**Title page**

Title: Assessing Toxicity of Waterpipe Tobacco Smoking
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contents: IRB Protocol Approval; Study Protocol and Statistical Analysis Plan; Informed Consent Form for each Arm

**Study Protocol and Statistical Analysis Plan**
Document Date: July 13, 2016
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**Informed Consent Form (Intervention Arm – Smoker)**
Document Date: July 1, 2016
Date of upload: March 20, 2025

**Informed Consent Form (Control Arm – Non-Smoker)**
Document Date: July 1, 2016
Date of upload: March 20, 2025

# Study Protocol and Statistical Analysis Plan

**Graduate and Research Affairs**
*Division of Research Affairs*
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SAN DIEGO STATE UNIVERSITY

# Full Committee Approval

Reg.: 45 CFR 46.111(a) 46.116 (a) – greater than minimal risk

Submit Report of Progress by: **June 12, 2017**

July 13, 2016

**Principal Investigator:** Dr. Nada Kassem
**Department:** Public Health
**Contract/grant number:** NIH / 1 R01 DA042471-01
**vIRB Number:** 2445100

Re: *Assessing Toxicity of Waterpipe Tobacco Smoking in Laboratory and Naturalistic Settings.*

Dear Dr. Nada Kassem,

The above referenced protocol was reviewed and presented on June 21, 2016 during a full convened meeting of the board in accordance with SDSU's Assurance and federal requirements pertaining to human subjects protections within the Code of Federal Regulations (45 CFR 46). This approval applies to the conditions and procedures described in your protocol. Please notify the IRB office if your status as an SDSU-affiliate changes while conducting this research study (you are no longer an SDSU faculty member, staff member or student). **This approval expires July 12, 2017**

- Please submit a Report of Progress by: June 12, 2017
- The following approved consent form and other related documents approved can be accessed within the vIRB system, within the Supporting Documents section:
    - *Kassem_2445100_STAMPED Consent - Hookah Smoker - Home Settings - approved 7.13.2016(7.12.2017)*
    - *Kassem_2445100_STAMPED Consent - Non Smoker - Home Settings - approved 7.13.2016(7.12.2017)*
    - *R01 DA - Recruitment Script Hookah Smokers - 7.12.16*
    - *R01 DA - Recruitment Script Non-Smokers - 7.12.16*
    - *R01 DA - Recruitment flyer - hookah smoker - project hookah - 6.29.16*
    - *R01 DA - Recruitment flyer - non-smoker - project hookah - 6.29.16*
    - *R01 DA - Questionnaire Tobacco Use history - 4.14.16*
    - *SpirobankII*
    - *R01 DA - Screen form*
    - *R01 DA - Tobacco Smoking Session Day and Exposure form - 4.14.16*
    - R01 DA - 7 Day Self-Report - 4.14.16
    - *11 R01 - Appendix K. Saliva Test Procedure - kassem - 1.3.13*

**Graduate and Research Affairs**
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Sincerely,

Ramona Pérez
Chair, Institutional Review Board

**A research project to determine the differential effects of waterpipe (WP) smoking practices on biomarkers of toxicants and carcinogens related to waterpipe tobacco smoking in naturalistic settings – urine analyses by Drs. S Hecht and Hoh laboratories.** (Table 1)

We will compare exhaled CO, and urinary levels of cotinine, NNAL, SPMA, and 1-HOP in first void WP smoker urine samples the morning of, and the morning after a WP smoking session across 3 configurations, as shown in Table 1.

Table 1. Three waterpipe (WP) smoking sessions.

| 3 WP smoking Confiurations at home | Heat Source | WP Jar Contents |
|---|---|---|
| WP smoking session 1 | Charcoal [2] | Water [4] |
| WP smoking session 2 | Charcoal | Ice cubes [5] |
| Training for session 3 [1] | Electric [3] | Water |
| WP smoking session 3 | Electric | Water |

[1] Smokers will be trained on using the Electric WP head
[2] Quick-light charcoal *Three Kings* (40mm)
[3] Charcoal-free electrically heated WP head
[4] Room temperature drinking water in the WP jar
[5] Adding ice cubes in the water in the WP jar
For all 3 configurations, flavored WP tobacco, *Starbuzz* (10g), Double Apple flavor will be smoked at home, Aluminum sheet (9x9cm) will be used to cover the WP head, volume of water & ice cubes will be standardized.

**Study Design and Target Population**

We will employ a repeated measures design. We will recruit a sample of 50 adult male and female exclusive WP smokers and a control sample of 25 male and female non-smokers. Participants will be recruited via intercept interviews from San Diego County, CA communities. Over a 4-week study period, WP smokers will smoke one WP tobacco head (10g) of *Starbuzz* during 3 separate sessions with a 7-day washout period before each session in their home in a naturalistic setting: *Week 1-Session 1,* WP smokers will smoke using 1 quick-light charcoal and room temperature water in the WP jar, *Week 2-Session 2,* WP smokers will smoke using 1 quick-light charcoal but adding ice cubes to the water in the WP jar, *Week 3-Training for session 3*, RAs will train participants on how to use a charcoal-free electrically heated WP head to heat the tobacco, and *Week 4-Session 3,* WP smokers will smoke WP tobacco without charcoal using the electric WP head and room temperature water. We will collect a) Tobacco Use History; b) 4-week Tobacco Use and Exposure Diaries; c) WP Smoking Session Day and Exposure Form; d) CO exposure: Micro+ Smokerlyzer® CO monitor will be used for exhaled CO two minutes pre and post each smoking session; and e) 6 first morning urine samples: pre and post the 3 sessions to measure urinary cotinine, NNAL, 1-HOP, and SPMA. We will measure urinary Furan-BDA-NAL in WP smokers who smoke WP tobacco as commonly practiced using quick-light charcoal and room temperature water in the WP jar (urine samples from Session1).

**Inclusion Criteria for Participants**

Adult waterpipe smoker: A male or female, 18 years or older, who smokes WP tobacco exclusively, smokes at least 1 WP head per month, and smokes at least 1 WP head per WP smoking session, smokes WP at home, agrees to smoke *Starbuzz* WP tobacco, and lives in a 'smoker home'. Adult non-smoker: A male or female, 18 years or older, who lives in a 'non-smoker home'.

