings will then be averaged for the subsequent analysis. The geometric mean and the corresponding 95% confidence interval for the biomarkers will be calculated for each brand. The one-way analysis of variance (ANOVA) will be used to compare the mean toxicant levels between all six brands. If this global test is significant, then the Tukey's studentized range procedure will be used for pair-wise comparisons between brands, while maintaining an overall significance level of 5%.

2.To determine if toxicant and nicotine exposure is related to product factors such as the levels of these constituents (nicotine, NNK and NNN) in the products and patterns of use such as tins per week, dips per day, duration per dip, total dip duration (dip per day x duration per dip), weight per dip.

Pearson or Spearman correlations will assess the relationship between toxicant and nicotine exposure and the level of toxicants in the tobacco products. Furthermore, regression models will determine the degree of association between exposure and patterns of use, while controlling for the different ST brands. Specifically, total nicotine equivalents will be correlated with the free nicotine levels of the products and patterns of use; NNAL will be correlated with the NNK levels of the product and patterns of use; NNN exposure will be correlated with the amount of NNN in the product and patterns of use; and 1-HOP exposure will be correlated with the amount of pyrene and patterns of use.

Secondary Aims

3. To determine if exposure is also moderated by individual factors such as degree of dependence, nicotine metabolism, age of initiation and duration of use, negative affect and stress, amount of alcohol use and perception of ST harm and environmental and social factors such as use among social network, cost of the product, bans on tobacco use.

Analysis of covariance (ANCOVA) and regression methods will measure the impact of level of tobacco constituents (e.g., free nicotine, NNK, NNN, pyrene), ST Dependency score, nicotine metabolite ratio, age of initiation, duration of use, negative affect and depressed mood (PANAS negative affect score and CESD), stress (Perceived Stress Scale), amount of alcohol use (NIAAA Alcohol Use Questionnaire), perception of ST harm (Perceived Health Risk), social network (percent of social network who uses ST and who smokes), price of product and income, and tobacco bans (smoking and ST bans at work, school or home) on exposure measures (total nicotine equivalents, total NNAL, total NNN). Potential confounding factors such as site and as assessed by the Exposure and Lifestyle measures will be examined and controlled. The ST brand will be included in the model to access the main effects adjusted for product brand and to explore interactions between individual, environmental and social factors and brands on exposure measurements. For example, does a high ST Dependency score increase exposure to toxicants for some brands more than others?

4. To estimate the overall toxicant burden in the population by examining the extent of carcinogen exposure of the products that have the greatest market shares.

The analysis for this aim is an extension of primary aim 1. The market share for each of the six ST products will be represented as the percent of the total. Market share information will be obtained from the Maxwell Report. Using the mean toxicant exposure level for the six brands from the one way ANOVA, a weighted mean value will be calculated across the brands, with the percent of the market as the weight. This will be done for each biomarker. Thus, the more popular brands will have more influence on the overall exposure level.

C2. Power Considerations:

The goal is to enroll 100 subjects for each of the six brands listed in Table 2. The mean toxicant uptake level is expected to vary between brands; however, the standard deviation (SD) should

be similar for all brands. This is the common SD and is equivalent to the root of the Mean Square Error (MSE) from the one-way ANOVA. With a total of 600 subjects, this F-test will have 92% power to detect a significant difference at the 0.05 level if the brands with the largest and smallest means differ by at least one-half the common SD. Therefore, if the common SD for the biomarker was 10 units, then there would be a 92% chance that we find a significant difference between brands if the biggest and smallest means were at least 5 units apart. This analysis was done using Power Analysis and Sample Size (PASS) 2005 by NCSS, Inc.

D. References

[1] Gray N. The consequences of the unregulated cigarette. Tobacco Control. 2006;**15**(5):405-8 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16998176

[2] Burns DM, Dybing E, Gray N, Hecht S, Anderson C, Sanner T, et al. Mandated lowering of toxicants in cigarette smoke: a description of the World Health Organization TobReg proposal. Tobacco Control. 2008;**17**(2):132-41 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18375736

[3] Hatsukami D, Ebbert JO, Feuer RM, Stepanov I, Hecht SS. Changing smokeless tobacco products: new tobacco delivery systems. American Journal of Preventive Medicine. 2007;**33**(6S):S368-S78 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18021912

[4] Richter P, Hodge K, Stanfill S, Zhang L, Watson C. Surveillance of moist snuff total nicotine, pH, moisture, un-ionized nicotine, and tobacco-specific nitrosamine content. Nicotine & Tobacco Reseach. 2008;**10**(11):1645-52 http://www.informaworld.com.floyd.lib.umn.edu/10.1080/14622200802412937.

[5] Stratton K, Shetty P, Wallace R, Bondurant S, eds. Clearing the Smoke: Assessing the Science Base for Tobacco Harm Reduction. Institute of Medicine. Washington, DC: National Academy Press 2001.

