Human Smoking Behavior Study

(Cigarette Yield and Body Burden of Smoke Toxins)

OMB No. 0920-0736

Request for Reinstatement with Change

SUPPORTING STATEMENT: PART B

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B.1 Respondent Universe and Sampling Methods

Participants will be established smokers, defined as smoking daily for at least two years, smoking a **minimum of 6 and a maximum of 60 cigarettes per day**, and legal smokers aged 18 or older. In addition, participants must be current smokers of brands in the most popular U.S. cigarette categories (for at least 3 months)

Data collection was approximately two-thirds complete as of the expiration date of the original OMB approval (0920-0736, exp. 3/31/2010). CDC is requesting two years of OMB approval to Reinstate and complete the information collection.

The study will be conducted in the Human Exposure Assessment Laboratory of Battelle Centers for Public Health Research and Evaluation, in Baltimore, MD. We estimate screening approximately 300 participants to yield the remaining 122 who complete both visits. Additional participants will be recruited as needed to account for drop outs, "no shows" and non-compliance. In the event that a respondent completes part of the protocol, then decides not to continue, (s)he will be replaced with another eligible respondent.

The overall study plan requires collection of information from 360 respondents, with equal representation of smokers who use cigarettes across a wide range of smoking-machine determined toxin deliveries (as determined by machine-smoked tar levels). Based on power analyses we project that information collection from 90 respondents in each group will be sufficient to detect small correlations with power of at least .80 and a significance level of .05. Additional detailed information on study design is provided below.

***Overview of Analysis Plan*.**

Power Analysis. Power analyses were conducted in two ways: Specificity analysis of sample size needed to detect differences across the range of effect sizes and a post-hoc approach providing a sample size parameter (N = 360) and determining the level of power associated with each level of effect.

Specificity Analysis. A specificity analysis of sample sizes needed to detect differences across the range of effect sizes was conducted for Aim 1. Cohen (1988) recommends that statistical power for clinical research should be equal to or greater than .80; therefore this standard was employed.[18] With a two-tailed test, an alpha level of .05, and a power of .80, the ability to detect small, medium and large effects is estimated to require 779, 82, 26 individuals, respectively (Table 1). This range of samples sizes will accurately detect the effects of machine-smoked tar level on body burden if the relationship is linear, with 95% confidence. Table 1. Sample size estimates by effect sizes needed to detect a correlation between body burden of toxins and machine-smoked tar levels.

Effect sizes	N
Small (.10)	779
Moderate (.30)	82
Large (.50)	26

*Based on an alpha level of .05, power of >.80, and two-sided test

Post-hoc Power Determination. A post-hoc approach was used to determine the power to detect a significant correlation given the proposed sample of 360 subjects. With a two-tailed test, an alpha level of .05, and n = 360, the power to detect small, medium and large effects is .48, >.99, >.99, respectively (Table 2). The power to detect a small effect (p=.48) is below standard conventions, but is adequate to determine moderate to strong effects (>.99). This range of samples sizes will accurately detect the effects of machine-smoked tar levels on body burden if the relationship is linear, with 95% confidence.

Table 2. Sample size estimates by effect sizes needed to detect a correlation between body burden of smoke toxins and machine-smoked tar levels.

Effect sizes	Power level
Small (.10)	.48
Moderate (.30)	>.99
Large (.50)	>.99

*Based on N = 360, an alpha level of .05, and two-sided test

Because our first hypothesis is directional, that is, that we hypothesize a positive correlation between machine-smoked tar level and body burden of smoke toxins, a one-sided test is more appropriate. In the case of a one-sided test, the sample of 360 is sufficient for detecting a correlation coefficient as low as 0.13 with power of .80 and significance level of .05.

Aim 2 seeks to determine if the relationship between the body burden of smoke toxins and the machine-smoked tar and nicotine levels is modified by smoking behavior. For this aim, we conducted a power analysis for multiple regression. For this analysis, we will control for potential confounding variables (i.e., age, gender, and number of cigarettes smoked per day). A power analysis for ordinary least-squares (OLS) multiple regression with 6 predictor variables, a significance level of .05, a baseline R^2 of 0.35, and power of at least .80, indicates that a sample size of 359 will detect an R^2 difference as small as 0.014.

