RESEARCH PROTOCOL

Waterpipe tobacco additives and their effect on human puffing behavior, toxicant exposures, pulmonary function, and appeal

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I. Objectives

The study addresses the following FDA Scientific Interest Areas: Chemistry and Engineering, Toxicity, Behavior and Addiction; in that it will determine how specific WP tobacco additives affect users puffing behavior, liking, the quantities of toxicants they inhale and take up into their bodies, and acute pulmonary affects from WP tobacco smoking. These data will inform potential FDA product standards, or maximum limits for additives in WP tobacco, to curb the harm, addictiveness and appeal of WP tobacco smoking. Our multidisciplinary team includes experts in the conduct of clinical trials, WP smoking behavior, WP emissions testing, tobacco product analytical chemistry, psychophysical measurement, and tobacco use risk perception. In the research conducted at The Ohio State University (OSU), we will conduct the study according to the following aims. Note that Aim 3 is the only aim that involves the use of human participants.

Aim 1: Characterize select harmful and potentially harmful constituents (HPHCs) and sugar content of four WP tobaccos (one brand prepared four different ways to vary glycerol and sugars). A commercial WP tobacco brand will be modified to produce four different tobaccos:

(1) Medium glycerol (60 mg/g), Medium sugars (350 mg/g)
(2) High glycerol (120 mg/g), Medium sugars (350 mg/g)
(3) Medium glycerol (60 mg/g), High sugars (510 mg/g)
(4) High glycerol (120 mg/g), High sugars (510 mg/g)

Aim 2: Characterize select HPHCs and sugars yield in mainstream smoke generated from the four WP tobaccos characterized under Aim 1 using machine smoking of the research-grade waterpipe, and a standardized WP puffing regimen. *Hypothesis*: Mainstream smoke generated from WP tobacco with higher concentrations of glycerol and sugars will have higher concentrations of HPHCs.

Aim 3: Determine how WP tobacco content impacts puffing behaviors, CO biomarker, pulmonary function, nicotine uptake, and perceived sensory attributes and appeal of WP smoking. Experienced WP smokers will participate in four laboratory sessions to smoke the research-grade waterpipe(s) *ad libitum* using the four different WP tobacco preparations characterized under Aim 1. *Hypotheses*: Participants will inhale a greater total puff volume and report greater appeal when smoking tobacco with medium glycerol and high sugars; nicotine uptake will not differ across the four tobaccos. Participants will report lower risk perceptions and greater satisfaction when smoking tobacco with medium glycerol and high sugars.

Aim 4: Determine the HPHC exposure ranges from the average puffing behaviors measured under Aim 2 for each tobacco. Topography data collected for all participants for each tobacco type will be averaged to produce four human-derived puffing regimens. Machine smoking will be conducted for each tobacco type, using the associated human-derived puffing regimens, to determine the range of HPHCs in the mainstream smoke. *Hypothesis*: Toxicant yields will be highest in the mainstream smoke generated from the medium glycerol and high sugars tobacco.

II. Background and Rationale

The ***scientific premise*** of this study includes understanding who smokes waterpipe, how the harm of using a tobacco product is codified by the FDA, and how sweet WP tobacco additives may contribute to the harm of WP tobacco smoking.

II.1. Who Smokes Waterpipe?

Just within the past 16 years, the global spread of waterpipe (WP) tobacco smoking has exceeded predictions.[1,29] This worldwide epidemic has increasingly spread among youth, primarily in the Middle East and North Africa.[29] During 2011-2014, U.S. middle and high school student use substantially increased each year with an estimated 1.6 million adolescent WP users.[30] Longitudinal studies indicate the majority of experimentation begins in high school and early college,[31,32] with up to 13.8% never-users initiating WP in the first month of college.[33] Because of inconsistent indoor air legislation across the U.S., hookah lounges that support and thrive on early social experimentation with WP and other combustible tobacco products are prevalent near college campuses.[34-36] The 1990s introduction and widespread availability of heavily sweetened WP tobacco,[1,37] and the persistent perception that WP usage is a safer alternative to cigarette smoking,[2,4,6-8] are thought to be two of the main contributing factors leading to the rise in popularity of WP tobacco smoking among youth. As cigarette smoking declines, more novel tobacco products that contain sweet flavors that mask the harshness of tobacco smoke are the products most often tried by youth.[1,38]

II.2. How Do We Codify Harm?

