Understanding the Effect of Waterpipe Size on Smoking Behavior, Toxicant Exposures and Subjective Experiences

Study Protocol and Statistical Analysis Plan

NCT Number: NCT05705375

2025.05.07

The University of Texas at Arlington

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Version date: May 07, 2025

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A. SIGNIFICANCE

Waterpipe (WP) smoking is increasingly popular among young adults in the U.S. and is associated with nicotine dependence, carbon monoxide (CO) exposure, and other adverse health effects. WP devices vary widely in size and design, which can influence smoking behavior, toxicant exposure, and perceptions of harm. Smaller WPs, often marketed to beginners, may produce higher toxicant levels and contribute to misperceptions of safety. Understanding the impact of WP size (Figure 1) on user behavior and exposure is critical for informing regulatory policies and protecting public health.

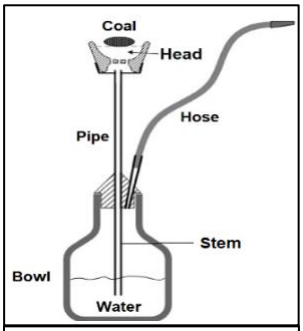

Figure 1. The main parts

B. OBJECTIVES

This study aims to evaluate the impact of WP size on smoking behavior, subjective experiences, and toxicant exposure among WP users. Specifically, the study will:

1. Characterize smoking behavior across small, medium, and large WP devices, including puff topography, session duration, and inhalation patterns.
2. Assess subjective experiences, such as perceived satisfaction, enjoyment, and harm perception, associated with different WP sizes.
3. Quantify toxicant exposure, focusing on CO and nicotine levels generated by each WP configuration.
4. Examine the relationship between WP size, user perceptions, and toxicant exposure, providing evidence for regulatory policy.

C. STUDY DESIGN

This study uses a within-subjects experimental design (crossover design), where participants will smoke WP devices of three different sizes (small, medium, large) in separate, randomized sessions (Figure 5). This design allows direct comparison of behavior and exposure within individuals, controlling for between-subject variability. The project will be conducted in the Nicotine and Tobacco Research laboratory at the University of Texas at Arlington (UTA).

Figure 5. Three WP study conditions (Large, medium and small)

C1. Participants and recruitment:

We will recruit healthy adults (21-39 years old), who are regular WP smokers (at least once a week for the past 6 months). Interested individuals will complete an online or phone screening questionnaire to determine initial eligibility, followed by an in-person screening to confirm eligibility and provide written informed consent. Eligible individuals will be scheduled for laboratory visits. We aim to recruit study participants from the substantially large catchment area of the Dallas-Fort Worth (DFW) metroplex with a population of over 7 million, making it the largest metropolitan area in Texas and the fourth-largest in the U.S. According to the recruitment method we followed in a previous similar lab study, recruiting participants from a larger catchment area will ensure reaching the desired sample sizes within the allotted timeframe and will likely increase the generalizability of the study findings. We will utilize a combination of *online*, *offline* and *in-person* recruitment methods to ensure successful completion of our study. Offline items such as posters, flyers, and study inserts will be created, posted and distributed on UTA campus, nearby off-campus locations, and around tobacco shops in DFW Metroplex. Online recruitment will include listservs, Facebook and social media. In person recruitment will be conducted by handing out flyers and word-of-mouth. Participants will be scheduled to attend three laboratory sessions and will be compensated with $150 for completing all study sessions. The study will be conducted in the Nicotine and Tobacco Research Laboratory at the University of Texas at Arlington (UTA), located The University of Texas at Arlington, Maverick Activities Center (MAC), 500 W Nedderman Dr, Arlington, TX 76013.

C2. Inclusion and exclusion criteria:

Inclusion criteria: 1) Young adults (21-39 yrs.); 2) Regular WP smokers (smoking WP at least once a week for the past 6 months); 3) generally healthy and 4) able to provide written informed consent; and willing to attend the 3 lab sessions as required by the study protocol.

Exclusion criteria: 1) history of chronic diseases such as diabetes, high blood cholesterol, hepatic or renal disease; 2) respiratory chronic diseases 3) cardiovascular diseases including low or high blood pressure (BP) (systolic BP>150 mm Hg, or diastolic BP>100 mm Hg); 4) regular use of prescription medications (other than vitamins/birth control); 5) breastfeeding or testing positive for pregnancy (urine testing) at the screening and 6) Regular use (> 5 times/month) of other tobacco products (e.g., e-cigarettes, cigarette, cigars).