For Aim 3, to determine if a positive relationship exists between the machine-smoked tar level and solanesol levels in spent cigarette filters, we conducted a power analysis for correlation with one-sided test. Again, because our hypothesis is uni-directional (to test for a positive correlation between the two measures), one-sided tests are most appropriate. The sample size of 360 should be sufficient for detecting a correlation coefficient as small as .13 with power of at least .80 and a significance level of .05. A sample of 360 subjects is more than adequate to detect even small correlations and changes in a baseline R^2.

Exploratory analyses. Missing values will be identified and verified as truly missing by comparison with source documents. The distributions of the continuous variables will be explored for shape (skew, spread, and normalcy) through stem and leaf plots and Q-Q plots. Outliers will be assessed with box plots and stem and leaf plots. All outliers will be checked against source documents.

Demographic distributions (e.g., age, gender) will be summarized using descriptive statistics (i.e., frequencies, percentages). Descriptive statistics will also be conducted to determine means, ranges, confidence bounds and standard errors of study variables.

Bi- and Multivariable Modeling.

Aim 1: To determine if biomarkers of exposure to cigarette-delivered toxins and measures of cardiovascular reactivity vary in proportion to machine-smoked tar levels.

Pearson Product Moment Correlations (PPMC) will be conducted to determine the relation between FTC machine yield tar and 22 identified biomarkers of smoke exposure. Correlations will also be run on machine-smoked tar yields and measures of cardiovascular reactivity. In order to correct for the inflation of alpha with multiple comparisons, the individual alpha level for each comparison will be adjusted using the Bonferroni correction approach. Proposed conventions for effect sizes based on correlation are: .10 = small, .30 = moderate, .50 = large.[18]

Aim 2: To determine if measures of how the cigarette is smoked (i.e., puff parameters and inhalation patterns) moderate the effects of machine-smoked yields of tar on biomarkers of exposure.

Given that there is a significant relation between machine-smoked tar yield generated by smoking machines under FTC conditions and individual biomarkers of exposure, the analytic technique of multiple regression will be used to determine if smoking behavior modifies that relationship. For this analysis, the predictor variables, FTC machine yield tar and smoking behavior variables (e.g. total puff volume) will be entered in a multiple regression model, controlling for potential confounder variables (e.g., age, gender, cigarettes per day). The dependent variable for this analysis will be the levels of specific biomarkers. The first step in this process will be to center first order predictor variables to zero. Then a cross product term of those predictor variables will be created. A multiple regression model, based on the cross product of those two variables, will then be conducted to determine if there is an interaction between measures of smoking behavior and level of tar on the level of biomarkers. The presence

of a significant interaction for this cross-product would indicate the presence of moderation for the relation between FTC tar and biomarker exposure. Further examination of the data would occur, examining simple effects. This will allow us to determine how smoking behavior may affect the outcome of biomarker body burden at differing levels of FTC machine based tar (e.g., low, middle, high). In order to correct for the inflation of alpha with multiple comparisons, the individual alpha level for each comparison will be adjusted using the Bonferroni correction approach. Proposed conventions for effect sizes based on the multiple regression approach are: .02 = small, .15 = moderate, .35 = large.[18]

Aim 3: To determine if there is a significant relation between cigarette yield category expressed as machine-smoked yields of tar and solanesol levels in human-smoked cigarette filters (a measure of mouth level exposure to tobacco smoke), and if solanesol levels are significantly associated with levels of carcinogenic and toxic biomarkers.

The approach to analyze Hypothesis 3 is similar to that used for Hypothesis 1, such that Pearson Product Moment Correlations (PPMC) will be conducted to determine the statistical relationship between machine-smoked yields of tar and levels of solanesol derived from human-smoked cigarette filters. Proposed conventions for effect sizes based on correlation are: .10 = small, .30 = moderate, .50 = large.[18]

Potential Covariates. The published literature supports that some smoking behavior differs as a function of gender and ethnicity. [19-22] Other factors such as number of cigarettes smoked per day may also impact smoking behavior. Multiple regression allows for statistical control of potential covariates such as these. Based on the above literature, age, gender, and cigarettes smoked per day will be introduced as covariates in the analyses to test Hypothesis #2. These analyses are designed to reduce the amount of error that can be accounted for by the covariate, resulting in increased statistical power.[23]

B.2 Procedures for the Collection of Information

This study will examine the influence of cigarette yield category on smoke exposure through biomarkers, smoking characteristics, inhalation parameters and physiologic measures, such as heart rate and blood pressure, and solanesol (a chemical trapped in cigarette filter butts that provides an estimate of total smoke intake). Data will be collected from cigarettes from the three major yield categories while smoked in both naturalistic and laboratory settings. The study is designed to detect both immediate and longer-term smoking-related changes in smokers. The laboratory session provides essential data that will allow us to capture short-lived biomarkers of exposure, observe smoking behavior, and investigate the agreement between solanesol levels in the butts of cigarettes smoked under both naturalistic and laboratory settings.