**Inclusion Criteria for Homes**

A Smoker Home: is a home in which residents have smoked WP tobacco exclusively indoors/outdoors at least in the last month. A Non-smoker Home: is a home where to the knowledge of the residents no smokers have lived in and no visitors had smoked indoors/outdoors in the past month, and where a potential nonsmoker participant who has not been exposed to any secondhand smoke at least in the last month resided full-time.

**Exclusion Criteria**

Adults with major physical/psychiatric illnesses that are judged by the research assistants (RAs) to interfere with ability to give informed consent or to successfully complete an interview will not be included in the study. Daily WP smokers will be excluded because they may not be able to adhere to the wash-out periods before the 3 WP sessions. Pregnant women are also excluded.

**Screening Potential Participants**

The PI, study coordinator (SC), RAs and volunteering student interns will take part in all phases of the study. We have a collaborative relationship with SDSU internship faculty advisors. Potential participants will be approached at random in the community by the RAs accompanied by interns, and will obtain a verbal consent to complete a brief voluntary screening form which will be used to recruit individuals and determine their initial eligibility to participate in a SDSU research study. The PI will work closely with the SC and RAs to qualify participants. The RAs will contact initially qualified potential participants by phone and further determine their qualification by completing an eligibility checklist of inclusion and exclusion criteria. Qualified potential participants will be invited for an Office Visit at our research center for enrollment in the study.

**The 4-Week Study period: comprised of 3 WP smoking sessions & 7-day activities per session** (Table 2)

Waterpipe Smoking Session 1

Table 6. Forms and measures for the 7-Day Data Collection Schedule for each of the 3 smoking sessions.

| | Office visit | | | phone call 1 | | phone call 2 | Home visit 1 | Home visit 2 |
|---|---|---|---|---|---|---|---|---|
| | *Day 1* | *Day 2* | *Day 3* | *Day 4* | *Day 5* | *Day 6* | *Day 7* | *Day 8* |
| **Waterpipe Smoking Session Day** | | | | | | | X | |
| Study Protocol and Consent Form | X | | | | | | | |
| Tobacco Use History Questionnaire | X | | | | | | | |
| 7-Day 24 hour Tobacco Use Diary | X | X | X | X | X | X | X | X |
| 7-Day 24 hour Tobacco Exposure Diary | X | X | X | X | X | X | X | X |
| WP Smoking Session Day & Exposure Form | | | | | | | X | |
| Provide First Morning Urine Samples | | | | | | | X | X |
| RAs Measure CO in exhaled breath | | | | | | | X | |
| Illnesses & Medical Care History Form | | | | | | | | X |
| RAs pick up urine samples | | | | | | | X | X |

***Day 1 - Office Visit:*** The Office Visit is expected to take between 60 and 90 minutes. Trained by the PI, the SC and RAs will explain the 7-day study period activities for the 3 smoking sessions and consent form, answer all questions, and obtain a signed consent form. Thereafter, the RAs will give a saliva test to nonsmokers; interview participants face-to-face using the *Tobacco Use History Questionnaire*; explain how to complete the diaries on a daily basis; explain the urine collection protocol (2 first morning urine samples/smoking session); provide each participant with 2 coded empty urine cups (no names on urine cups and forms), gloves, 2 zip-lock bags/urine cup; ask participants to store the double sealed urine samples once obtained in his/her personal refrigerator (freezer section) until pickup by RAs the next day. Arrange for the smoking session and home visit on day 7 of the first week study period. The PI and SC will accompany the RAs to their first two homes (more if needed) for hands-on training for data collection. Wash-out period: smokers will abstain from smoking and exposure to any tobacco smoke for 7 days prior to each smoking session. WP smokers will choose a WP smoking day to start the 4-week study period; the RAs will coordinate the Office Visit accordingly. Non-smokers will choose any day to provide 2 first urine samples on two consecutive days. Saliva Test: Trained by the PI, the RAs will use NicAlert to validate non-smoking status, and as a bogus pipeline technique, which we used successfully in our previous studies. NicAlert, a commercial semi-quantitative instant saliva cotinine test, is accurate and valid with high sensitivity (93-100%) and specificity (95-96%) Non-smoking participants will be informed about the purpose of the NicAlert test during the informed consent process. *(Appendix K. Saliva Test Procedure)* ***Days 2 - 6:*** The RAs will contact participants by phone on the 4$^{th}$ day to confirm adherence to the 7-wash-out period and diaries, and on the 6$^{th}$ day to remind participants to collect the first morning urine sample on day 7, and to arrange for a home visit for the first WP smoking session. ***Day 7: Waterpipe smoking session 1.*** WP smokers will provide one first morning urine sample and store it in their refrigerator (freezer section). Participants from our previous WP studies stored their urine samples, double bagged in their refrigerator. WP smokers will smoke WP tobacco (10 grams) as they normally do using charcoal and room temperature water in the water jar. The RAs will collect expired CO levels 2 minutes before and 2 minutes after concluding smoking. ***Day 8:*** Participants will provide a first morning urine sample. The RAs will pick up the 2 urine samples (frozen), place them in a cooler (to transfer them to our research center laboratory for aliquoting and storage in a freezer at -20ºC to be sent out for analyses); pick up the diaries and *Waterpipe Smoking Session Day and Exposure From*, arrange for the 2$^{nd}$ WP smoking session home visit, provide forms, urine cups package for week 2 of the study.

Waterpipe Smoking Session 2. The 7-Day study activities: Days 1 through 7 are similar to session 1 with the exception that the RAs will add one tray of ice cubes and water in the WP jar. The RAs will arrange for a training session to use the charcoal-free electrically heated WP head.

Waterpipe Smoking Session 3. The 7-Day study activities: ***Training for session 3.*** Any day during the third week, the RAs will arrange to visit the participants to train them on the charcoal-free electrically heated WP head, and arrange for the 3rd smoking session using the electric head. ***Smoking session 3***. Days 1 through 7 are similar to session 1 using room temperature water in the WP jar, however using the charcoal-free electrically heated WP head instead of using charcoal. During the last visit, the day after the 3rd smoking session, the RAs will interview participants to complete the *Illnesses and Health Care History Form* (completed during the last day of the study period to avoid biasing smoking behavior), pick up the final urine samples, thank the participants and inform them that they will receive a check from SDSU in the amount of $250 by mail.

