

The Impact of Waterpipe Tobacco Flavors on Waterpipe Smoking Intentions, Perceptions, Patterns, and Toxicant Exposure

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A. SPECIFIC AIMS

Waterpipe (WP) smoking in the US is on the rise and is beginning to overtake cigarettes as the most popular method of tobacco use among young adults.^{1,2} A potential reason for the appeal of WP tobacco is that it is almost exclusively flavored.³ Added sweeteners may also flavor the tobacco. The Family Smoking Prevention and Tobacco Control Act which banned flavors, except menthol, from cigarettes due to scientific evidence indicating that flavors increased youth initiation and maintained use among adults, does not currently apply to WP tobacco.⁴ As a result, WP tobacco is unregulated and comes in hundreds of flavors. In order to regulate WP tobacco effectively, the FDA requires clear scientific evidence to support regulations.⁴ **The present study will provide direct evidence to the FDA as to whether flavorings contribute to the initiation and maintenance of WP smoking, influence WP smoking patterns, and impact the uptake of toxicants.**

WP smoking involves inhaling smoke through a hose, causing air to be pulled over burning charcoal, heating the tobacco and producing smoke that travels through the waterpipe, the water, and finally the hose to the user.⁵ Mistakenly, WP users believe that the water filters out toxicants; however, WP users are exposed to the same toxicants that are present in combustible cigarette smoke, such as heavy metals, benzene, polycyclic aromatic hydrocarbons, tar, carbon monoxide, and nicotine, but often at *higher* levels.^{6,7} As such, **WP smoking is associated with many of the same negative health outcomes as combustible cigarette use such as cancer, lung disease, respiratory illness, and cardiovascular disease.**⁸

Both the frequency of WP use and the manner with which it is smoked contributes to the development and maintenance of nicotine dependence.⁹⁻¹¹ WP smoking delivers pharmacologically active doses of nicotine to the user.^{12,13} Although most WP smokers in the U.S. smoke WP intermittently, some eventually move on to more frequent use.^{14,15} **If flavorings are determined to influence users to initially try WP, improve their perceived satisfaction from use of the product, and/or make it more palatable to puff more frequently or take longer/deeper puffs, then they would be contributing to the initiation and maintenance of WP dependence.** Moreover, current level of WP dependence may moderate the impact of flavorings on smoking patterns. Unflavored WP tobacco is more difficult to find and purchase, and internet customer reviews suggest that few WP users try and even fewer continue to use unflavored tobacco (“didn’t taste good”, “may be a little hard for less experienced smokers”); however, some product reviews from WP aficionados are more positive (“This is a blend to be sipped and enjoyed slowly”), suggesting that current WP dependence level may be an important factor in the role of flavors.¹⁶

The overall aim of the current study is to determine if flavorings contribute to the initiation and maintenance (i.e., abuse liability) of WP smoking and also influence how a WP is smoked (i.e., smoking topography), which has implications for both risk of dependence but also smokers’ level of exposure to tobacco-related toxicants. Using a randomized crossover-design, 76 current WP smokers (38 low dependent, 38 high dependent) will complete four counterbalanced WP smoking sessions (preferred flavored-sweetened, preferred flavored-very low sweetened, unflavored-sweetened, unflavored-very low sweetened) that are preceded by 12 hours of tobacco/nicotine abstinence. Sessions will be separated by a standard 48-hour washout period.

Aim 1: To understand how flavorings impacts WP initiation, smoking behaviors, abuse liability, and exposure to tobacco-related toxicants. (H1a) *Flavored-sweetened WP tobacco will be associated with longer and more frequent puffing resulting in the greatest overall levels of smoke inhalation (mL of smoke inhaled), (H1b) highest abuse potential, and (H1c) greatest levels of exposure to nicotine and carbon monoxide (CO), followed by unflavored-sweetened WP, then flavored-very low sweetened and lastly unflavored-very low sweetened WP.* (H1d) *A majority of WP smokers will report having initiated WP smoking with flavoredsweetened tobacco and report flavoring as an important reason for trying WP.*

Aim 2: To understand whether level of WP dependence influences the impact of WP flavoring on smoking behaviors, intentions for continued use, abuse liability, and exposure to tobacco-related toxicants. Compared to high dependence users smoking unflavored WP tobacco, low dependence users will show greater declines in (H2a) puff frequency and duration resulting in lower levels of smoke inhalation, (H2b) intentions of continued use, (H2c) abuse liability, and (H2d) their exposure to nicotine and CO while smoking unflavored-sweetened and unflavored-unsweetened (greatest decline) vs. flavored-sweetened WP tobacco.

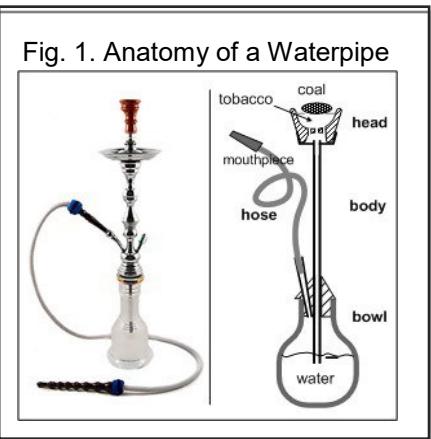


Our team is uniquely suited to conduct this investigation. Understanding how WP smokers and smoking would be influenced by a flavoring ban is critically important. This novel study addresses seven of the FDA's research priorities and will contribute knowledge urgently needed by the FDA and health officials to effectively regulate WP tobacco in a way that best serves public health.