The U.S. Food and Drug Administration (FDA), through the Family Smoking Prevention & Tobacco Control Act,[39,40] classifies harm from tobacco products in terms of the chemical constituents that can be inhaled, ingested, or absorbed into the bodies of users.[10,11] These harmful and potentially harmful constituents (HPHCs) have been measured in WP tobacco smoke,[12] and thus have the potential to cause direct harm via delivery of carcinogens, pulmonary, cardiovascular and developmental toxicants, and addictive chemicals to the body,[12] or indirect harm by facilitating tobacco initiation,[41,42] increasing progression to cigarette smoking,[43-48] impeding cessation of cigarette smoking,[48,49] and/or increasing the intensity of tobacco product use.[50]

II.3. How Can Sweet WP Tobacco Additives (Sugars and Glycerol) Contribute to Direct Harm?

WP tobacco smoking is associated with increased risk for lung, oral, esophageal, and head and neck cancers, and cardiovascular and pulmonary disease.[51-54] Despite its growth in popularity over the past 30 years, there is little data available on the ingredients in WP tobacco, as the bulk of research has focused on toxicant yields in the smoke that is inhaled.[12] WP tobacco is very different from cigarette or even pipe tobacco[15,16] in that initial reports show that sweet tasting additives such as sugars and glycerol can comprise up to 70% of WP tobacco by weight.[13]

Glycerol, which is not a sugar but still tastes sweet, can comprise 23–63% of the weight of the tobacco.[13,22] Sugars such as sucrose, glucose, and fructose can also make up more than a third of WP tobacco weight.[16] These same compounds are added to cigarettes, but at much lower levels (1-5%),[55,56] to impart "smoothness" to the smoke and act as moisturizing agents, preservatives and solvents for flavor application.[57,58] During the heating process, these sweet additives form volatile carbonyls, compounds that are known carcinogens and pulmonary toxicants such as acrolein, formaldehyde (IARC Group 1 carcinogen) and acetaldehyde (IARC Group 2B).[59] During smoking, glycerol can dehydrate to acrolein;[60,61] thus it is not surprising that, given WP tobacco is heavily fortified with glycerol, acrolein levels in WP smoke exceed those in cigarette smoke by a factor of 15 (see Table 3).[12] Acrolein is ciliatoxic and can inhibit both the immune system and lung clearance.[62,63] When heated, glycerol can also produce formaldehyde and acetaldehyde,[64,65] which are both contributors to lung cancer in cigarette smokers and chronic obstructive pulmonary disease.[66] Moreover, acetaldehyde increases the abuse liability of nicotine, contributing to the addictiveness of tobacco smoke.[67,68] Short-term effects from exposure to these carbonyls results in pulmonary irritation and edema.[69,70] After their authorities were extended to more novel tobacco products like WP,[40] FDA proposed adding glycerol to the HPHC list.[11]

Semivolatile furans, including furfuryl alcohol (FFA), furfural, and 5-hydroxymethylfurfural (HMF), are another group of carcinogens and respiratory toxicants, that are substantially more abundant in mainstream WP smoke than cigarette smoke (see Table 3) due to the abundance of added sugars in WP tobacco. Simple sugars are comprised of molecules that contain six carbon atoms (hexoses) and five carbon atoms (pentoses). Hexoses can be chemically transformed via thermal dehydration into 5-(hydroxymethyl)-2-furaldehyde (HMF).[71] Similarly, pentoses can be thermally dehydrated into furfural.[72] Concerningly, there are few data available regarding the toxicological implications of acute and chronic human inhalation of these compounds. In long-term inhalation studies, FFA (IARC Group 2B) showed carcinogenic activity in the noses of male rats, and the kidneys (renal tubules) of male mice.[22,73] Furfural shows carcinogenicity in experimental animals via oral administration, but is classified as an IARC Group 3 carcinogen due to the inadequacy of human evidence.[74] However, FDA Center for Tobacco Products considered existing evidence strong enough to propose furfural also be added to the HPHC list.[11] Humans can metabolize both HMF and FFA to form genotoxic and mutagenic compounds (5-sulfoxymethyfurfural[75] and furfural sulphate,[76] respectively) in the body via sulfotransferase enzymatic activity.[77] For this reason, WHO has recommended HMF be given high priority for carcinogenic evaluation.[78]

II.4. How Can Sweet WP Tobacco Additives Contribute to Indirect Harm?