Participants will be recruited through a variety of print advertisements and word-of-mouth, screened for eligibility via a brief telephone interview, consented, and enrolled in the study. Each subject will participate in 2 consecutive laboratory clinic visits separated by about 30 hours. The first visit will take place between 8 am and 11 am, and the second visit will take place between 1 pm and 5 pm. Subjects will be asked to arrive at the laboratory clinic “wanting” a cigarette. This method should catch all participants at the same relative stage of craving. Because craving

states vary by individual (i.e. two hours to one individual may not elicit much craving, whereas 2 hours to another individual may elicit strong craving), it is not appropriate to demand a pre-determined period of abstinence prior to appointments.

Urine and saliva samples will be collected from participants upon their arrival at the clinic and stored for later determination of levels of biomarkers of exposure with long half lives (carcinogens, nicotine metabolites, heavy metals). Biomarkers with long half-lives reflect subjects' usual smoking patterns under every day conditions. For example, the elimination half-life of NNAL, a carcinogen and metabolite of the tobacco-specific nitrosamine NNK, is approximately 40 days.[24] Cotinine, the major metabolite of nicotine, has a half-life of 15-40 hours.[25] Biomarkers of acute exposure, expired air carbon monoxide boost (the difference between CO levels before and after smoking a cigarette), and markers of cardiovascular reactivity (blood pressure, heart rate), change quickly in response to smoking and must be measured in a clinic environment. **Attachment C** contains a matrix of the biomarkers proposed for this study. The Human Exposure Assessment Laboratory (Battelle) has the capability of measuring carbon monoxide boost and markers of cardiovascular reactivity and of collecting all biomarker samples, and the Division of Laboratory Science, Centers for Disease Control and Prevention laboratory has the capability of testing all biomarkers proposed for this study.

Participants will be asked to don a vest that measures inhalation and heart rate through sensors imbedded in the vest, and smoking behavior will be measured by having the subject smoke one of his/her own cigarettes through a holder connected to a CreSS® puff analyzer. In addition, smoking sessions will be video taped, and all cigarette butts will be collected over the approximate 30-hour test period between visits for analysis of solanesol levels, ventilation hole blocking behavior, and as a check for compliance. Collected butts will be used to estimate the persistence of ventilation hole blocking behavior over several cigarettes. Ventilation hole blocking of these cigarettes will be approximated using the filter stain technique of analyzing the stain pattern of the filter tip. Urine samples, saliva samples, and collected butts will be shipped to the CDC in Atlanta, Georgia for analyses.

Battelle, contractor, has standard procedures for training study personnel, including training regarding data collection, recording, tracking and scanning processes, working with human subjects, biohazard and pathogen certification, International Air Transport Association (IATA) certification, and study-specific training. A study-specific comprehensive training and procedures session will be developed to include sections for training staff in screening calls, collecting biological samples and puff data, heart rate and respiration data, operating the smoking behavior machine and coordinating the study. Staff will be trained and activities monitored through a series of Standard Operating Procedures. This training and monitoring will include sampling and analysis procedures for urine, saliva, as well as procedures for sample storage and shipping. Measurement and documentation procedures for expired-air carbon monoxide and smoking behavior will also be included. Standard Operating Procedures will be printed in a manual stored at the laboratory clinic. All recruitment and data collection forms, along with scripts, answers to frequently asked questions, and suggestions for handling problems that may arise, will be included. Standard quality control procedures will be implemented to monitor study progress and staff performance.

B.3 Methods to Maximize Response Rates and Deal with Nonresponse

We will recruit smokers in Baltimore, Maryland, by placing study advertisements in the local newspapers and posting study flyers on public bulletin boards at local places of business, college campuses and libraries (**Attachment F – Recruitment Materials**). In order to accommodate recruitment of hard-to-reach subjects, placement of advertisements will be timed to help assure a steady flow of volunteers.

For this project, we may also seek the cooperation of student organizations at Towson University and similar groups in the Baltimore community.