SIGNIFICANCE

The rapidly growing popularity of WP smoking: While traditional tobacco product use (cigarettes, cigars, etc.) continues to decline, WP smoking is on the rise in the United States.^{1,17} From 2011 to 2014, current (past 30-day) cigarette use declined from 15.8% to 9.2% among high school students, while during the same period, current WP use increased from 4.1% to 9.4%.¹ Additionally, WP has overtaken cigarettes as the most frequently tried tobacco product among young adults (46.6%)¹⁸ and is frequently the first tobacco product ever tried (12.0%).² Recent estimates of current and ever WP use among adults also indicates an upward trend, with current and ever use rates currently at their highest (1.5% and 9.8%, respectively).¹⁹

The negative health effects of WP smoking: WP smoking involves the inhalation of tobacco smoke after it has been drawn through water (see figure 1).^{5,20} WP smoking behavior (i.e., topography) is significantly different than cigarette smoking behavior. A cigarette smoker will inhale approximately 500ml of smoke per cigarette (50 ml/puff x ~10 puffs); a WP smoker will inhale approximately 500ml of smoke *per puff*.²¹⁻²⁷ **One puff of WP delivers as much smoke as one cigarette.**²⁸⁻³⁰ To make matters worse, a typical WP session involves smoking for 45-60 minutes, with users inhaling ~90,000ml of smoke—the equivalent of smoking 9 packs of cigarettes.²¹⁻²⁷ As a result, WP smoking delivers the same toxicants present in cigarette smoke but often at higher levels: volatile aldehydes that cause lung disease, polycyclic aromatic hydrocarbons and heavy metals that cause cancer, carbon monoxide that contributes to heart disease, and nicotine that causes dependence.^{22-24,27,29,31-38} Consequently, smoking tobacco through a WP is linked to the same adverse health conditions as smoking cigarettes.⁸



The Potential Role of Flavorings in WP Smoking Initiation and Maintenance: Flavors in tobacco cigarettes were banned due to their proven role in the initiation and maintenance of smoking. Specifically, flavors were demonstrated to 1) specifically target youth and young adults as flavors had a strong appeal to naïve and younger smokers;³⁹⁻⁴³ 2) make it easier to smoke tobacco, increasing rates of initiation and nicotine dependence;^{44,45} and 3) maintain smoking as smokers reported that flavors were an important component in their continued use of cigarettes and also made the experience of smoking more satisfying.⁴⁶ However, **no research has experimentally explored the impact of flavors among WP smokers.**

The extant WP research indicates that flavors have played a significant role in the spread of WP smoking.⁴⁷ In a nationally representative sample, 59% of WP smokers reported use of flavored tobacco, more than any other tobacco product.⁴³ Participants in qualitative research emphasize the importance of flavors in WP smoking stating, "I think it has to do with the flavor that got me to smoke it. It was a lot of variety" and "I like it, it was flavored and it was fun after a while".⁴⁸ Individuals also cite flavors as giving the impression that WP smoking in general is relatively harmless,⁴⁸ and that flavored WP tobacco is "healthier" than unflavored WP tobacco.⁴⁹ Anecdotal evidence of the importance of flavors in WP can be found in online reviews. Consumers indicate the significance of flavors in their reviews of unflavored shisha and state, "it was too strong" and "didn't taste good." One established user stated, "It is not for the new smokers," indicating the role of flavors in the initiation of WP smoking among naïve users. Making matters worse, emerging evidence indicates that WP use not only increases smokers' intentions and interest in using cigarettes,^{50,51} but longitudinal and prospective research shows that WP may also act as a gateway product to regular cigarette use.⁵²⁻⁵⁴ For these reasons, researchers and health officials have begun calling for a ban on flavored WP tobacco.³

Humectants, like glycerine, act as sweeteners and may enhance WP tobacco flavors. Regulators have limited the amount of glycerin (≤5%) in WP tobacco sold in Germany; substantially less than the levels sold in the U.S. (~25-50%).^{85,86} Unfortunately, Germany has not regulated the separate sale of bottles of WP-specific glycerin additive which are now sold alongside WP tobacco on German WP websites. The sale of these bottles

indicates the importance of glycerin to WP tobacco users. Thankfully, if the present study's hypotheses are supported, the Tobacco Control Act grants the FDA regulatory authority over these component products.

Potential role of flavorings among highly dependent vs low dependent WP users: Research indicates that like cigarette smokers, WP smokers are sensitive to nicotine and its subjective effects. In a laboratory study of high (high dependence) and low frequency (low dependence) smokers administered active or a placebo (zero-nicotine) WP, high dependence smokers exhibited changes in puff topography between products.⁵⁵ Specifically, when smoking the placebo WP, high dependence smokers took more frequent and longer puffs relative to the active WP session.⁵⁵ A similar pattern is possible in the way high vs. low dependence WP users are affected by a lack of flavorings. More dependent users may be highly motivated to decrease craving or withdrawal and therefore, are less affected by the lack of flavoring. Alternatively, low dependence users, less motivated by craving or withdrawal and rather, motivated by the experience provided by flavored WP, may be more affected by the lack of flavorings. To date, no research has explored the interaction between level of WP dependence and the impact of WP tobacco flavorings.