**Standardized Setting of the Waterpipe Device, Smoking Protocol, Waterpipe Tobacco and Charcoal**

To reduce variance of factors extraneous to our configurations of WP smoking practices, we will standardize setting the WP device, aluminum foil, and amount, type and brand of WP tobacco and quick-light charcoal. We will determine and standardize the volume and commercial brand of drinking water to be placed in the WP jar, and volume of water and added ice cubes to be standardized.

Waterpipe Device. We will purchase 10 new unused WPs similar in specification to the Battelle Research-grade WP: 2L glass water jar, 28.6 cm length of body. All WPs will be the same size, style, and brand. In the kitchen of the participants, before and after each use, the RAs will thoroughly clean the stem and glass jar with soap and water between sessions and between participants. Three new hoses will be used for each smoker.

Smoking Protocol. During each WP smoking session, participants will be asked to smoke one WP tobacco head at their home as they normally do, not to share their WP with other smokers nor be in the presence of another smoker. WP smokers usually smoke 1 WP head per smoking session, ranging 30-60 minutes per session. At home of the participants, the RAs will set up the WP following a standardized protocol. Briefly, the RAs, wearing gloves at all times, will place room temperature commercial drinking water in the WP jar for sessions 1 and 3, and one tray of ice cubes and a standardized volume of water for session 2. The RAs will weight (using an analytical balance) and load one head of WP tobacco (10 gram Two Apples flavor, *Starbuzz* brand, U.S.), and cover it with a single (9x9 cm) aluminum sheet (manufacturer pre-perforated punch pattern). Using a portable lighter, the RAs will light a single quick-light charcoal (40 mm, *Three Kings* brand, Holland), and place it on the loaded WP head. At the conclusion of the smoking session, the RAs will weigh the remaining tobacco and charcoal, and complete the WP session observation form, such as length of time smoking, etc.

Waterpipe Tobacco Protocol and Brand - *Starbuzz*: All smokers will smoke the WP brand *Starbuzz* during the 3 WP sessions. We will purchase for each participant one package of *Starbuzz* tobacco to be used for the 3 smoking sessions and the training session. Following opening the package for session 1, the RAs will place the package in a sealed Ziploc bag and ask the participant to store it in a cabinet at room temperature and away from any direct sunlight until use the following smoking session. The majority of WP smokers use flavored WP tobacco *Maassel* (Table 2). We identified *Starbuzz*, a flavored WP tobacco brand, that is manufactured in the U.S. and is popular and accessible to WP smoker consumers based on: (1) a search of 'online vendors' such as hookah-shisha.com, hookahwholesalers.com, thehookah.com, and Amazon.com who provide a customer review section for WP tobacco products; (2) a survey of point-of-sale display of the most frequent WP tobacco brands in 6 Middle Eastern markets and smoke shops that sell WP tobacco in the San Diego area; (3) our preliminary unpublished WP data; and (4) brands most served at 5 WP lounges. We will use a popular flavor 'Double Apple' for all WP smoking sessions as reported in the literature and by our unpublished data (*Appendix L. Preferred WP tobacco Flavors & Brands – unpublished data).*

Waterpipe Charcoal Protocol and brand - *Three Kings* quick-light charcoal (40mm)*:* Quick-light charcoal is manufactured to light fast and has been used in previously published studies in naturalistic and laboratroy settings. A standardized protocol will be used in lighting and placing the charcoal on the tobacco-covered head. *Three Kings* quick-light charcoal (Holland) is an internationally recognized brand, and customarily sold where WP tobacco is sold in Smoke shops and Middle Eastern markets in the U.S. and online. Smokers use them for convenience, since lighting only requires a match or lighter held to it for a few seconds with only a small flame, and usually stay lit up throughout the smoking session.

**Questionnaires, Diaries, Forms** will be developed based on our previous WP studies.

Screening Form (face-to-face intercept interviews) will collect the name, e-mail address, telephone number, age, gender, smoking habits, and willingness to participate in the study. The RAs will request permission to contact them by phone to confirm eligibility using the inclusion and exclusion criteria. Consent Form is explained in detail in the 'Protection of Human Subjects form'. Tobacco Use History Questionnaire (face-to-face interview using a standardized protocol) Past 7 and 30 days use; average number of cigarettes and/or WP heads and length of time smoked per typical work and non-work day; past 7 and 30 days secondhand exposure, e.g. average number of WPs and/or cigarettes exposed to per day at home, at work and elsewhere. Exposure will be defined as "in the same room or car with someone who has at least one puff of a cigarette or WP." WP tobacco and charcoal brands use (point of purchase, cost/gram, preferred brands and flavors and reasons for preference). Location at which most often smoked WP at home. Demographics: gender, age, race, ethnicity, age of initiation of WP use and any other tobacco product, education, income, family size and number smokers**;** height and weight measurement for Body Mass Index calculation (BMI = weight [kg]/height squared [$m^2$]). Illness and Medical Care History Form (face-to-face interview), family history of pulmonary and cardiovascular diseases, cancer, lifetime and past year tobacco-related symptoms, e.g. headaches, dizziness; past year frequency of emergency room, hospitalization & other health services utilization. The 2 following forms will be used for the 3 WP smoking sessions: 7-Day Tobacco Use and Exposure Diaries (Self-administered), a 7-day 24-hour time-diary for participants on frequency, length of time and # of WP heads and/or other tobacco products smoked/exposed to indoors/outdoors by location: home/other location. WP Smoking Session Day & Exposure Form (Self-administered, RAs will complete the WP observation section), frequency, length of time and number of WPs smoked or exposed to indoors/outdoors. Location of smoking: inside home/on patio. Overall description of house: number of rooms, ventilation, windows opened, apartment/house. For WP sessions 2 and 3, open-ended questions on experience, attitudes towards and intention to future use of ice cubes in WP jar and/or use of the charcoal-free electric WP head.