By masking the unpleasant, bitter taste of nicotine,[79] sweet additives play a powerful role in initiation of and addiction to tobacco smoking.[80] Tobacco industry documents reveal that cigarette manufacturers, in an attempt to maximize the appeal of cigarette smoke, conducted human studies to determine the optimal ratio of added sugars to nicotine content.[58] Adding sugars increased the concentration of carbonyls in the smoke, which, as acids, reduced the smoke pH, resulting in nicotine being inhaled predominantly in its protonated form.[58] Protonated nicotine is more appealing for first-time users because it is less harsh than freebase nicotine and is therefore easier to inhale.[81,82] Nicotine is a highly addictive compound, and WP tobacco

smoking delivers enough nicotine to support nicotine addiction.[83] Moreover, glycerol can enhance the transfer of nicotine to the smoke increasing nicotine delivery to the user,[17] and both glycerol and sugars reduce the harshness and increase the appeal of WP tobacco smoke.[27,28] There is evidence that WP tobacco smoking may function as a gateway for initiation[45-48] and regular use of combustible cigarettes.[43,84]

II.5 Why Use Investigational Tobacco Products?

Some crossover studies have examined WP tobaccos that contain varying levels of glycerol and presence/absence of characterizing flavors and found differences in smokers puffing behaviors, toxicant exposure and appeal when using these products.[27,85] However, the measured differences cannot be related to the level of specific additive in those studies because the WP tobacco brands tested had numerous other differences that were not quantified. To systematically examine the direct and indirect harm associated with specific additives it is necessary to precisely fortify the WP tobacco with specific levels of the additives. As the initial raw material for the manipulated tobacco, we will use a popular unflavored WP tobacco with low glycerol content.

To ensure the ITPs are ecologically valid, we will fortify the unflavored WP tobacco with the correct type and concentration of sugars. There is a common belief that WP tobacco is sweetened by adding honey, perhaps because "ma'assel" is taken from the Arabic "muassel," which means "honeyed".[86] About 70% of honey is made up of two sugars, glucose and fructose, that are present in roughly equal amounts.[87,88] Given the low cost of WP tobacco ($3 for a pack that has the same amount of tobacco as an $8 pack of cigarettes), and that the worldwide market for it exceeded $1.9B in 2017,[89] it is more likely that inexpensive syrups are used.[88,90] Table II.1 shows the sweeteners that comprise honey and sugar, and less expensive syrups such as high fructose corn syrup (HFCS) and molasses, in comparison to the sugar content data we collected on flavored and unflavored WP tobacco.[16] Based on these data, the unflavored WP tobacco may have been sweetened with a combination of sugar, honey and molasses; but molasses and HFCS 55 are the more likely candidates given their availability and low cost. For the flavored WP tobacco, sucrose was not detected and thus neither molasses nor sugar were used. Molasses and HFCS 42 were most likely used to sweeten the flavored WP tobacco, given their availability and low cost. To more precisely isolate the additives of interest, we will fortify the unflavored tobacco with food grade (>99% purity) glycerol, glucose and fructose to produce sweetness and glycerol levels that do not exceed those measured in the flavored tobacco.

TABLE II.1. SUGAR CONTENT OF SWEETENERS, AND FLAVORED AND UNFLAVORED WP TOBACCO.

Sweetener	Hexoses, wt %			Glycerol, wt %
	Sucrose	Glucose	Fructose	
Corn Syrup	-	100	-	-
HFCS 55	-	45	55	-
HFCS 42	-	58	42	-
Honey		32	39	-
Molasses	34	8	8	-
Sugar	99.7	-	-	
Flavored WP Tobacco[a]	-	30	29	38
Unflavored WP Tobacco[a]	25	34	37	4

" - " = negligible; [a] = wt % expressed as a fraction of the sum of the primary sweeteners sucrose, glucose, fructose and glycerol.

Not all sugars have the same sweetening power. A sweetness hierarchy for sugars has been determined empirically by asking participants to detect sweetness in progressively increasing concentrations of single sugars.[91,92] Among the most highly fortified sweeteners added to WP tobacco, sucrose and fructose have the highest relative sweetness.[92] A power function to predict the relative sweetness of sugars based on their concentration and individual sweetening power was determined empirically from human trials.[92] Because the unflavored WP tobacco that is our starting material contains sucrose, it is important to tally the relative sweetness contributions of all the sugars and glycerol in the ITPs to produce medium and high sweetness levels, while also producing medium and high glycerol levels.