The role of the U.S. FDA and regulation of flavoring: The current prohibition against characterizing flavors established in the Tobacco Control Act applies only to cigarettes and will not generalize to other tobacco products even if the product itself is deemed to be under the authority of the FDA.⁴ In order for the FDA to institute a flavor ban on WP tobacco, strong scientific data would be needed to support such an action.⁴ As a result, waterpipe tobacco, a product potentially as addictive and as harmful as cigarettes, is currently sold in hundreds of flavors and enjoying an unfettered market and a naïve consumer base, similar to that enjoyed by cigarette manufacturers before the 1964 Surgeon General's report.⁵⁶ **The present study will provide direct evidence to the FDA as to whether flavorings contribute to the initiation and maintenance of WP smoking, influence WP smoking patterns, and impact the uptake of tobacco-related toxicants.**

INNOVATION:

One of the main appeals of WP smoking is that it is almost exclusively flavored, yet no study to date has experimentally investigated the impact of WP tobacco flavorings. While utilizing standardized and accepted methods of measurement for smoking behavior, abuse liability, behavioral intentions, and tobacco-related toxicant delivery required to inform regulations, the present study will be the first to examine the importance of WP flavorings on: 1) the puff topography of the user; 2) the abuse liability of WP tobacco; 3) the behavioral intentions to continue use of WP; 4) the delivery of addictive nicotine; and 5) exposure to CO. This study will also be the first to evaluate if a user's level of WP dependence influences the impact of smoking flavorings. This study will break new ground and provide a rich data source that is necessary for the FDA to establish a product standard prohibiting flavoring of WP tobacco.

APPROACH

Investigators: Dr. Theodore Wagener (PI) is an Assistant Professor at the University of Oklahoma Health Sciences Center (OUHSC) and the Associate Director of Training at the Oklahoma Tobacco Research Center. Although a new investigator, he received excellent training at Brown Medical School as a clinical psychology resident and T32 postdoctoral fellow in cardiovascular behavioral medicine (NHLBI T32HL076134). He has continued this momentum over the last 4 years, gaining significant experience conducting state and locallyfunded prospective, randomized control trials, clinical laboratory studies, and survey studies examining noncigarette tobacco products such as WP, e-cigarettes, and dissolvable tobacco.⁵⁷⁻⁶¹ He also has been the PI of a NIH R21, a randomized clinical trial (n=114) examining the provision of smoke-free, nicotine-containing products to parents as a method to reduce their child's exposure to secondhand smoke, which he recently completed with excellent retention (84%). Over the last year, he has completed one WP cessation study,⁶¹ and is currently conducting two clinical laboratory investigations of WP using similar methodology as the present study (i.e., topography, subjective effects, eCO, and plasma nicotine). Overall, Dr. Wagener has the experience, methodological skill, and knowledge necessary to successfully conduct the proposed study. Dr. Thomas Eissenberg (Consultant) is the leading international expert in the study of the behavioral and pharmacological effects of WP smoking. He has been PI on numerous NIH grants on WP smoking and is the PI of an FDA-funded Tobacco Centers of Regulatory Science grant investigating the behavioral and toxicological effects of alternative tobacco products. He has published over 60 papers directly related to waterpipe tobacco and serves as a member of the FDA's Tobacco Products Scientific Advisory Committee. Dr.

Alan Shihadeh (Consultant) is an internationally recognized expert in WP tobacco smoke emissions and research methods and has developed novel technologies to study human puff topography. He developed the novel WP topography device that will be used for the proposed study. Dr. Shihadeh has been funded by the NIH and FDA, and serves as a scientific expert to the World Health Organization's Study Group on Tobacco Product Regulation. Dr. Kai Ding (Biostatistician/Co-I) is a biostatistician and will be responsible for assisting with randomization procedures, database management, and conducting statistical analyses.

Design Overview: Dr. Wagener and his team will conduct a randomized crossover-design clinical laboratory trial at the Oklahoma Tobacco Research Center. WP smokers will complete four (preferred flavored sweetened, unflavored-sweetened, preferred flavored-very low sweetened, unflavored-very low sweetened), 2hr long counterbalanced WP smoking sessions that are preceded by 12 hours of overnight tobacco abstinence, biochemically verified by exhaled carbon monoxide (eCO<20ppm). Sessions will take place in a separately ventilated experimental smoking room within the OTRC and will be separated by a standard 48-hour washout period. Due to the variability in nicotine content between different brands and flavors of WP tobacco,⁶² nicotine levels will be tested prior to study recruitment by Dr. Shihadeh to ensure the same level of nicotine between WP tobacco. Outcome measures will include smoking behavior, toxicant exposure, and subjective effects.

Participants: A total of 94 current WP smokers (47 low dependent, 47 high dependent) will be recruited from the general community via internet advertisements (i.e., Craigslist, Facebook, and Twitter) as well as flyers and word-of-mouth advertising at local WP bars and lounges in the Oklahoma City Metropolitan Area. Based on our team's previous studies we conservatively assume a 20% attrition rate; thus, we will need to recruit 94 participants to have 76 complete all four sessions. WP smokers will contact a study representative and be screened over the telephone or self-screened online via computer based questionnaire. Consistent with other laboratory studies of waterpipe smoking,^{21,28,30,32,55,63,64} participants who meet the following eligibility criteria will be asked to take part in the study. Inclusion criteria: 1) a current WP smoker for at least the past 6 months, 2) smoke WP ≥ 3 times over the past 6 months, 3) Lebanese Waterpipe Dependence Scale-11 (LWDS-11) score of ≤ 9 (Low Dependence) or ≥ 10 (High Dependence), 4) between 18-50 years old, 5) willing to provide informed consent and 6) abstain from all tobacco, nicotine, and marijuana use for at least 12 hours prior to each of the four sessions. Exclusion criteria: 1) self-reported diagnosis of lung disease including asthma, cystic fibrosis, or chronic obstructive pulmonary disease, 2) history of cardiac event or distress within the past 3 months, 3) currently pregnant, planning to become pregnant, or breastfeeding (will be verified with urine pregnancy test at first session visit), and 4) any use of other illicit drugs during the last 30 days. Note: There are high levels of poly-tobacco use (35-45%) among WP smokers;^{1,18} as such, consistent with previous WP studies, the current eligibility criteria allows some flexibility in the use of other tobacco.