[6] Benowitz N, Henningfield J. Establishing a nicotine threshold for addiction. New England Journal of Medicine. 1994;**331**:123-5 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7818638

[7] Hatsukami D, Lemmonds C, Tomar SL. Smokeless tobacco use: harm reduction or induction approach? Preventive Medicine. 2004;**38**(3):309-17 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14766113

[8] Fant RV, Henningfield JE, Nelson RA, Pickworth W. Pharmacokinetics and pharmacodynamics of moist snuff in humans. Tobacco Control. 1999;**8**(4):387-92. http://www.ncbi.nlm.nih.gov/htbin-post/Entrez/query?db=m&form=6&dopt=r&uid=10629244 http://www.tobaccocontrol.com/cgi/content/full/8/4/387 http://www.tobaccocontrol.com/cgi/content/abstract/8/4/387.

[9] Kotlyar M, Mendoza-Baumgart MI, Li ZZ, Pentel PR, Barnett BC, Feuer RM, et al. Nicotine pharmacokinetics and subjective effects of three potential reduced exposure products, moist snuff and nicotine lozenge. Tobacco Control. 2007;**16**(2):138-1342

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17400953

[10] Hatsukami D, Severson HH. Oral spit tobacco: addiction, prevention and treatment. Nicotine & Tobacco Research. 1999;**1**(1):21-44 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11072386

[11] Scientific Committee on Emerging and Newly Identified Health Risks. Health effects of smokeless tobacco products. Preliminary report; 2007 June 21-22.

[12] International Agency for Research on Cancer. Smokeless tobacco and some tobacco-specific N-nitrosamines. Lyon, France: IARC 2007.

[13] MacCoun RJ, Reuter P. Drug War Heresies: Learning From Other Vices, Times and Places. Cambridge, England: Cambridge University Press 2001.

[14] Hecht SS, Hatsukami D. Tobacco induced cancers and their prevention. In: Hong WK, Bast RC, Kufe DW, Weischselbaum RR, Hait WN, Pollock R, et al., eds. *Cancer Medicine, Edition 8*. Hamilton, Ontario, Canada: BC. Decker, Inc in press.

[15] Henningfield JE, Fant RV, Tomar SL. Smokeless tobacco: an addicting drug. Advances in Dental Research. 1997;**11**(3):330-5 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9524433

[16] Rao Y, Hoffmann E, Zia M, Bodin L, Zeman M, Sellers EM, et al. Duplications and defects in the CYP2A6 gene: identification, genotyping, and in vivo effects on smoking. Molecular Pharmacology. 2000;**58**(4):747-55 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10999944

[17] Strasser AA, Malaiyandi V, Hoffmann E, Tyndale RF, Lerman C. An association of CYP2A6 genotype and smoking topography. Nicotine & Tobacco Research. 2007;**9**(4):511-8 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17454707

[18] Ho MK, Tyndale RF. Overview of the pharmacogenomics of cigarette smoking. The Pharmacogenomics Journal. 2007;**7**(2):81-98 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17224913

[19] Mwenifumbo JC, Tyndale RF. Genetic variability in CYP2A6 and the pharmacokinetics of nicotine. Pharmacogenomics. 2007;**8**(10):1385-402 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17979512

[20] Malaiyandi V, Sellers EM, Tyndale RF. Implications of CYP2A6 genetic variation for smoking behaviors and nicotine dependence. Clinical Pharmacology and Therapeutics. 2005;**77**(3):145-58 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15735609

[21] Johnstone E, Benowitz N, Cargill A, Jacob R, Hinks L, Day I, et al. Determinants of the rate of nicotine metabolism and effects on smoking behavior. Clinical Pharmacology and Therapeutics. 2006;**80**(4):319-30 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17015050

[22] Benowitz N. Clinical pharmacology of nicotine: implications for understanding, preventing, and treating tobacco addiction. Clinical Pharmacology and Therapeutics. 2008;**83**(4):531-41 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18305452

[23] Bonnie RJ, Stratton K, Wallace RB, eds. Ending the Tobacco Problem. A Blueprint for the Nation. Washington, DC: The National Academies Press 2007.

[24] Mueller LL, Munk C, Thomsen BL, Frederiksen K, Kjaer SK. The influence of parity and smoking in the social environment on tobacco consumption among daily smoking women in Denmark. European Addiction Research. 2007;**13**(3):177-84 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17570914

[25] Lynch BS, Bonnie RJ, eds. Growing Up Tobacco Free: Preventing Nicotine Addiction in Children. Washington, DC: National Academy Press 1994.

[26] Warner KE, ed. Tobacco Control Policy, Robert Wood Johnson Foundation. San Francisco, CA: Jossey-Bass 2006.

[27] National Cancer Institute. The Role of the Media in Promoting and Reducing Tobacco Use. *Tobacco Control Monograph No 19*. Bethesda, Maryland: U.S. Department of Health and Human Services, National Institutes of Health, National Cancer Institute 2008.