**Exhaled Carbon Monoxide (CO) Concentrations.** We will measure CO exposure using a Micro+ Smokerlyzer® CO monitor for exhaled CO two minutes before lighting the charcoal and 2 minutes at conclusion of each smoking session. CO is a smoke toxicant that reduces the blood's ability to transport oxygen to various organs, including the brain, and can cause dizziness, headache, syncope and nausea. An acute increase in exhaled CO/COHb, and CO poisoning were demonstrated in WP smokers. Post 50 minutes of smoking, mean expired-air CO concentration increased to 32.9 ± 2.7 ppm in WP smokers, and to 7.4 ± 0.5 ppm when smoked cigarettes.

**Urinary Biomarkers of Exposure to Waterpipe Tobacco Smoke.** Urinary cotinine, NNAL, SPMA, and 1-HOP, will be determined in WP tobacco smokers to assess comparative risk related to WP tobacco smoking across 3 configurations, as shown in Table 1. We expect that this panel of biomarkers will be higher in smokers who add ice cubes in the WP jar; and SPMA and 1-HOP will be lower in smokers who replace charcoal with charcoal-free electric WP head. We will also assess levels of WP smokers' exposure to furan by quantifying its urinary metabolite Furan-BDA-NAL among WP, in which we expect to find an increase post smoking.

**Cotinine** will be measured as an index of nicotine consumption in WP smokers using emerging WP smoking practices. On average, 70%-80% of nicotine entering the body is converted into its metabolite, cotinine. Daily use of WP tobacco produced a 24-hour mean (95%CI) urinary cotinine level of 785 ng/ml (578-991ng/ml) equivalent to smoking 10

cigarettes/day. Post smoking WP, mean (95% CI) urinary cotinine increased: 11.8 (7.21-19.2) to 55.3 (33.9-90.1) ng/mg creatinine, N=55.

**NNAL** will be measured as an important tobacco-specific lung carcinogen. In 55 WP smokers NNAL about doubled (2.1-fold) post WP smoking: 1.32 to 2.84 pg/mg creatinine. Higher urinary mean levels (uncorrected) were found in WP smokers in Syria (N=24) [mean (95% CI), 33.0 pg/ml (21.6-50.6 pg/ml)].

**SPMA** will be measured as an index for exposure to benzene. We found that urinary SPMA levels in 50 WP smokers increased significantly 2.2 times post a home WP smoking event [geometric mean (GM)(95%CI): 0.26 pmol/mg creatinine (0.17-0.41) to 0.58 pmol/mg creatinine (0.40-0.83)]. A crossover study clinical study found that exposure to benzene when smoking WP tobacco was 2.5 times higher than when smoking cigarettes (N=13) [PMA:GM(95%CI)=1.73 µg/24h (0.76-3.93) vs. 0.695 µg/24h, p=0.03, respectively].

**1-HOP** will be measured as an index for exposure to pyrene. Most PAHs are considered genotoxic carcinogens. A crossover study demonstrated that exposure to pyrene when smoking WP tobacco was 1.5 times higher than when smoking cigarettes (N=13), [1-HP: GM (95%CI)=115 pmol/24h (87-150) vs. 81 pmol/24h (66-101), P=0.01, respectively].

**Furan-BDA-NAL** Furan, a possible human carcinogen (group 2B), is found in processed food, pollution, car exhaust, and cigarette smoke. The contribution of canned and processed foods to furan exposure is about 0.3 µg/kg/day. Smokers may be exposed to larger amounts of furan than nonsmokers since cigarette smoke contains significant levels of furan (20-40 µg/ cigarette). Heating of a carbohydrate-rich matrix results in chemical conversions, including sugar caramelization, which may result in generation of furanic compounds. Flavored WP tobacco is typically manufactured through the fermentation of tobacco with molasses and sweetened fruit flavors, when smoked produced the smell of caramelized sugars. Using a smoking machine, furanic compounds were generated during one WP smoking session such as up to 62.3±11 mg of 5-(hydroxymethyl)-2-furaldehyde (HMF).
Furan is oxidized to a reactive α,β-unsaturated dialdehyde, *cis*-2-butene-1,4-dial (BDA), a toxic metabolite of this liver toxicant and carcinogen. It has been determined that there is a strong relationship between Furan-BDA-NAL and smoking. Levels of urinary Furan-BDA-NAL, a BDA derived metabolite of furan, were higher in cigarette smokers relative to those in nonsmokers. We will measure sulfides 2 and 4; sulfoxides 3 and 5 and BDA-NAL: 1 and 6. In small sample sizes (N=5, N=16), furan metabolite sulfoxide 3 ranged from 8.7 to 69 pmol/mg creatinine among cigarette smokers and from 1.4 to 6.5 pmol/mg creatinine, with LOQ of <.02. We will be the first to determine the levels of Furan-BDA-NAL in WP smokers, thereby assessing WP smokers' potential health risks associated with furan exposure.

**Laboratory Analyses for the Proposed Urinary Biomarkers.**

Dr. S. Hecht laboratory at The Masonic Cancer Center, University of Minnesota, will conduct the following laboratory urine analyses, as described previously: NNAL with a LOQ of 1.672 pg/mL (0.01 pmol/mL), 1-HOP with a LOQ of (0.05 pmol/mL), SPMA with a LOQ of 0.03 pmol/mL, Furan-BDA-NAL with a LOQ of <.02 pmol/mg creatinine). Dr. Eunha Hoh at SDSU will conduct laboratory urine analyses for cotinine with a LOQ of 0.1 ng as described previously; and will conduct laboratory analyses for creatinine for normalization.

**Gender Representation.** Efforts will be made to recruit equal samples of males and females.

**Participants Retention and Incentives.** Flexible scheduling for study activities where applicable. Following completion of study activities, a $75 check issued by SDSU will be mailed to non-smokers, and $250 to smokers: $100/smoking session and $25/ training session. Proration will apply for completed activities.