Recruitment Feasibility and Retention: Recruitment: We intend to recruit 94 current WP smokers (and assume 20% attrition) over a 16-month period (24 total months – 4 months of study preparation – 4 months of data cleaning, analysis, manuscript prep). Therefore, we need ~6 participants to be recruited and complete study procedures each month to achieve our goal; we are confident that our recruitment approaches will yield sufficient numbers. For example, in a recently conducted WP cessation inducement study in Dr. Wagener's lab, we were able to recruit 109 WP smokers and have them complete study procedures in a 6 month period.⁶¹ Retention: Participants will receive \$50 per completed session and a \$50 bonus after completing all four sessions, for a total of \$250. We will also facilitate study visits by offering weekend appointments as well as additional retention strategies including: 1) multiple sources of contact (email, cell phone, significant others), 2) call/text/email reminders to participants prior to appointments, and 3) requiring that in addition to a functional phone number, participants must have a home address so that they can be contacted by mail if necessary. These methods are consistent with our team's previous studies and have resulted in excellent retention rates.

WP Materials: Waterpipe: As used in previous laboratory WP studies,⁸⁷⁻¹⁰⁰ a one-hose, research quality WP, purchased from MYA (www.myasaray.com) will be used—a standard high-quality chrome-plated waterpipe and glass bowl that is fitted with seals at all joints to ensure leak-free operation, and which is manufactured to tight tolerances for consistent operation. For safe measure, a stock of WPs will be purchased and will be tested for leaks in the aerosol lab at American University of Beirut. WPs which are found to have a measurable leak (i.e. 0.05 lpm or greater at 5 cm H₂O) will be excluded (we anticipate no leaky WPs from this manufacturer based on our long experience with their products). In addition, a stock of leather hoses will be purchased from MYA and screened for air infiltration rate according to a standard protocol.¹⁰¹ Hoses whose infiltration rates fall within

a specified interval (e.g. 1.5 +/- 0.2 lpm at 5 cm H₂O) will be retained for the study. This will assure that WPs used throughout the study are research qualified, while constructed of materials typical of what is used in the natural environment. All aspects of the WP apparatus will be held constant (water level, type of foil, disposable mouthpieces, and hoses). In every session, the waterpipe hose will be tipped with a new, sterile, disposable mouthpiece. Participants accompanying the index participant, will smoke from a second Mya Saray WP.

Charcoal: We will use self-lighting charcoal briquettes. Each piece of charcoal will be pre-weighed and will be placed on top of the foil. Tobacco: For the flavored-sweetened WP smoking session, participants will be able to choose their preferred flavor from a selection of the most popular Nakhla brand flavors that we will keep on hand to ensure availability. For the unflavored-sweetened session, participants will be provided Nakhla Zaghloul and for the flavored-very low sweetened and unflavored-very low sweetened session, the German version of Nakhla Shisha. Nakhla was chosen because it is a popular brand and is one of the few that has a flavored, unflavored-sweetened, flavored-very low sweetened and unflavored-very low sweetened tobacco product.

WP Smoking Session Procedures: After completing the initial screening over the phone or online, participants will be informed of the study procedures and invited to the lab to complete the four WP smoking sessions. To ensure 12-hr abstinence, upon their arrival at the lab, participants will perform exhaled carbon monoxide testing (eCO<20ppm) and confirm that they have not used any nicotine/tobacco/marijuana products over the last 12 hours. Pregnancy exclusion will also be confirmed with a urine test (n.b.: pregnancy tests will be completed at each visit throughout the study). Along with a member of the study staff, the participant will review informed consent to ensure they understand their rights as a participant. Participants will be randomized and complete study procedures in self-selected dyads. The sessions will be counterbalanced and include: 1) smoking preferred flavored-sweetened WP tobacco 2) smoking unflavored-sweetened WP Tobacco and 3) smoking preferred flavored-very low sweetened WP tobacco and 4) smoking unflavored-very low sweetened WP tobacco. Participants will complete all four study visits in the laboratory. Pre-session abstinence from tobacco for at least 12 hours will be mandatory for all participants. Abstinence will be confirmed via participant self-report and an exhaled carbon monoxide monitor (< 20 ppm) for tobacco use. Participant dyads will first complete a 10-minute puffing session separated by 30-second intervals with the prepared hookah and then smoke *ad libitum* for up to 1 hour. This procedure will be completed for all visits. Participants will also complete a minimum 48-hour washout period between sessions.

Participants will not be allowed to eat, drink or use their phone during the session. Initially, one piece of charcoal will be lit and placed on top of the foil. Our preliminary studies as well as other WP laboratory trials, indicate that one piece of charcoal will not be sufficient for one WP session; thus, participants will be provided additional pre-weighed pieces to use as they wish. Use of additional charcoal will be recorded. Participants will smoke *ad libitum* and a WP puff topography device will discretely record smoking behavior throughout the session, including puff duration, number of puffs, puff volume, puff flow rate, and time between puffs. Blood samples (15mL per sample) and eCO will be collected immediately pre- and post-waterpipe session and during the 10-minute puffing session (one after each 5-minute puffing period). Self-report measures will be administered as indicated in Table 1.