[28] Wyckham RG. Smokeless tobacco in Canada: deterring market development. Tobacco Control. 1999;**8**(4):411-20 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10629248

[29] NIH. NIH State-of-the-Science Conference Statement on Tobacco Use: Prevention, Cessation, and Control. Annals of Internal Medicine. 2006;**145**:839-44 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16954353

[30] Idris AM, Ibrahim SO, Vasstrand EN, Johannessen AC, Lillehaug JR, Magnusson B, et al. The Swedish snus and the Sudanese toombak: are they different? Oral Oncology. 1998;**34**(6):558-66. http://www.ncbi.nlm.nih.gov/htbin-post/Entrez/query?db=m&form=6&dopt=r&uid=9930371.

[31] Osterdahl BG, Jansson C, Paccou A. Decreased levels of tobacco-specific N-nitrosamines in moist snuff on the Swedish market. Journal of Agricultural and Food Chemistry. 2004;**52**(16):5085-8 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15291479

[32] Hatsukami D, Lemmonds CA, Zhang Y, Murphy SE, Le C, Carmella SG, et al. Evaluation of carcinogen exposure in people who used "reduced exposure" tobacco products. Journal of the National Cancer Institute. 2004;**96**(11):844-52 http://www.ncbi.nlm.nih.gov/sites/entrez?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15173268.

[33] Lee PN. Circulatory disease and smokeless tobacco in Western populations: a review of the evidence. International Journal of Epidemiology. 2007;**36**(4):789-804 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17591642

[34] Connolly GN, Winn DM, Hecht SS, Henningfield JE, Walker B, Jr., Hoffmann D. The reemergence of smokeless tobacco. New England Journal of Medicine. 1986;**314**(16):1020-7 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3515184

[35] Russell MA. Low-tar medium-nicotine cigarettes: a new approach to safer smoking. British Medical Journal. 1976;**1**(6023):1430-3 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=953530

[36] Benowitz N, Jacob P, 3rd, Herrera B. Nicotine intake and dose response when smoking reduced-nicotine content cigarettes. Clinical Pharmacology and Therapeutics. 2006;**80**(6):703-14 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17178270

[37] Benowitz N, Hall SM, Stewart S, Wilson M, Dempsey D, Jacob P, 3rd. Nicotine and carcinogen exposure with smoking of progressively reduced nicotine content cigarette. Cancer Epidemiology, Biomarkers and Prevention. 2007;**16**(11):2479-85 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18006940

[38] Hatsukami D, Ebbert JO, Anderson A, Lin H, Le C, Hecht SS. Smokeless tobacco brand switching: A means to reduce toxicant exposure? Drug and Alcohol Dependence. 2007;**87**(2-3):217-24 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16996230

[39] Garcia-Closas M, Vermeulen R, Sherman ME, Moore LE, Smith MT, Rothman N. Application of biomarkers in cancer epidemiology. In: Schottenfeld D, Fraumeni Jr JF, eds. *Cancer Epidemiology and Prevention*. 3rd ed: Oxford University Press 2006:70-88.

[40] Boyle RG, Gerend MA, Peterson CB, Hatsukami DK. Use of smokeless tobacco by young adult females. Journal of Substance Abuse. 1998;**10**(1):19-25 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9720003

[41] Lemmonds CA, Hecht SS, Jensen JA, Murphy SE, Carmella SG, Zhang Y, et al. Smokeless tobacco topography and toxin exposure. Nicotine & Tobacco Research. 2005;**7**(3):469-74 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16085515

[42] Hatsukami D, Anton D, Callies A, Keenan R. Situational factors and patterns associated with smokeless tobacco use. Journal of Behavioral Medicine. 1991;**14**(4):383-96 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1942016

[43] Severson HH, Eakin EG, Lichtenstein E, Stevens VJ. The inside scoop on the stuff called snuff: an interview study of 94 adult male smokeless tobacco users. Journal of Substance Abuse. 1990;**2**(1):77-85

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2136105

[44] Hatsukami D, Keenan RM, Anton DJ. Topographical features of smokeless tobacco use. Psychopharmacology. 1988;**96**(3):428-9
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3146780

[45] Ayo-Yusuf OA, Swart TJ, Pickworth WB. Nicotine delivery capabilities of smokeless tobacco products and implications for control of tobacco dependence in South Africa. Tobacco Control. 2004;**13**(2):186-9
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15175538

[46] Richter P, Spierto FW. Surveillance of smokeless tobacco nicotine, pH, moisture, and unprotonated nicotine content. Nicotine & Tobacco Research. 2003;**5**(6):885-9
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14668072

[47] Boyle RG, Jensen J, Hatsukami DK, Severson HH. Measuring dependence in smokeless tobacco users. Addictive Behaviors. 1995;**20**(4):443-50
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7484325