**Study Timeline.** (Table 3) In the first 3 quarters of Year 1 we will develop forms and procedure manuals, hire and train staff, pilot test and refine protocols and study measures, and obtain IRB approval. Sequential recruitment of participants, data collection, urine aliquoting, data entry, cleaning and coding will start during the 4th quarter of Year 1, and continue through the end of year 2. We expect we will need about 15 months to complete data collection in naturalistic settings. Data analysis and manuscript preparation will start in year 3. Dissemination of results will start in Year 3.

| Table 3. Timeline | 1 | | | 2 | 3 |
|---|---|---|---|---|---|
| Forms Procedures manual development | | | | | |
| Hire and train staff | | | | | |
| IRB approval, pilot testing of data collection | | | | | |
| Refine data collection & procedures manual | | | | | |
| Recruitment of participants | | | | | |
| Measures:<br>Study Questionnaire, Forms & Diaries<br>CO concentrations, Urine samples | | | | | |
| Battelle - Laboratory analyses | | | | | |
| Urine aliquoting, shipping samples to labs | | | | | |
| Data processing-coding, entry, cleaning | | | | | |
| Data analysis and manuscript preparation | | | | | |
| Dissemination of Results *(continues beyond year 3)* | | | | | |

**General Statistical Analysis Strategy.** Dr. Kassem will oversee data processing and**.** Statistical analyses will be determined by Drs. Kassem, Hovell, Lui, Brinkman and Liles. Final analyses will be conducted using SPSS and Stata software by Dr. Kassem, Mr. Liles, and Ms. Jackson. Type I error rate will be set at $\alpha$=.05, and corrections for Type I error will be performed as necessary (e.g., Bonferroni). We will begin with a comprehensive descriptive analysis of levels of toxicants, CO, and urinary biomarkers and their distribution in the various smoking configurations. Arithmetic means and standard deviations, geometric means and 95% confidence intervals, medians and 25th and 75th percentiles, and minimum and maximum levels will be computed for each outcome measure, and for uncorrected and creatinine-corrected values of urinary biomarkers. Correlations between biomarkers will be determined by Pearson's *r*. Measures will be log-transformed as necessary, to control for skewed distribution, the undue influence of outliers, and violations of assumptions for statistical inference. Dependent or independent samples t-tests, as appropriate, will compare pairs of machine smoking configurations, smokers post-session vs. pre-session, and smokers vs. non-smokers. Missing data from the questionnaire will be handled using multiple imputation in Stata.

**Aim 1** calls for comparing pre/post session change in exhaled CO, and in urinary levels of cotinine, NNAL, SPMA, and 1-HOP in first morning urine samples the morning of, and the morning after, each of 3 WP smoking sessions at home: (Session 1) using charcoal to heat the tobacco and only water in the jar; (Session 2) using charcoal to heat the tobacco and adding ice cubes in the jar; and (Session 3) using electrically heated tobacco and only water in the jar. Among smokers, differences in pre/post change on CO and each biomarker between sessions 1 & 2, and between sessions 1 & 3, will be assessed by mixed regression models to handle repeated measures on each participant. Demographics, duration of smoking, and possible confounding factors, such as number of smokers inside the home and years smoked, will be included as covariates.

**Power analysis.** As no data exist on effect sizes of differences between session configurations for Aim 2, in Table 4 we show estimated power to detect difference for a range of potential intra-class correlations (ICC) and an effect size of .40 (medium by Cohen's criteria), based on paired z-test, n=50, two-sided alpha=.05. Estimates are conservative, since addition of covariates and adjustment for pre-session CO or urinary biomarker levels in the analyses will increase power.

Table 4. Power estimates

| Effect Size | ICC | Power |
|---|---|---|
| 0.40 | 0.25 | 0.637 |
| 0.40 | 0.50 | 0.807 |
| 0.40 | 0.75 | 0.979 |

**Aim 2** will examine levels of urinary Furan-BDA-NAL following the analysis strategy described in Aim 1, but using data only from Session 1, the configuration commonly practiced in naturalistic settings, using charcoal to heat the tobacco, and room temperature water in the jar. We will begin with a comprehensive descriptive analysis of demographics and Furan-BDA-NAL levels and their distribution in WP smoker participants. As in Aim 1, analyses will identify differences in metabolite levels between smokers vs. non-smokers, and between pre vs. post WP smoking Session 1, for the sample overall as well as by demographic categories (age, gender, BMI). Pearson's correlations will be conducted between urinary levels of Furan-BDA-NAL and values of other metabolites (cotinine, NNAL, SPMA, and 1-HOP) quantified for WP smoking Session 1.

# Informed Consent Form for Hookah Smoker (intervention arm)

San Diego State University (SDSU)
**INFORMED CONSENT FORM**
**Consent form version date: July 1, 2016**

Participant ID _____

**R01 DA – Hookah Project - Hookah Smoker at Home**

You are being asked to participate in a research study. Before you give your consent to volunteer, it is important that you read the following information and ask as many questions as necessary to be sure you understand what you will be asked to do.

## WHO ARE THE INVESTIGATORS?

Dr. Nada Kassem, from San Diego State University (SDSU), Center for Behavioral Epidemiology and Community Health (CBEACH), is conducting a study funded by the U.S. Food and Drug Administration (FDA) Center for Tobacco Products (CTP) and administered by the National Institutes of Health (NIH). Other investigators include Dr. Melbourne Hovell, Dr. Eunha Hoh, and Dr. Kung-jong Liu.

## WHO SHOULD I CONTACT IF I HAVE QUESTIONS?

Principal Investigator: Dr. Nada Kassem
Study Coordinator: Noura Kassem, MPH
SDSU Department: CBEACH Address: 9245 Sky Park Court, Suite 230, San Diego CA 92123

## WHAT IS THE PURPOSE OF THIS RESEARCH?

This study involves research. The purpose of this study is to learn about the effects of hookah tobacco smoking practices. Approximately 50 hookah smoker participants will be included at SDSU.

## ELIGIBILITY CRITERIA

- ✓ You should be a male or female, age 18 years or older
- ✓ Current weekly or occasional smoker of only hookah tobacco
- ✓ Do not use any form of tobacco products and nicotine replacement products except hookah tobacco
- ✓ In apparently good physical and mental health with no unstable medical condition

## HOW LONG WILL I BE IN THIS RESEARCH?

Your participation will last 4 weeks consisting of one visit to our office at SDSU-CBEACH, and 7 visits to your home by two research assistants to collect data.