Protocol Adherence and Quality Control: All research staff will have completed Human Subjects and HIPAA training. Standard operating procedures (SOP) have already been developed for a similar but much smaller version of the proposed study. We will spend the first month of the study refining this SOP. All staff will be trained to ensure adherence to the SOP. As is standard practice for our team's current studies, each visit will have its own checklist of specific measures to be completed and the order in which they are to be administered. To reduce data entry errors, we will use secured computer-based questionnaires for participants to complete. All blood samples collected for biomarker analysis will be given individualized bar codes for each participant. All key on-site personnel will meet face-to-face weekly throughout the entire study. Drs. Eissenberg and Shihadeh too will participate in these meetings on a biweekly basis throughout the study. During these meetings, recruitment, enrollment, data collection, data monitoring results, and concerns will be discussed.

[Baseline and Outcome Measures (see Table 1):]

All breath and blood collections will be administered by a trained research assistant at the OTRC during the four study visits. All self-report measures will be self-administered using a computer-based questionnaire. All measures have been previously used by Dr. Wagener's lab and are reliable and valid.

Demographics: Measures will assess participant age, sex, marital status, ethnicity, employment status, occupation, years of education, and socioeconomic status.

Smoking Behavior Measure: WP smoking puff topography will be measured using a pressure transducer integrated into the WP hose, whereby inhalation-induced pressure changes are amplified, digitized, and sampled.⁶⁵ Software converts signals to airflow (ml/s) and integrates the flow data, producing measures of puff volume, total puff volume, puff number, and inter-puff-interval (IPI; i.e., amount of time between puffs).⁶⁵ Puff topography is a validated and sensitive measure of drug self-administration (i.e., a behavioral measure of abuse liability) that has been used for years in both the cigarette and WP smoking literature.⁷⁹ A smoker's puff topography is highly stable and associated with users' level of dependence and predicts their level of exposure to harmful tobacco-related toxicants.^{55,80-82}

Self-report Measures: WP Use History and Dependence: WP dependence will be measured using the *Lebanese Waterpipe Dependence Scale-11*.⁶⁶ This validated scale is correlated significantly with nicotine

exposure, CO, and WP use frequency.⁶⁶ We will also ask participants at what age they first used WP, if it was flavored/unflavored, importance of flavor to their desire to use WP, average number of WP sessions per month, quantity of tobacco smoked per typical WP session, usual brand, preferred flavor, number of WP quit attempts and other tobacco, nicotine and marijuana use. Abuse Liability of Product will be measured across several domains 1) experiences positive/negative drug effects, 2) behavioral-economic choice tasks, and 3) craving for and suppression of craving and withdrawal. An adapted version of the *Drug Effects/Liking*

| Table 1. Baseline and Outcome Measures | | | Baseline | Pre-Session | After 5min Puffing Session | After 10min Puffing Session | During Smoking Sessions | Post-Session |
|---|--|---|----------|-------------|----------------------------|-----------------------------|-------------------------|--------------|
| Self-Report Measures | | | | | | | | |
| Sociodemographic Measures | | X | | | | | | |
| Tobacco Products Quantity/Frequency | | X | | | | | | |
| WP Use History – AWS (Maziak et al 2016) | | X | | | | | | |
| Lebanon Waterpipe Dependence Scale-11 | | X | | | | | | |
| Hookah Readiness Rulers | | X | | | | | | |
| Hooked on Nicotine Checklist – Hookah | | X | | | | | | |
| Hookah Knowledge Questions | | X | | | | | | |
| VAS Current Feelings | | | X | | | | | X |
| Drug Effects/Liking Questionnaire (2item) | | | | | | | | X |
| Adapted Drug Effects/Liking Questionnaire (10-item) | | | | | | | | X |
| CUDIT-R | | | | | | | | X |
| Cigarette Purchase Task (modified) | | | | | | | | X |
| Adapted Multiple Choice Procedure (modified) | | | | | | | | X |
| Questionnaire of Smoking Urges: Brief Form (modified) | | | X | | | | | X |
| Minnesota Nicotine Withdrawal Scale | | | X | | | | | X |
| Behavioral Intentions | | | | | | | | X |
| Physiological Measures | | | | | | | | |
| Blood draw (plasma nicotine) | | | X | X | X | | | X |
| Exhaled carbon monoxide | | | X | | | | | X |
| Smoking Behavior Measure | | | | | | | | |
| Smoking topography | | | | | | | X | |