C3. Experimental procedure:

Participants who are deemed eligible by phone will attend the lab for an additional in-person screening visit to confirm eligibility and provide written informed consent. Participants will be asked to abstain from any tobacco use ≥12 hours prior to study visit. Abstinence will be confirmed by breath CO<5 ppm (Ben Taleb et al., 2020). Participants will attend the lab for three sessions (~2-h each), separated by a 48 hours washout period. The session order will be counterbalanced to account for the first-order carryover effect between the study conditions (WP sizes). The first session will be conducted at the end of screening if the CO < 5 ppm criterion is met. Following a 15 min adaptation period and before WP smoking, participants will respond to subjective measures, and 2 ml of saliva will be collected. They will then begin a maximum 45 min WP smoking session. Based on the commonly marketed WP sizes, we will use the classic *Khalil Mammon* WP devices for large WP (~90 cm), medium WP (~60cm) and small WP (~30cm) (Figure 5). For standardization, the type of tobacco, flavor, charcoal used, and the hose length will be held constant across the three WP size conditions. Prior to each session, the lab staff will clean the WP bowl and fill it with water maintaining 4 cm of WP body stem immersion (Shihadeh, 2003), which is ideal to sustain unrestricted airflow (Hookah-Shisha, 2014). The research staff will pack the WP head with 15 g of tobacco preparation and cover it with perforated aluminum foil. Tobacco will be heated with quicklight charcoal disks. Participants will smoke *ad libitum* and at the end of the smoking period 2 ml of saliva will be collected again, CO will be measured, and subjective measures will be

assessed. The session will terminate 30 minutes after the last puff. To facilitate natural smoking behavior similar to WP lounges, entertainment will be available during the session (e.g. movie selections/background music).

C4. Study outcomes:

1) Toxicant exposure: This study includes measurement of expired air CO and saliva nicotine as listed below.

- *Exhaled CO:* High levels of exhaled CO (major respiratory toxicant) has been shown to compromise cardiopulmonary regulation and is a major risk for cardiovascular. Expired air CO has been shown to be a key marker of toxicant exposure in WP smokers. Expired CO levels will be measured at baseline, and within 10 min after the WP smoking session via Breath-CO monitor.
- *Saliva nicotine:* Participants will receive instructions by the staff to collect ~2 ml of saliva via a sterile disposable collection kit, before and immediately after the end of each smoking session. Saliva samples will be stored at -80°C and will be later analyzed using GC-MS by UTA Shimadzu Institute (see letter of support). While plasma nicotine is commonly used as a measure of nicotine exposure, based on a thorough examination of the literature, the collection of plasma samples is not necessary for achieving the aims of this study. Saliva nicotine is relevant to WP acute effects and has been used in studies assessing subjective experiences and toxicant exposure in tobacco smokers. Moreover, saliva and plasma nicotine levels were found to be highly correlated with saliva being potentially more sensitive than plasma. Furthermore, in studies that assess mood-related responses, such as satisfaction and craving, plasma collection could present a possible confounding factor because of the anxiety and pain involved in venipuncture. Unlike plasma, collecting saliva can be done noninvasively and therefore is considered an ideal sampling site in clinical studies evaluating subjective experiences.

2) Puff topography: Parameters of puffing topography are important indicators of toxicants exposure among WP smokers including exposure to nicotine and CO. In this study, puff topography will be recorded using a device integrated into the WP hose, that was developed by our collaborator (Dr. Shihadeh), and validated in several studies of WP smoking. Briefly, Inhalation-induced pressure changes will be amplified, digitized, and sampled. Software converts signals to air flow (mL/sec) and integrates the flow data, producing measures of total smoking time, total puff time, puff duration, interpuff interval, number of puffs, total volume inhaled, and average puff volume.

3) Subjective measures: In this project, we will utilize 5 subjective measures. The selection of these measures was driven by the suitability for our study's aims and the prior use in similar studies assessing WP tobacco products. Participants will use a computer tablet to respond to these measures for each study condition using the *QuestionPro* software. Individual items for each measure are summarized below.

- *Harm perception:* We will use the 3 items adapted from (Popova & Ling, 2013). The direct measure will be; "Compared to the WP size that you usually use for smoking, this WP size is…" with answers on a 7-point scale ranging from −3, a lot less harmful; to 0, equally as harmful; to +3, a lot more harmful. To measure perceptions of WP's relative risk compared to cigarettes and e-cigarette, we will ask 2 separate questions: "In your opinion, how harmful is the WP to general health?" and "In your opinion, how harmful is smoking cigarettes for health?" The answers to both will be noted on 7-point scale ranging from 1 (not at all harmful), to 7 (extremely harmful). These questions will be administered *after* the WP smoking session.
- *The Cigarette/WP Evaluation Scale (WES):* Is an 11-item questionnaire that will be adapted from cigarette and modified for the WP. This scale assesses participants' perception of the smoked WP for: (1) Satisfying; (2) Tastes good; (3) Makes you dizzy; (4) Calms you down; (5) Makes you concentrate; (6) Feels more awake; (7) Reduces hunger for food; (8) Makes you nauseous; (9) Feels less irritable; (10) Did you enjoy the

sensations of the smoke in your throat and chest?; and (11) Did it immediately reduce your craving for smoking?. WES will be answered on a 7-point Likert scale as the DSQ. WES will be administered *after* the WP smoking session.