Because we anticipate difficulty recruiting participants for the lowest tar category, we will implement strategies to discover and invite this population of smokers. One strategy will include recruiting this population at bingo parlors, bus stops, malls, bowling alleys and other areas where smokers gather. Referral incentives will be provided for subjects referring friends, who fit the eligibility criteria and enrolls in the study.

To compensate each study subject for his/her time and inconvenience, remuneration will be according to the schedule shown in Table A.9-1. Because completion of each visit represents a considerable investment of study resources, and subjects who drop out or are non-compliant after one or two visits must be replaced entirely, we plan on escalating reimbursements for each completed visit.

B.4 Tests of Procedures or Methods to be Undertaken

The study activity protocol and procedures for implementing it were reviewed and approved by the IRB committees of both CDC and Battelle. All but two techniques were used and established in our previous study (Menthol Crossover conducted between November 2003 and July 2004). The standard operating procedures for all techniques are attached to the protocol and are separately placed in the laboratory clinic. The screening instruments (**Attachments D and E**-1) have been evaluated in order to determine that questions are worded clearly and can be easily understood by subjects of different socio-economic levels and that questions are comprehensible and unambiguous. Enough questions are asked to provide adequate material for analysis without making the questions burdensome. Questions are designed to obtain a definite response without influencing the subject's answer.

One procedure that was not done previously is a method for determining mouth level exposure to smoke toxicants based on solanesol levels in the used cigarette filter butt. Solanesol is a long-chain terpenoid naturally present in tobacco. A 1-cm portion of the cigarette filter, measured from the mouth end, is removed from the cigarette butts for analysis. Solanesol is extracted from the filter butt and then analyzed using liquid chromatography coupled with a single-quadrupole mass analyzer. Mouth-level exposure to smoke toxicants such as nicotine and tobacco-specific nitrosamines may be estimated by their relation to the solanesol retained in the cigarette filter.

The second procedure not included in the previous study is measurement of heart rate and respirations with sensors imbedded in a vest. The system allows real time assessment of an array of physiologic parameters. An electrocardiogram is recorded by means of three electrodes placed directly onto the skin on the upper chest and on the lateral surface of the abdomen. This standard configuration provides a single lead for heart rate and ECG waveform determinations. R-spikes in the ECG are detected, and the R-R intervals are converted to instantaneous heart rate. Bands around the chest and abdomen use impedance plethysmography to register respirations. The system's on-board recorder continuously encrypts and stores physiologic data on a compact flash memory card. VivoLogic™, a proprietary PC-based software, decrypts and processes recorded data, and provides viewing and reporting features for researchers and clinicians to view the full-disclosure, high-resolution waveforms. The system has been approved by the Food and Drug Administration for use in adults and children older than age 6.

All data collection methods have undergone in-house pre-testing, where Battelle staff experienced in biologic and environmental sample data collection and preparation, have reviewed and used these methods in simulated data collection situations. Detailed notes have been kept of all pre-testing activities and used to evaluate and revise the forms, where necessary.

B.5. Individuals Consulted on Statistical Aspects and Individuals Collecting and/or Analyzing Data

The Principal Investigator, Patricia Richter, PhD, National Center for Chronic Disease Prevention and Health Promotion (770-488-5825; pir1@cdc.gov) has overall responsibility for the design, conduct, and analysis of the study. Co-Investigators, David Ashley, PhD, National Center for Environmental Health, (770-488-7962; dla1@cdc.gov), Clifford Watson, PhD, National Center for Environmental Health (770-488-7638; cow1@cdc.gov), and Gregory Polzin, PhD, National Center for Environmental Health (770-488-7292; gpolzin@cdc.gov) assisted with design and will assist with conduct and analysis of the study.

The questionnaire, sampling and data collection procedures, and analysis plan were designed by CDC in collaboration with researchers at Battelle Centers for Public Health Research and Evaluation through Contract #HHSP23320045006XI. The Battelle Project Leader, Jennifer Malson, M.A. (410-372-2724; malsonj@battelle.org), has overall technical and financial responsibility for the study at Battelle. The Battelle Study Manager is Lauren Canlas, BS (410-372-2741; canlasl@battelle.org) who will supervise the field work and data collection.

The filter butts, saliva and urine samples will be analyzed by the CDC's Division of Laboratory Sciences. Expired-air carbon monoxide samples will be read and recorded at the field site.