[48] Ebbert JO, Patten CA, Schroeder DR. The Fagerstrom Test for Nicotine Dependence-Smokeless Tobacco (FTND-ST). Addictive Behaviors. 2006;**31**(9):1716-21
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16448783

[49] Ferketich AK, Wee AG, Shultz J, Wewers ME. Smokeless tobacco use and salivary cotinine concentration. Addictive Behaviors. 2007;**32**(12):2953-62
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17604914

[50] Thomas JL, Ebbert JO, Patten CA, Dale LC, Bronars CA, Schroeder DR. Measuring nicotine dependence among smokeless tobacco users. Addictive Behaviors. 2006;**31**(9):1511-21
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16386854

[51] Schroeder KL, Chen MS, Jr., Iaderosa GR, Glover ED, Edmundson EW. Proposed definition of a smokeless tobacco user based on "potential" nicotine consumption. Addictive Behaviors. 1988;**13**(4):395-400
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3239472

[52] Hecht SS, Carmella SG, Edmonds A, Murphy SE, Stepanov I, Luo X, et al. Exposure to nicotine and a tobacco-specific carcinogen increase with duration of use of smokeless tobacco. Tobacco Control. 2008;**17**(2):128-31
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18375734

[53] Walsh MM, Ellison J, Hilton JF, Chesney M, Ernster VL. Spit (smokeless) tobacco use by high school baseball athletes in California. Tobacco Control. 2000;**9 Suppl 2**:II32-9

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10841589

[54] Mumford EA, Levy DT, Gitchell JG, Blackman KO. Tobacco control policies and the concurrent use of smokeless tobacco and cigarettes among men, 1992-2002. Nicotine & Tobacco Research. 2005;**7**(6):891-900
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16298724

[55] Ohsfeldt RL, Boyle RG, Capilouto E. Effects of tobacco excise taxes on the use of smokeless tobacco products in the USA. Health Economics. 1997;**6**(5):525-31
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9353656

[56] Morrell HE, Cohen LM, Bacchi D, West J. Predictors of smoking and smokeless tobacco use in college students: a preliminary study using web-based survey methodology. Journal of American College Health. 2005;**54**(2):108-15
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16255323

[57] Coffey SF, Lombardo TW. Effects of smokeless tobacco-related sensory and behavioral cues on urge, affect, and stress. Experimental and Clinical Psychopharmacology. 1998;**6**(4):406-18
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9861555

[58] Vander Weg MW, DeBon M, Peterson AL, Sherrill-Mittleman D, Klesges RC, Relyea GE. Prevalence and correlates of lifetime smokeless tobacco use in female military recruits. Nicotine & Tobacco Research. 2005;**7**(3):431-41
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16085511

[59] Cohen-Smith D, Severson HH. A comparison of male and female smokeless tobacco use. Nicotine & Tobacco Research. 1999;**1**(3):211-8
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11072417

[60] Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nature Reviews Cancer. 2003;**3**(10):733-44
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14570033

[61] Hatsukami D, Benowitz NL, Rennard SI, Oncken C, Hecht SS. Biomarkers to assess the utility of potential reduced exposure tobacco products. Nicotine & Tobacco Research. 2006;**8**(4):599-622
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16920658

[62] Stepanov I, Hecht SS. Tobacco-specific nitrosamines and their pyridine-N-glucuronides in the urine of smokers and smokeless tobacco users. Cancer Epidemiology, Biomarkers and Prevention. 2005;**14**(4):885-91
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15824160

[63] Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. Chemical Research in Toxicology. 1998;**11**(6):559-603
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9625726

[64] Hecht SS. Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. Carcinogenesis. 2002;**23**(6):907-22
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12082012

[65] Chen L, Wang M, Villalta PW, Luo X, Feuer R, Jensen J, et al. Quantitation of an acetaldehyde adduct in human leukocyte DNA and the effect of smoking cessation. Chemical Research in Toxicology. 2007;**20**(1):108-13
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17226933

[66] Carmella SG, Chen M, Zhang Y, Zhang S, Hatsukami D, Hecht SS. Quantitation of acrolein-derived (3-hydroxypropyl)mercapturic acid in human urine by liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry: effects of cigarette smoking. Chemical Research in Toxicology. 2007;**20**(7):986-90
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17559234

[67] Hatsukami D, Slade J, Benowitz NL, Giovino GA, Gritz ER, Leischow S, et al. Reducing tobacco harm: research challenges and issues. Nicotine & Tobacco Research. 2002;**4 Suppl 2**:S89-101
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12573171

[68] Idris AM, Prokopczyk B, Hoffmann D. Toombak: a major risk factor for cancer of the oral cavity in Sudan. Preventive Medicine. 1994;**23**(6):832-9
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7855117

[69] WHO. Tobacco or health: a global status report; country profiles by region, 1997. *WHO Tobacco or Health Programme*. Geneva 1997.