## WHAT WILL HAPPEN IN THIS RESEARCH?

**Office Visit and Study Forms:** During the office visit you will be asked to complete a screen form about your illnesses and medical care history. If you are not eligible to participate, the information obtained from you during the screening will be omitted from this study and shredded to protect your privacy.

If you qualify you will be asked to complete a Tobacco Use History questionnaire, and will be trained study on (1) completing the 7-Day 24-hour Tobacco Use and Exposure form, (2) Tobacco Smoking Session Day and Exposure form (3) to exhale in a hand held Micro+ Smokerlyzer CO monitor to measure the level of Carbon monoxide (CO) exhaled, and (4) to exhale in a hand held spirometer/oximeter for pulmonary testing.

**Study Activities at Home:** You will be asked to smoke, where you normally do at home, once a week for 4 consecutive weeks with a 7-day washout period before each smoking session. During each smoking session you will smoke one hookah tobacco head (10g) of *Starbuzz*, a U.S. made product, with 3 different smoking practices using *three Kings* quick-light charcoal (where applicable).

Participant ID _____

**Week 1-Session 1**, you will smoke using 1 quick-light charcoal and room temperature water in the hookah jar.
**Week 2-Session 2,** you will smoke using 1 quick-light charcoal and ice cubes in the water in the hookah jar.
**Week 3-Training for session 3,** RAs will train you how to use a charcoal-free electrically heated hookah head**.**
**Week 4-Session 3,** you will smoke using the charcoal-free electric hookah head and room temperature water.
**Home Visits and Measures:** Two research assistants will visit you at home during each of the 3 hookah smoking sessions to:

(1) Set up the hookah and standardize the amount of charcoal and hookah tobacco to be smoked

(2) Measure the following before and after each smoking session:

a. Carbon monoxide (CO) exposure by exhaling in a Micro+ Smokerlyzer® CO monitor
b. Pulmonary function testing (PF) and blood oxygen saturation (SO2) by exhaling in a spirometer/oximeter
c. Your vital signs: Blood pressure (BP), heart rate (HR), and respiratory rate (RR)

(3) You will also be asked to provide 2 first morning urine samples per week for a total of 6 samples as follows:

Days of urine collection will be on the day of and the day after each of the three hookah smoking sessions. You will be trained how to catch a clean urine sample. You will be provided with empty cups, gloves, zip-lock bags and asked to store the sealed urine samples in your personal refrigerator for approximately 48 hours. You will be asked to schedule an appointment to drop off your samples to the CBEACH laboratory, where urine samples will be stored, aliquoted, and sent to Drs. Stephen Hecht and Eunha Hoh's laboratories for analyses. If needed, we will arrange for someone to collect your urine samples (See Table 1).

Table 1. Forms and measures for the 7-Day Data Collection Schedule for each of the 3 hookah smoking sessions.

| The research assistants (RAs) will arrange for an office visit, obtain consent, train on study activities and measures, and schedule the 4-week study activities. | Office visit | | | phone call 1 | | phone call 2 | Home visit 1 | Home visit 2 |
|---|---|---|---|---|---|---|---|---|
| | *Day 1* | *Day 2* | *Day 3* | *Day 4* | *Day 5* | *Day 6* | *Day 7* | *Day 8* |
| Study Protocol and Consent Form | X | | | | | | | |
| Screen form about illnesses & medical care history | X | | | | | | | |
| Tobacco Use History Questionnaire | X | | | | | | | |
| 7-Day 24-hour Tobacco Use Diary | X | X | X | X | X | X | X | X |
| 7-Day 24-hour Tobacco Exposure Diary | X | X | X | X | X | X | X | X |
| **Hookah Smoking Session Day** | | | | | | | **X** | |
| Hookah Smoking Session Day & Exposure Form | | | | | | | X | |
| Provide first morning urine samples | | | | | | | X | X |
| RAs measure carbon monoxide (CO) in exhaled breath | | | | | | | X | |
| RAs measure pulmonary function testing | | | | | | | X | |
| RAs measure blood pressure, heart & respiratory rate | | | | | | | X | |
| RAs pick up urine samples | | | | | | | X | X |

None of the procedures used in this study are experimental in nature. The only experimental aspect of this study is the gathering of information for the purpose of analysis.

## WHAT ARE THE RISKS OR DISCOMFORTS INVOLVED IN THE RESEARCH?

You may feel discomfort when smoking using ice cubes in the water jar as the smoke may become thicker, thus you may be at risk of exposure to higher levels of smoke components. If you feel uncomfortable, you can stop smoking at any time.

You may feel discomfort with the CO exposure and pulmonary function testing as you have to forcefully exhale into a spirometer and a Smokerlyzer CO monitor. You can rest between measurements.

If you become pregnant, the smoking and testing procedures may involve risks to the embryo or fetus which are currently unforeseeable, therefore you will be asked to stop participating in the study.

There may be new findings developed during this research which may relate to your willingness to continue to participate in this study. These new findings will be shared with you.

**ARE THERE ANY BENEFITS TO PARTICIPATION?**

There are no benefits for participating in this research; however, by participating you are helping to provide information which may benefit science and society.

**ARE THERE ANY ALTERNATIVES TO PARTICIPATION?**

An alternative is to not participate.

**WILL MY INFORMATION BE PRIVATE?**

Confidentiality will be maintained to the extent allowed by law. However, the study team members are required by California law to report suspected child or elder abuse to the appropriate authorities.

Research records will be stored in a locked office in a locked file cabinet and will only be accessible to the study team members. Research data will be destroyed three years after the end of this study. Any electronically saved information and/or data will be encrypted (saved with a password), and after three years will be deleted.

Any shared data with other researchers will be de-identified.

The results of your participation in this study may be used for publication or for scientific purposes, but the results will not include any information that could identify you.

**WHAT WILL HAPPEN IF I AM HURT OR INJURED?**

If any complications arise as a direct result of participation in this study, we will assist you in obtaining appropriate attention. If you need treatment or hospitalization as a result of being in this study, you are responsible for payment of the cost for that care. If you have insurance, you may bill your insurance company. You will have to pay any costs not covered by your insurance. San Diego State University and San Diego State University Research Foundation will not pay for any care, lost wages, or provide other financial compensation. However, if you feel you have a claim that you wish to file against the State [or the Foundation], please contact Graduate and Research Affairs - Division of Research Administration at (619) 594-6622 to obtain the appropriate claim forms.