Questionnaire⁶⁷ will assess the desire and liking of all three WP tobacco products, positive and negative effects (i.e., side effects), and perceived strength and effectiveness. The *modified Cigarette Purchase Task*^{68,69} will ask participants how much they would be willing to pay (ranging from 0¢ to \$1,120) to smoke the four WP tobacco preparations (modified by substituting “1 cigarette” for “45-minute session of waterpipe smoking”), “How much would you be willing to pay for...a 45-minute session of waterpipe smoking [with the current waterpipe tobacco]?”). Willingness to spend more will indicate greater abuse liability. Smoking urges/craving will be measured using a modified version (replacing the word “cigarette” with “waterpipe”) of the *TiffanyDrobes Questionnaire of Smoking Urges: Brief Form*.⁷⁰ This is a 10-item measure where participants rate smoking-related items (“All I want right now is a cigarette.”) on a 7-point Likert scale ranging from ‘strongly agree’ to ‘strongly disagree’. Similar to previous WP studies, we will collapse the items into two previously identified factors (Factor 1: strong desire and intention to smoke; Factor 2: anticipation of relief from withdrawal symptoms). Nicotine withdrawal will be assessed using the empirically validated 15-item version of the *Minnesota Nicotine Withdrawal Scale*.⁷¹ This measure assesses smoking craving, anger/irritability, anxiety, depressed mood, restlessness/difficulty concentrating, increased appetite, sleep problems, and somatic symptoms (nausea, constipation, sore throat, dizziness, coughing). Behavioral intentions is the best proximate predictor of behavior and changes in behavioral intentions are associated with changes in related behavior.⁷² Behavioral intentions of continued WP use will be evaluated at the end of the WP session using a 4-item, 7-point Likert scale ranging from 1 (“extremely unlikely”) to 7 (“extremely likely”) where they will answer “If this was the only flavor available, how likely are you to: 1) try this product again, 2) pay to smoke this product at a waterpipe lounge/bar, 3) purchase this product for personal use, 4) use this product regularly. Other self-report measures that will be administered during the baseline, pre-session, and/or post-session visits include tobacco use and frequency, waterpipe readiness rulers, hooked on nicotine checklist, waterpipe knowledge, VAS current feelings, Cannabis Use Disorders Identification Test-Revised (CUDIT-R), and finally, adapted multiple choice procedure questions.

Physiological Measures: Plasma Nicotine was chosen as a biomarker because it is the major known addictive constituent in tobacco smoke,⁷³ and is a key ingredient in WP tobacco. Nicotine content can greatly influence the category of users to which WP smoking will appeal, and also affects the extent and pattern of WP use, thus driving exposures to other constituents in the given product. Less than fifteen mL of blood will be collected just before the onset of smoking, after the first 5-minute and 10-minute puff sampling, and immediately after the smoking session (within 5 minutes of the last puff). Study staff will centrifuge samples and freeze two separate aliquots of plasma immediately at -80C for later analysis of nicotine concentrations; analyses will be completed by VCU’s Tobacco Biomarkers Core. Samples will be analyzed according to standardized methods.⁷⁴ Exhaled Carbon Monoxide (eCO) will be collected immediately before and after the smoking session. CO is a cardiotoxin that is rapidly absorbed into the bloodstream when smoke is inhaled into the lungs. WP users inhale much larger amounts of CO than a cigarette smoker, with some reports of acute CO poisoning requiring hospitalization following WP smoking.⁷⁵

Study Design Considerations & Potential Concerns: Placebo WP Tobacco: We considered adding two placebo conditions (tobacco-free, flavor matched WP herbal product, such as Soex Herbal) to further isolate the effect of flavor from the effect of nicotine; however, a tobacco-free, unflavored (or even tobacco flavored) WP herbal product is not currently available. Even if we were to find the product for sale, we were concerned about the feasibility of completing a five session crossover study within the budget and time period of a R03. If any of these products become available, and the current study indicates a pattern of results that would suggest that flavorings impact the outcomes of interest, then we plan to submit a much larger grant to examine WP tobacco flavorings and nicotine on smoking behavior, toxicant exposure and subjective effects.

Rationale for certain inclusion criteria: We chose to recruit WP smokers who had been smoking WP for at least 6 months and at least 3 times over the past 6 months as this is consistent with many previous WP clinical laboratory trials including Dr. Eissenberg’s and Shihadeh’s, and to ensure that we would be able to enroll WP smokers across a wide range of dependence levels. We will recruit WP smokers whose LWDS-11 scores are ≤ 9 (Low Dependence) or ≥ 10 (High Dependence). Specifically, High Dependence users take longer, lower velocity puffs at a higher frequency compared to Low Dependence users. As a result, they inhale more smoke per session and are exposed to greater amounts of nicotine and tobacco-related toxicants. Also, these two groups may be impacted differently by WP tobacco flavoring (or the lack thereof).



Can the study be completed within the two year limit? Yes. We have IRB approval, developed a SOP, and are currently piloting a much smaller, 2-session version (n=15) of this same study in Dr. Wagener's lab, which will allow us to address and revise any potential obstacles before we begin the proposed study. Dr. Wagener has a fully functioning laboratory, with all of the equipment and staff necessary to successfully execute the study and a successful history of recruiting WP smokers.

Power and Data Analytic Plan: Power - Aim 1 (assumes 20% attrition of 82 participants recruited): Overall levels of smoke inhalation is our primary outcome; thus, we will estimate power based on this outcome. As no study to date has investigated the impact of WP flavoring, assumptions are based on a clinical laboratory investigation of percent changes in smokers' topography while smoking menthol vs. non-menthol cigarettes.⁷⁷ According to Chow et al.,⁷⁶ 60 WP users in a Williams 6×4 crossover design achieve >87.5% power to detect a decrease of ≥23L (or ≥20% decrease for unflavored-very low sweetened vs. flavored-sweetened) and ≥18L (unflavored-very low sweetened vs. unflavored-sweetened) in total volume of smoke inhaled using two-sided tests with 0.025 alpha level (used to adjust for the aforementioned 2 pairwise comparisons), conservatively assuming a mean total volume of 115L, 110L, 100L, and 92L for flavored-sweetened, unflavored-sweetened, flavored-very low sweetened, and unflavored-very low sweetened condition, respectively, a common standard deviation of 30L, and a within-subject correlation varying from 0.1 to 0.8. Power - Aim 2: Based on a comparison study of puff topography between high vs. low dependent WP smokers,⁶³ we assume a decrease of 10L (unflavored-very low sweetened vs. flavored-sweetened) and 5L (unflavored-very low sweetened vs. unflavored-sweetened) in total volume of smoke inhaled among high dependence WP users, and a decrease of 44L (or 40% decrease for unflavored-very low sweetened vs. flavored-sweetened) and 39L (unflavored-very low sweetened vs. unflavored-sweetened) in total volume of smoke inhaled among low dependence WP users. As in the power calculation for Aim 1, we assume a common standard deviation of 30L in total volume and a within-subject correlation varying from 0.1 to 0.8. Based on these assumptions, we estimate that 30 high dependence and 30 low dependence WP users achieve >83% power to detect the difference between high and low dependence groups in terms of Δ total volume (unflavored-very low sweetened vs. flavored-sweetened or unflavored-very low sweetened vs. unflavored-sweetened), using a two-sided, two-sample t-test with an adjusted alpha level of 0.025.