[70] Stepanov I, Hecht SS, Ramakrishnan S, Gupta PC. Tobacco-specific nitrosamines in smokeless tobacco products marketed in India. International Journal of Cancer. 2005;**116**(1):16-9
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15756678

[71] Hatsukami D, Anton D, Keenan R, Callies A. Smokeless tobacco abstinence effects and nicotine gum dose. Psychopharmacology. 1992;**106**:60-6
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1738794

[72] Hatsukami D, Grillo M, Boyle R, Allen S, Jensen J, Bliss R, et al. Treatment of spit tobacco users with transdermal nicotine system and mint snuff. Journal of Consulting and Clinical Psychology. 2000;**68**(2):241-9
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10780124

[73] Hatsukami D, Jensen J, Allen S, Grillo M, Bliss R. The effects of behavioral and pharmacological treatment on smokeless tobacco users. Journal of Consulting and Clinical Psychology. 1996;**64**(1):153-61
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8907095

[74] Stepanov I, Jensen J, Hatsukami D, Hecht SS. Tobacco-specific nitrosamines in new tobacco products. Nicotine & Tobacco Research. 2006;**8**(2):309-13
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16766423

[75] Stepanov I, Jensen J, Hatsukami D, Hecht SS. New and traditional smokeless tobacco: comparison of toxicant and carcinogen levels. Nicotine & Tobacco Reseach. in press.

[76] Hecht SS, Carmella SG, Ye M, Le KA, Jensen JA, Zimmerman CL, et al. Quantitation of metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone after cessation of smokeless tobacco use. Cancer Research. 2002;**62**(1):129-34
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11782369

[77] Hatsukami D, Edmonds A, Schulte S, Jensen J, Le C, Losey L, et al. Preliminary study on reducing oral moist snuff use. Drug and Alcohol Dependence. 2003;**70**(2):215-20
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12732415

[78] Hatsukami D, Ebbert JO, Edmonds A, Li C, Lin H, Le C, et al. Smokeless tobacco reduction: preliminary study of tobacco-free snuff versus no snuff. Nicotine & Tobacco Research. 2008;**10**(1):77-85
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18188748

[79] Hatsukami D, Giovino GA, Eissenberg T, Clark PI, Lawrence D, Leischow S. Methods to assess potential reduced exposure products. Nicotine & Tobacco Research. 2005;**7**(6):827-44
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16298718

[80] Hecht SS, Carmella SG, Le K, Murphy SE, Li YS, Le C, et al. Effects of reduced cigarette smoking on levels of 1-hydroxypyrene in urine. Cancer Epidemiology, Biomarkers and Prevention. 2004;**13**(5):834-42
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15159317

[81] Hecht SS, Murphy SE, Carmella SG, Zimmerman CL, Losey L, Kramarczuk I, et al. Effects of reduced cigarette smoking on the uptake of a tobacco-specific lung carcinogen. Journal of the National Cancer Institute. 2004;**96**(2):107-15
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14734700

[82] Murphy SE, Link CA, Jensen J, Le C, Puumala SS, Hecht SS, et al. A comparison of urinary biomarkers of tobacco and carcinogen exposure in smokers. Cancer Epidemiology, Biomarkers and Prevention. 2004;**13**(10):1617-23
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15466978

[83] Hecht SS, Carmella SG, Yoder A, Chen M, Li ZZ, Le C, et al. Comparison of polymorphisms in genes involved in polycyclic aromatic hydrocarbon metabolism with urinary phenanthrene metabolite ratios in smokers. Cancer Epidemiology, Biomarkers and Prevention. 2006;**15**(10):1805-11 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17035385

[84] Hatsukami D, Kotlyar M, Allen S, Jensen J, Li S, Le C, et al. Effects of cigarette reduction on cardiovascular risk factors and subjective measures. Chest. 2005;**128**(4):2528-37 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16236919

[85] Hatsukami D, Rennard S, Jorenby D, Fiore M, Koopmeiners J, de Vos A, et al. Safety and immunogenicity of a nicotine conjugate vaccine in current smokers. Clinical Pharmacology and Therapeutics. 2005;**78**:456-67 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16321612

[86] Hecht SS, Murphy SE, Carmella SG, Li S, Jensen J, Le C, et al. Similar uptake of lung carcinogens by smokers of regular, light, and ultralight cigarettes. Cancer Epidemiology, Biomarkers and Prevention. 2005;**14**(3):693-8 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15767351

[87] Hecht SS. Tobacco smoke carcinogens and lung cancer. Journal of National Cancer Institute. 1999;**91**:1194-210 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10413421

[88] Hecht SS, Hoffmann D. Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. Carcinogenesis. 1988;**9**(6):875-84 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3286030.