**DO I HAVE TO PARTICIPATE?**

You do not have to participate in this research study. If you choose not to participate, there is no penalty or loss of benefits to which you are otherwise entitled. Additionally, you may choose to stop participating at any time without penalty or loss of benefits to which you are otherwise entitled. Any data collected during the process of which a participant decided to stop the study will be destroyed and/or deleted.

Under FDA regulations, data collected on subjects up to the time of withdrawal from the clinical investigation must remain in the study database. See 21 CFR 312.62(b) and 812.140(a)(3). If a subject withdraws from a study, removal of data that were already collected may undermine the scientific, and therefore the ethical, integrity of the research.

San Diego State University (SDSU)
**INFORMED CONSENT FORM**
**Consent form version date: July 1, 2016**

Participant ID _____

If an investigator conducting research under the provisions of FDA regulations, the investigator is required to inform subjects that their data cannot be destroyed.

**WILL I BE TOLD ABOUT THE STUDY RESULTS?**

We will not contact you with results of this study after this study is completed. There may be new findings developed during this research which may relate to your willingness to continue to participate in this study. These new findings will be shared with you.

**WILL IT COST ME ANYTHING TO PARTICIPATE?**

You are responsible for travel costs for one visit to our research center for training on the study activities.

**WILL I BE PAID FOR MY PARTICIPATION IN THE RESEARCH?**

You will receive four checks issued by SDSU that will be mailed to you totaling $250: $75 per smoking session (including completing the study forms, providing six urine samples, exhaling into a Smokerlyzer CO monitor, exhaling into a spirometer, and having your vital signs taken such as, blood pressure, heart rate and respiratory rate), and $25 per training session. Proration will apply for completed activities.

**WHAT IF I HAVE QUESTIONS REGARDING THIS STUDY**?

If you have any questions about the research now, please ask. If you have questions later about the research, you may contact Dr. Nada Kassem at 619-370-7488. If you have any questions about your rights as a participant in this study, or in the event of a research related injury, you may contact the Division of Research Affairs at San Diego State University (telephone: 619-594-6622; email: irb@mail.sdsu.edu). At any time during the research you can contact the IRB for questions about research rights, to discuss problems, concerns, or suggestions, or to offer input.

**CONSENT TO PARTICIPATE:**

The San Diego State University Institutional Review Board (SDSU IRB) has approved this consent form, as signified by the Board's stamp. The consent form must be reviewed annually and expires on the date indicated on the stamp. Your signature below indicates that you have read the information in this document and have had a chance to ask any questions you have about the study. Your signature also indicates that you agree to be in the study and have been told that you can change your mind and withdraw your consent to participate at any time. The investigator Dr. Nada Kassem or a member of her research team has provided you with a copy of this consent form with information about who to contact in the event you have questions.

Name of Participant (please print) Date

Signature of Participant Date

Signature of Investigator Date

SAN DIEGO STATE UNIVERSITY

Institutional Review Board

Approval Expires: July 12, 2017
Study Number: 2445100

**Informed Consent Form for Non-Smoker (control arm)**

San Diego State University (SDSU)
**INFORMED CONSENT FORM**
**Consent form version date: July 1, 2016**

Participant ID _____

**R01 DA – Hookah Project - Non-Smoker at Home**

You are being asked to participate in a research study. Before you give your consent to volunteer, it is important that you read the following information and ask as many questions as necessary to be sure you understand what you will be asked to do.

## WHO ARE THE INVESTIGATORS?

Dr. Nada Kassem, from San Diego State University (SDSU), Center for Behavioral Epidemiology and Community Health (CBEACH), is conducting a study funded by the U.S. Food and Drug Administration (FDA) Center for Tobacco Products (CTP) and administered by the National Institutes of Health (NIH). Other investigators include Dr. Melbourne Hovell, Dr. Eunha Hoh, and Dr. Kung-jong Liu.

## WHO SHOULD I CONTACT IF I HAVE QUESTIONS?

Principal Investigator: Dr. Nada Kassem
Study Coordinator: Noura Kassem, MPH
SDSU Department: CBEACH Address: 9245 Sky Park Court, Suite 230, San Diego CA 92123

## WHAT IS THE PURPOSE OF THIS RESEARCH?

This study involves research. The purpose of this study is to learn about the effects of hookah tobacco smoking practices. Approximately 25 non-smoker participants will be included at San Diego State University.

## ELIGIBILITY CRITERIA

- ✓ You should be a male or female, age 18 years or older
- ✓ **Non-smoker** who does not live with a smoker
- ✓ Do not use any form of tobacco products and nicotine replacement products
- ✓ Do not allow any tobacco product to be smoked at home indoors or outdoors on patio during the 7- day study period
- ✓ In apparently good physical and mental health with no unstable medical condition

## HOW LONG WILL I BE IN THIS RESEARCH?

Your participation will last one week. You will come to SDSU-CBEACH for one office visit for one hour. Two research assistant will visit your home two times. First home visit will last one hour to collect data, the second home visit is to pick up your urine samples and completed forms.

## WHAT WILL HAPPEN IN THIS RESEARCH?

### Office Visit and Study Forms:

During the office visit you will be asked to (1) complete a screen form about your illnesses and medical care history, and (2) receive a saliva test by a trained research assistant using NicAlert, a commercially available test, to validate non-smoking status.

If you are not eligible to participate, the information obtained from you during the screening will be omitted from this study and shredded to protect your privacy.

San Diego State University (SDSU)
**INFORMED CONSENT FORM**
**Consent form version date: July 1, 2016**

Participant ID _____

If you qualify you will be asked to complete a Tobacco Use History questionnaire, and will be trained study on (1) completing the 7-Day 24-hour Tobacco Use and Exposure form, (2) to exhale in a hand held Micro+ Smokerlyzer CO monitor to measure the level of Carbon monoxide (CO) exhaled, and (3) to exhale in a hand held spirometer/oximeter for pulmonary testing.