Data Analytic Plan: Data will be summarized using mean (SD). Both overall and stratified (by dependence level) statistics will be reported. For Aim 1, we will use linear mixed effects models (with a random subject effect) to assess the main effect of flavor on total volume of smoke inhaled, abuse liability score, nicotine and CO exposure levels. In addition, period and carryover effects will be considered in the model. All pairwise comparisons for the main effect of flavor will be conducted and Bonferroni-adjusted p-values reported. For Aim 2, we will further include dependence level (high vs. low) and its interaction with flavoring in the linear mixed effects models (as in Aim 1) for outcomes including total volume of smoke inhaled, interest in continued WP use, abuse liability score, and nicotine and CO exposure levels. If the interaction term is significant, flavoring group differences in the outcome for high and low dependence subjects will be compared using contrasts, again adjusting for multiple comparisons with Bonferroni's method. Data will be transformed as appropriate. Missing data will not be imputed and likelihood based linear mixed models will yield valid statistical inference under missing at random (MAR) and ignorable missing mechanism assumptions. A two-sided p-value of <0.05 defines statistical significance. The SAS software (v9.3, Cary, NC) will be used for all data analyses.

Predicted Results: Aim 1: Flavored-sweetened WP tobacco will result in the greatest number of puffs as well as longer, lower velocity puffs, ultimately resulting in greater total puff volume per session. Because of greater total puff volume, participants will be exposed to more nicotine and CO. Unflavored-sweetened will expose users to the next greatest amount, followed then by the flavored-very low sweetened WP, and unflavored-very low sweetened WP will expose users to the least. Self-report measures will indicate greatest abuse liability for flavored-sweetened WP tobacco, and its importance for initiation. Aim 2: Compared to low dependence users, high dependence users will have a greater total puff volume, nicotine, and CO across all sessions. Moreover, compared to high dependence users, low dependence users will show significantly greater declines in total puff volume, CO, nicotine, abuse liability and interest in continued use when using unflavored-very low sweetened (greatest declines) and flavored-very low sweetened vs. unflavored-sweetened vs. flavored-sweetened WP.

Timeline: The first 4 months will be for any additional training required for staff, product/supplies ordering, and preparation of materials. Conservatively, we will need approximately 16 months to recruit and run the needed



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~4 participants per month. Our final participant session will be in month 20. We will clean data, conduct data analysis, and write up results in months 21-24.

5.1. Protection of Human Subjects Human Subjects Involvement, Characteristics, and Design

This section describes how the proposed project is designed and will be conducted to maximize the protection of the human participants.

Inclusion/Exclusion criteria: Waterpipe smokers will contact a trained study representative and be screened over the telephone or self-screened online via computer based questionnaire to minimize inconvenience to ineligible individuals. Those who meet the following eligibility criteria will be invited to attend the first baseline visit. Inclusion criteria: 1) a current WP smoker for at least the past 6 months, 2) smoke WP at least 3 times over the past 6 months, 3) Lebanese Waterpipe Dependence Scale-11 (LWDS-11) score of ≤ 9 (Low Dependence) or ≥ 10 (High Dependence), 4) between 18-50 years old, and 4) willing to provide informed consent and abstain from all tobacco, nicotine, and marijuana use for at least 12 hours prior to each of the four sessions. Exclusion criteria: 1) self-reported diagnosis of lung disease including asthma, cystic fibrosis, or chronic obstructive pulmonary disease, 2) history of cardiac event or distress within the past 3 months, 3) currently pregnant, planning to become pregnant, or breastfeeding (will be verified with urine pregnancy test at first session visit), and 4) any use of other illicit drugs during the last 30 days. To protect research participants' data from legal subpoena, a Certificate of Confidentiality will be obtained.

Recruitment: All WP smokers will be recruited from the general community via internet advertisements (i.e., Craigslist, Facebook, and Twitter) as well as flyers and word-of-mouth advertising at local WP bars and lounges in the Oklahoma City Metropolitan Area. Participants will receive \$50 per completed session and a \$50 bonus after completing all four sessions, for a total of \$250. We will also facilitate study visits by offering weekend appointments as well as additional retention strategies including: 1) multiple sources of contact (email, cell phone, significant others), 2) call/text/email reminders to participants prior to appointments, and 3) requiring that in addition to a functional phone number, participants must have a home address so that they can be contacted by mail if necessary.