[89] Hoffmann D, Hecht SS, Ornaf RM, Wynder EL. N'-nitrosonornicotine in tobacco. Science. 1974;**186**(4160):265-7 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=4414773

[90] Hecht SS, Carmella SG, Murphy SE, Riley WT, Le C, Luo X, et al. Similar exposure to a tobacco-specific carcinogen in smokeless tobacco users and cigarette smokers. Cancer Epidemiology, Biomarkers and Prevention. 2007;**16**(8):1567-72 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17684130

[91] Severson HH. Adolescent social drug use: School prevention programs. School Psychology Review. 1984;**13**(2):150-61.

[92] Severson HH, Glasgow R, Wirt R, Brozovsky P, Zoref L, Black C, et al. Preventing the use of smokeless tobacco and cigarettes by teens: Results of a classroom intervention. Health Education Research. 1991;**6**(1):109-20.

[93] Severson HH, Lichtenstein E. Smoking prevention programs for adolescents: Rationale and review. In: Krasnegor N, Araseth J, Cataldo M, eds. *Child Health Behavior*. New York: John Wiley 1986.

[94] Eakin E, Severson H, Glasgow RE. Development and evaluation of a smokeless tobacco cessation program: A pilot study. NCI Monograph. 1989;**8**:95-100
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2716855

[95] Severson HH, Andrews JA, Lichtenstein E, Gordon JS, Barckley M, Akers L. A self-help cessation program for smokeless tobacco users: comparison of two interventions. Nicotine & Tobacco Research. 2000;**2**(4):363-70
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11197317

[96] Severson HH. Tobacco control research: a journey of discovery in prevention and cessation. American Journal of Health Behavior. 2006;**30**(6):567-79
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17096614

[97] Severson HH, Andrews JA, Lichtenstein E, Danaher BG, Akers L. Self-help cessation programs for smokeless tobacco users: long-term follow-up of a randomized trial. Nicotine & Tobacco Research. 2007;**9**(2):281-9
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17365759

[98] Hatsukami D, Kotlyar M, Hertsgaard LA, Zhang Y, Carmella SG, Jensen J, et al. Reduced nicotine content cigarettes: Effects on toxicant exposure, dependence and cessation. in submission.

[99] Mooney ME, Li ZZ, Murphy SE, Pentel PR, Le C, Hatsukami DK. Stability of the nicotine metabolite ratio in ad libitum and reducing smokers. Cancer Epidemiology, Biomarkers and Prevention. 2008;**17**(6):1396-400
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18559554

[100] Hatsukami D, Joseph AM, Lesage M, Jensen J, Murphy SE, Pentel PR, et al. Developing the science base for reducing tobacco harm. Nicotine & Tobacco Research. 2007;**9 Suppl 4**:S537-53
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18067031

[101] Severson HH, Andrews JA, Lichtenstein E, Danaher BG, Akers L. Self-help cessation programs for smokeless tobacco users: Long-term follow-up of a randomized trial. Nicotine & Tobacco Research. in press.

[102] Severson HH, Akers L, Andrews JA, Lichtenstein E, Jerome A. Evaluating two self-help interventions for smokeless tobacco cessation. Addictive Behaviors. 2000;**25**(3):465-70
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10890303

[103] Etter JF, Le Houezec J, Perneger TV. A self-administered questionnaire to measure dependence on cigarettes: the cigarette dependence scale. Neuropsychopharmacology. 2003;**28**(2):359-70
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12589389

[104] Spitzer RL, Kroenke K, Williams JB. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire. JAMA. 1999;**282**(18):1737-44

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10568646

[105] Radloff LS. The CES-D Scale: A self-report depression scale for research in the general population. Applied Psychological Measurement. 1977;**1**:385-401.

[106] Selzer ML. The Michigan alcoholism screening test: the quest for a new diagnostic instrument. American Journal of Psychiatry. 1971;**127**(12):1653-8 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=5565851

[107] Task Force on Recommended Alcohol Questions. National Council on Alcohol Abuse and Alcoholism recommended sets of alcohol consumption questions. Bethesda, MD: National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health; 2003 October 15-16.

[108] Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: The PANAS scales. Journal of Personality and Social Psychology. 1988;**54**:324-33 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3397865

[109] Kenford S, Smith S, Wetter D, Jorenby D, Fiore M, Baker T. Predicting relapse back to smoking: contrasting affective and physical models of dependence. Journal of Consulting and Clinical Psychology. 2002;**70**(1):216-27 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11860048

[110] Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. Journal of Health and Social Behavior. 1983;**24**(4):385-96 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=6668417

[111] Warner DO, Patten CA, Ames SC, Offord KP, Schroeder DR. Effect of nicotine replacement therapy on stress and smoking behavior in surgical patients. Anesthesiology. 2005;**102**(6):1138-46 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15915026

[112] Cohen S, Lichtenstein E. Perceived stress, quitting smoking, and smoking relapse. Health Psychology. 1990;**9**(4):466-78 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2373070

[113] Cohen S, Williamson G. Perceived stress in a probability sample of the United States. In: Spacapan S, Oskamp S, eds. *The Social Psychology of Health: Clarement Symposium on Applied Social Psychology*. Newbury Park, CA: Sage 1988:31-68.