- *Duke Sensory Questionnaire (DSQ):* The DSQ consists of nine items; (1) How much did you like the puffs?, (2) How satisfying were the puffs?, (3) How high in nicotine were the puffs?, (4) How similar to your usually smoked WP were the puffs?. Rate the strength of the puffs on (5) tongue, (6) nose, (7) back of mouth and throat, (8) windpipe and (9) chest? All questions will be answered on a 7-point Likert scale anchored at the extremes (1= not at all; 7=extremely). The DSQ will be administered *after* the WP smoking session.
- *Minnesota Nicotine Withdrawal Scale (MNWS):* The MNWS scale consists of 11 items; (1) urges to smoke, (2) irritability (3) anxious, (4) difficulty concentrating, (5) restlessness, (6) hunger, (7) impatient, (8) craving (9) drowsiness, (10) depression and (11) desire for sweets. These items are presented on Visual Analog Scale (0-100) with each item ranging from *not at all* to *extremely*. The MNWS will be administered pre and post WP smoking.
- *Questionnaire on Smoking Urges (QSU):* The QSU consists of 10 items on a 7-point Likert scale from 0 (strongly disagree) to 6 (strongly agree). Items are; (1) I have a desire to smoke now; (2) Nothing better than smoking now; (3) If it were possible, I probably would smoke now; (4) I could control things better now if I could smoke; (5) All I want now is to smoke; (6) I have an urge for a WP; (7) A WP would taste good now; (8) I would do almost anything for a WP now; (9) Smoking would make me less depressed; and (10) I am going to smoke WP as soon as possible. The QSU will be administered pre and post WP smoking

C5. Sample size: Because this is the first clinical study assessing the effect of WP size among smokers, there is no prior data available. Nonetheless, we built the calculation for the sample size based on our prior work on assessing the effects of health warning labels and tobacco flavor on WP subjective experiences (satisfaction), puffing topography (inhaled volume) and toxicant exposure (exhaled CO). The effect sizes corresponding to those outcomes ranged from 0.7 to 0.9, which is considered to be large using Cohen's criteria. To be conservative we chose a small-to-medium effect size of .4 using repeated measures ANOVA F test for within-subject factors. Accordingly, a sample size of 40 would be sufficient to detect this effect size with 80% power, assuming a two-sided significance level of 5% and accounting for expected attrition.

C6. Statistical analysis: Topography data will be processed automatically by the topography instrument software to remove closely spaced puffs (i.e., IPIs < 300 msec). Such puffs are considered as continuation of previous puff and the recorded volume and duration values will be added to that preceding puff. After this procedure, any puffs less than 5 ml will be automatically considered an artifact and discarded. Remaining data will be averaged for each participant in each condition using all remaining values for puff volume, duration, number, and IPI. For saliva nicotine, values below the limit of quantitation (LOQ) will be replaced with the value of the LOQ. Sample characteristics will be summarized with means and SDs or proportions. Means will be calculated for measures of harm perception, WES, and DSQ and will be compared by study condition (WP size) using two-tailed paired samples t-tests. For measurements of exhaled CO, saliva nicotine, QSU and MNWS, analyses will consist of repeated measures analysis of variance ANOVA with 2 within-subject factors: study condition (small WP vs. medium WP vs. large WP) and time (pre-post smoking). Significance levels will be adjusted for violations of the sphericity assumption using Huynh-Feldt corrections. The mean square error terms for the overall interaction will be used to conduct Tukey's HSD test, with comparisons at $P < 0.05$ considered significant. Statistical assumptions will be evaluated prior to analyses. Normality of continuous variables will be assessed using the Shapiro-Wilk test and visual inspection of histograms. ***The model will also control for potential confounding effects such as age, gender, history of smoking, and frequency of use. All***

analyses will be conducted in a blinded fashion toward the allocated tobacco product to thwart bias. All statistical analyses will be conducted using SPSS version 29.

D. TIMELINE:

This project will be conducted over three years. During the first 3 months of year one, we will equip the study lab, prepare study protocols and instruments, test the equipment and software, and obtain IRB approvals. Recruitment and enrollment of participants will start on month 4 and will continue to month 20. The final 4 months of the project will be dedicated to data analyses and reporting. However, we expect to start reporting of preliminary data beforehand. Please see Table 1 (above) for details.

Year 1 (months)	1	2	3	4	5	6	7	8	9	10	11	12
Prepare protocols and instruments												
Obtain IRB and equip the study lab												
Train study staff and students												
Enrollment of participants												
Year 2 (months)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Enrollment of participants												
Data analyses												
Reporting/manuscripts submissions												