**Home Visit and Measures:**

(1) Two research assistants (RAs) will visit you at home on the 7th day of the one-week study period to collect data as follows:

a. Carbon monoxide (CO) exposure by exhaling in a Micro+ Smokerlyzer® CO monitor
b. Pulmonary function testing (PF) and blood oxygen saturation (SO2) by exhaling in a spirometer/oximeter
c. Your vital signs: Blood pressure (BP), heart rate (HR), and respiratory rate (RR)

(2) You will also be asked to provide 2 first morning urine samples on two consecutive days. Days of urine collection will be on the 6th and 7th day of the 7-day study period. You will be trained how to catch a clean urine sample. You will be provided with empty cups, gloves, zip-lock bags and asked to store the sealed urine samples in your personal refrigerator for approximately 48 hours. You will be asked to schedule an appointment to drop off your samples to the CBEACH laboratory, where urine samples will be stored, aliquoted, and sent to Drs. Stephen Hecht and Eunha Hoh's laboratories for analyses. If needed, we will arrange for someone to collect your urine samples (See Table 1).

Table 1. Forms and measures for the 7-Day study period.

| The research assistants (RAs) will arrange for an office visit, obtain consent, train on study measures, and schedule the 1-week study activities. | Office visit | | | phone call 1 | | phone call 2 | Home visit 1 | Home visit 2 |
|---|---|---|---|---|---|---|---|---|
| | *Day 1* | *Day 2* | *Day 3* | *Day 4* | *Day 5* | *Day 6* | *Day 7* | *Day 8* |
| Study Protocol and Consent Form | X | | | | | | | |
| Screen form about illnesses & medical care history | X | | | | | | | |
| Tobacco Use History Questionnaire | X | | | | | | | |
| 7-Day 24-hour Tobacco Use Diary | X | X | X | X | X | X | X | X |
| 7-Day 24-hour Tobacco Exposure Diary | X | X | X | X | X | X | X | X |
| Provide first morning urine samples | | | | | | | X | X |
| RAs measure carbon monoxide (CO) in exhaled breath | | | | | | | X | |
| RAs measure pulmonary function testing | | | | | | | X | |
| RAs measure blood pressure, heart & respiratory rate | | | | | | | X | |
| RAs pick up urine samples | | | | | | | X | X |

None of the procedures used in this study are experimental in nature. The only experimental aspect of this study is the gathering of information for the purpose of analysis.

**WHAT ARE THE RISKS OR DISCOMFORTS INVOLVED IN THE RESEARCH?**

You may feel discomfort with the CO exposure and pulmonary function testing as you have to forcefully exhale into a Smokerlyzer CO monitor and a spirometer/oximeter. You can rest between measurements.

If you become pregnant, the testing procedures may involve risks to the embryo or fetus, therefore you will be asked to stop participating in the study.

There may be new findings developed during this research which may relate to your willingness to continue to participate in this study. These new findings will be shared with you.

San Diego State University (SDSU)
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Participant ID _____

## ARE THERE ANY BENEFITS TO PARTICIPATION?

There are no benefits for participating in this research; however, by participating you are helping to provide information which may benefit science and society.

## ARE THERE ANY ALTERNATIVES TO PARTICIPATION?

An alternative is to not participate.

## WILL MY INFORMATION BE PRIVATE?

Confidentiality will be maintained to the extent allowed by law. However, the study team members are required by California law to report suspected child or elder abuse to the appropriate authorities.

Research records will be stored in a locked office in a locked file cabinet and will only be accessible to the study team members. Research data will be destroyed three years after the end of this study. Any electronically saved information and/or data will be encrypted (saved with a password), and after three years will be deleted.

Any shared data with other researchers will be de-identified.

The results of your participation in this study may be used for publication or for scientific purposes, but the results will not include any information that could identify you.

## WHAT WILL HAPPEN IF I AM HURT OR INJURED?

If any complications arise as a direct result of participation in this study, we will assist you in obtaining appropriate attention. If you need treatment or hospitalization as a result of being in this study, you are responsible for payment of the cost for that care. If you have insurance, you may bill your insurance company. You will have to pay any costs not covered by your insurance. San Diego State University and San Diego State University Research Foundation will not pay for any care, lost wages, or provide other financial compensation. However, if you feel you have a claim that you wish to file against the State [or the Foundation], please contact Graduate and Research Affairs - Division of Research Administration at (619) 594-6622 to obtain the appropriate claim forms.

## DO I HAVE TO PARTICIPATE?

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## WILL I BE TOLD ABOUT THE STUDY RESULTS?

We will not contact you with results of this study after this study is completed. There may be new findings developed during this research which may relate to your willingness to continue to participate in this study. These new findings will be shared with you.

San Diego State University (SDSU)
**INFORMED CONSENT FORM**
**Consent form version date: July 1, 2016**

Participant ID _____

**WILL IT COST ME ANYTHING TO PARTICIPATE?**

You are responsible for travel costs for one visit to our research center for training on the study activities.

**WILL I BE PAID FOR MY PARTICIPATION IN THE RESEARCH?**

You will receive one check issued by SDSU that will be mailed to you totaling $75: which will include completing the study forms, providing two urine samples, exhaling into a Smokerlyzer CO monitor, exhaling into a spirometer, and having vital signs taken such as, blood pressure, heart rate and respiratory rate. Proration will apply for completed activities.

**WHAT IF I HAVE QUESTIONS REGARDING THIS STUDY**?

If you have any questions about the research now, please ask. If you have questions later about the research, you may contact Dr. Nada Kassem at 619-370-7488. If you have any questions about your rights as a participant in this study, or in the event of a research related injury, you may contact the Division of Research Affairs at San Diego State University (telephone: 619-594-6622; email: irb@mail.sdsu.edu). At any time during the research you can contact the IRB for questions about research rights, to discuss problems, concerns, or suggestions, or to offer input.

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Name of Participant (please print) Date

Signature of Participant Date

Signature of Investigator Date

SAN DIEGO STATE UNIVERSITY

Institutional Review Board

Approval Expires: July 12, 2017
Study Number: 2445100