[114] Hatsukami D, McBride C, Pirie P, Hellerstedt W, Lando H. Effects of nicotine gum on prevalence and severity of withdrawal in female cigarette smokers. Journal of Substance Abuse. 1991;**3**(4):427-40 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1821296

[115] Hughes JR, Gust SW, Skoog K, Keenan RM, Fenwick JW. Symptoms of tobacco withdrawal: a replication and extension. Archives of General Psychiatry. 1991;**48**:52-9

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1984762

[116] Hughes JR, Hatsukami D. Signs and symptoms of tobacco withdrawal. Archives of General Psychiatry. 1986;**43**:289-94
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3954551

[117] Hatsukami D, Huber M, Callies A. Physical dependence on nicotine gum: effect of duration on use. Psychopharmacology. 1993;**111**:449-56
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7870986

[118] Hatsukami D, Gust SW, Keenan R. Physiologic and subjective changes from smokeless tobacco withdrawal. Clinical Pharmacology and Therapeutics. 1987;**41**:103-7
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3802698

[119] Baer JS, Lichtenstein E. Classification and prediction of smoking relapse episodes: an exploration of individual differences. Journal of Consulting and Clinical Psychology. 1988;**56**(1):104-10
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3346434.

[120] Westman EC, Levin ED, Rose JE. Smoking while wearing the nicotine patch: Is smoking satisfying or harmful? Clinical Research. 1992;**40**:871A.

[121] Brauer L, Behm F, Lane J, Westman E, Perkins C, Rose J. Individual differences in smoking reward from de-nicotinized cigarettes. Nicotine & Tobacco Research. 2001;**3**:101-9
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11403723

[122] Rose JE, Behm FM. Inhalation of vapor from black pepper extract reduces smoking withdrawal symptoms. Drug and Alcohol Dependence. 1994;**34**:225-9
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8033760

[123] Rose JE, Behm FM, Westman EC. Acute effects of nicotine and mecamylamine on tobacco withdrawal symptoms, cigarette reward and ad lib smoking. Pharmacology, Biochemistry and Behavior. 2001;**68**:187-97
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11267622

[124] Routt Reigart J, Roberts JR. Environmental and occupational history. In: Routt Reigart JR, Roberts JR, eds. *Recognition and Management of Pesticide Poisonings*. Washington, DC: U.S. Environmental Protection Agency 1999:17-32.

[125] WHO. International Physical Activity Questionnaire (IPAQ). 1997.

[126] Asplund K. Smokeless tobacco and cardiovascular disease. Progress in Cardiovascular Diseases. 2003;**45**(5):383-94
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12704595

[127] Hergens MP, Ahlbom A, Andersson T, Pershagen G. Swedish moist snuff and myocardial infarction among men. Epidemiology. 2005;**16**(1):12-6
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15613940

[128] Hergens MP, Alfredsson L, Bolinder G, Lambe M, Pershagen G, Ye W. Long-term use of Swedish moist snuff and the risk of myocardial infarction amongst men. Journal of Internal Medicine. 2007;**262**(3):351-9
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17697156

[129] Hergens MP, Lambe M, Pershagen G, Ye W. Risk of hypertension amongst Swedish male snuff users: a prospective study. Journal of Internal Medicine. 2008;**264**(2):187-94
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18393959

[130] Malaiyandi V, Goodz SD, Sellers EM, Tyndale RF. CYP2A6 genotype, phenotype, and the use of nicotine metabolites as biomarkers during ad libitum smoking. Cancer Epidemiology, Biomarkers and Prevention. 2006;**15**(10):1812-9
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17035386

[131] Benowitz N, Pomerleau OF, Pomerleau CS, Jacob P, 3rd. Nicotine metabolite ratio as a predictor of cigarette consumption. Nicotine & Tobacco Research. 2003;**5**(5):621-4
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14577978

[132] Dempsey D, Tutka P, Jacob P, 3rd, Allen F, Schoedel K, Tyndale RF, et al. Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. Clinical Pharmacology and Therapeutics. 2004;**76**(1):64-72
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15229465

[133] Levi M, Dempsey DA, Benowitz NL, Sheiner LB. Prediction methods for nicotine clearance using cotinine and 3-hydroxy-cotinine spot saliva samples II. Model application. Journal of Pharmacokinetics and Pharmacodynamics. 2007;**34**(1):23-34
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17206525

[134] Carmella SG, Han S, Fristad A, Yang Y, Hecht SS. Analysis of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in human urine. Cancer Epidemiology, Biomarkers and Prevention. 2003;**12**(11 Pt 1):1257-61
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14652291