Involvement and Design: Participants' eligibility will be determined over the phone or online using a screener and a pregnancy test will be completed at the initial visit to ensure that the participant is not pregnant. Those who are eligible and willing to participate will be invited to sign informed consent and complete their baseline visit. Enrolled WP smokers will complete four, 2-hr long counterbalanced WP smoking sessions that are preceded by 12 hours of overnight tobacco abstinence, biochemically verified by exhaled carbon monoxide (eCO<20ppm). Sessions will be separated by a standard 48-hour washout period. Participants will first complete a controlled 10-minute puffing session separated by 30-second intervals with the prepared hookah and then smoke ad libitum. The WP puff topography device will discretely record smoking behavior throughout the session, including puff duration, number of puffs, puff volume, and time between puffs. Blood samples (15ml per sample), eCO, and self-report measures of craving and withdrawal will be recorded immediately before and after the smoking session with two blood samples (less than 10mL) collected during the 10-minute puffing session. Self-report measures of abuse liability, as well as interest and willingness to continue to use the WP product just smoked will be assessed within 5 minutes after completing the smoking session.

Sources of Materials

All data collection will follow HIPAA guidelines. Data will be collected directly from the participant by a research assistant. Data will include participant responses to computer-based survey questionnaires and collected blood and breath samples.

Access to Identifiable Information and Data Storage: Only research assistants who have completed training in the ethical conduct of research and the study PI (Dr. Wagener) will have access to individually identifiable private information about human subjects. All data will be treated as confidential and will never be stored or reported in association with identifying information. In the presence of hard copies of signed informed consent,



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locked filing cabinet will be used to keep these documents separate from other study related data. The Research Electronic Data Capture (REDCap) will be used to track study related data and administer all questionnaires. REDCap is a secure, web-based application designed to support data capture and utilizes a computer-administered self-interview format. This system is designed to comply with all HIPAA regulations.

Potential Risks and Protection against Risk

There are minimal risks associated with this protocol. The protocol requires adult waterpipe tobacco smokers to undergo 12 hours of tobacco/nicotine abstinence on four occasions. Tobacco/nicotine abstinence can lead to withdrawal symptoms that include irritability, anxiety, restlessness, hunger, and difficulty sleeping.⁷¹ The effects can be uncomfortable but are not dangerous. There is a slight risk of infection with blood draw; however, blood will be drawn by trained research staff (core staff at the OTRC). Sterile instruments will be used and the participants skin will be cleaned with an alcohol wipe at the site of the needle stick. Protection against loss of confidentiality and privacy will be maintained by numerically coding all data, disguising identifying information, and keeping data locked in file drawers or in a secure, password-protected database. All biospecimen samples are de-identified and stored in a freezer. Names of participants will be kept separate from participant data. Only study research assistants and the PI will have the information that connects participant's name and ID number. All electronic data will be numerically coded and stored in a password protected database, on a password protected computer in a secure research space. Participant information will be accessible only to research staff, who are pledged to confidentiality and complete training in the ethical conduct of research (i.e., both HIPAA and CITI trainings). Identifying information will not be reported in any publication.

Recruitment and Informed Consent

At first contact, all participants will be screened according to the study's inclusion/exclusion criteria. Those who are eligible will be given a brief verbal overview of the study and invited to participate. Informed consent (including a description of the nature, purpose, risks, and benefits of the study) will take place through both oral and written explanation of the study. The voluntary nature of the study and the participant's right to withdraw at any time will be stressed during the consent process; a copy of the informed consent will be provided to the participant at the time of consent for them to keep. Informed consent will be collected by IRB-approved study personnel. Recruitment script and materials, consent forms and all study procedures will be approved by the OU Health Sciences Center Institutional Review Board. All participants will provide consent before any study data is collected.

Potential Benefits of the Proposed Research

Whereas no assurance can be made to an individual participant that s/he will personally benefit from this research, the experience should be beneficial. The immediate benefits of this research are scientific in nature, which in the long-term should benefit society as a whole. The study will also benefit waterpipe smokers as a group by providing information as to the potential toxicant exposure and abuse potential of this method of smoking. Overall, it is expected that the potential benefits to participants in the proposed study outweigh the potential risks.

Importance of Knowledge to be Gained

This study is an innovative investigation that will have important public health implications given the rapid proliferation of waterpipe smoking. To date, no study has experimentally investigated the impact of waterpipe tobacco flavorings. Understanding the impact of a WP tobacco flavorings on user initiation, continued use, exposure to addictive nicotine, and to harmful toxicants such as carbon monoxide, will help immediately inform the science base needed for the FDA to impose appropriate product specific regulations.

Data and Safety Monitoring Plan

Because a Data and Safety Monitoring Board is only required for higher risk and/or multisite studies, and the proposed research is a single site pilot study with minimal risk we do not include a formal Data and Safety Monitoring Board. However, the OTRC in conjunction with the Stephenson Cancer Center does have a



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standing DSMB that we can utilize if the funding agency or the reviewers request one. However, we do have a detailed data and safety monitoring plan.

Adverse events will be assessed by study staff at each follow-up visit via participant self-report and managed immediately. All adverse events will be reported to the OUHSC IRB. We will monitor for risk of smoking waterpipe by screening participants for general medical precautions (pregnancy, cardiovascular disease). Participants will be given contact numbers of study personnel and PI. Any adverse events, breaks of confidentiality, or any other data or safety issues that arise will be discussed immediately between study personnel and Dr. Wagener. Dr. Wagener will be responsible for completing an Adverse Events Form should an event occur. He will report Serious Adverse Events to the OUHSC IRB within 24 hours of having received notice of the event. Dr. Wagener will gather any information needed to investigate the event and to determine subsequent action. Any subsequent action will be documented and reported to the OUHSC IRB and the Program Officer at NIH. Adverse event reports will be reviewed annually with the OUHSC IRB to ensure participant safety.

ClinicalTrials.gov Requirement

The proposed clinical trial will be registered at www.clinicaltrials.gov.

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