[135] Carmella SG, Le KA, Hecht SS. Improved method for determination of 1-hydroxypyrene in human urine. Cancer Epidemiology, Biomarkers and Prevention. 2004;**13**(7):1261-4
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15247141

[136] Derave W, Vanden Eede E, Hespel P, Carmella SG, Hecht SS. Oral creatine supplementation in humans does not elevate urinary excretion of the carcinogen N-nitrososarcosine. Nutrition. 2006;**22**(3):332-3

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16413752

[137] Carmella SG, Kagan SS, Kagan M, Foiles PG, Palladino G, Quart AM, et al. Mass spectrometric analysis of tobacco-specific nitrosamine hemoglobin adducts in snuff dippers, smokers, and nonsmokers. Cancer Research. 1990;**50**(17):5438-45
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2386948

[138] Critchley JA, Unal B. Is smokeless tobacco a risk factor for coronary heart disease? A systematic review of epidemiological studies. European Journal of Cardiovascular Prevention and Rehabilitation. 2004;**11**(2):101-12
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15187813

[139] Khurana M, Sharma D, Khandelwal PD. Lipid profile in smokers and tobacco chewers--a comparative study. Journal of the Association of Physicians of India. 2000;**48**(9):895-7
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11198789

[140] Gupta BK, Kaushik A, Panwar RB, Chaddha VS, Nayak KC, Singh VB, et al. Cardiovascular risk factors in tobacco-chewers: a controlled study. Journal of the Association of Physicians of India. 2007;**55**:27-31
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17444341

[141] Bolinder G, Alfredsson L, Englund A, de Faire U. Smokeless tobacco use and increased cardiovascular mortality among Swedish construction workers. American Journal of Public Health. 1994;**84**(3):399-404
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8129055

[142] Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA. 1998;**279**(18):1477-82
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9600484

[143] Hackam DG, Anand SS. Emerging risk factors for atherosclerotic vascular disease: a critical review of the evidence. JAMA. 2003;**290**(7):947-9
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12928471

[144] Folsom AR, Aleksic N, Catellier D, Juneja H, Wu K. C-reactive protein and incident coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) study. American Heart Journal. 2002;**144**:233-8
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12177639

[145] Folsom AR. "New" risk factors for atherosclerotic diseases. Experimental Gerontology. 1999;**34**:483-90
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11012298

[146] Wilkes HC, Kelleher C, Meade TW. Smoking and plasma fibrinogen. Lancet. 1988;**1**:307-8 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2893128

[147] Petitti D, Kipp H. The leukocyte count: associations with intensity of smoking and persistence of effect after quitting. American Journal of Epidemiology. 1986;**123**(1):89-5 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3940445

[148] Sunyer J, Munoz A, Peng Y, Margolick J, Chmiel JS, Oishi J, et al. Longitudinal relation between smoking and white blood cells. American Journal of Epidemiology. 1996;**144**(8):734-41. http://www.ncbi.nlm.nih.gov/htbin-post/Entrez/query?db=m&form=6&dopt=r&uid=8857822.

[149] Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PA, et al. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. Human Molecular Genetics. 2007;**16**(1):36-49 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17135278

[150] Bierut LJ, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau OF, et al. Novel genes identified in a high-density genome wide association study for nicotine dependence. Human Molecular Genetics. 2007;**16**(1):24-35 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17158188

[151] Alpert HR, Koh H, Connolly GN. Free nicotine content and strategic marketing of moist snuff tobacco products in the United States: 2000-2006. Tobacco Control. 2008;**17**(5):332-8 http://tobaccocontrol.bmj.com/cgi/content/abstract/17/5/332

[152] Andrews JA, Severson HH, Akers L, Lichtenstein E, Barckley M. Who enrolls in a self-help cessation program for smokeless tobacco? Addictive Behaviors. 2001;**26**(5):757-64 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11676385

[153] Mendes P, Kapur S, Wang J, Feng S, Roethig H. A randomized, controlled exposure study in adult smokers of full flavor Marlboro cigarettes switching to Marlboro Lights or Marlboro Ultra Lights cigarettes. Regulatory Toxicology and Pharmacology. 2008;**51**(3):295-305 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18565634

[154] Roethig HJ, Feng S, Liang Q, Liu J, Rees WA, Zedler BK. A 12-month, randomized, controlled study to evaluate exposure and cardiovascular risk factors in adult smokers switching from conventional cigarettes to a second-generation electrically heated cigarette smoking system. Journal of Clinical Pharmacology. 2008;**48**(5):580-91 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18319361

[155] Sobell LC, Sobell MB. Timeline follow-back. A technique for assessing self-reported alcohol consumption. In: Litten R, Allen J, eds. *Measuring Alcohol Consumption Psychosocial and Biochemical Methods*. New York: Springer-Verlag 1992:41-69.

[156] Roese NJ, Jamieson DW. Twenty years of bogus pipeline research: a critical review and meta-analysis. Psychological Bulletin. 1993;**114**(2):363-75.