Equally important to consider is the nicotine content of each of the proposed ITPs, not only because nicotine can drive use behavior, it also produces the bitterness that the sugars and glycerol are added to offset. Given that the additives can comprise more than half the weight of the WP tobacco, we must normalize the relative sweetness by the nicotine coming from the tobacco leaf itself. We define the term "Starter Product Index" (SPI) to assign a numerical quantity to the mass of sugars fortified in the tobacco to offset the bitter harshness of nicotine:

SPI = sum of the relative sweetness of the sugars and glycerol / mass of nicotine

The proposed additive concentrations in the ITPs are shown in Table II.2. For ecological validity, the SPI of the proposed ITPs are bracketed by the SPIs for the unflavored and flavored commercial WP tobaccos, also shown in Table II.2.

Table II.2. The proposed Investigational Tobacco Products (ITPs) are fortified with ecologically valid levels of sweet additives.

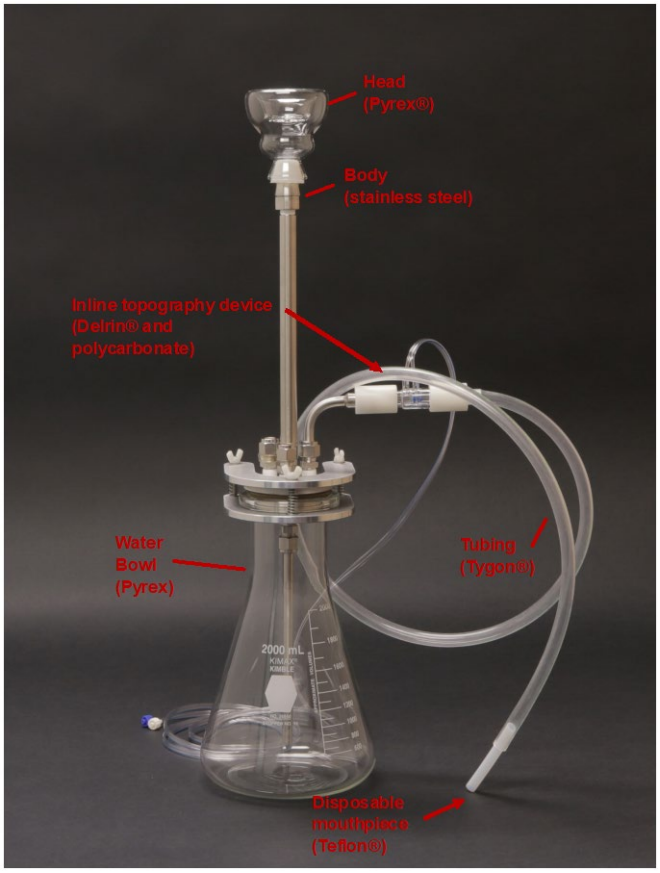
	WP Tobaccos	Concentration (mg additive/g of tobacco)				Relative Sweetness	SPI
		Glycerol	Sucrose	Glucose	Fructose		
Proposed ITPs	Medium Glycerol Medium Sugars	60	90	125	135	106	64
	High Glycerol Medium Sugars	120	90	125	135	101	68
	Medium Glycerol High Sugars	60	90	195	225	110	91
	High Glycerol High Sugars	120	90	195	225	103	99
Commercial Products	Unflavored WP Tob	13	90	125	135	106	59
	Flavored WP Tob	246	0	195	190	106	103

II.6. Why Use a Research-Grade Waterpipe?

Commercially available WPs and their components vary widely in design and durability, including differences in fabrication materials used for stems, bases, bowls, and hoses; sealing joint designs and degree of leak-tight fit; and diameter of flow path. All of these components can affect puffing intensity, and exposure to nicotine, CO and other harmful toxicants.[93-95] A rugged, reproducible WP is needed to reliably determine the variability in human WP puffing behavior (topography). Our team developed and qualified the use of standardized single- and dual-hose WPs (RWP and RWP2) equipped with puffing topography analyzers.[96] The RWP2 is shown in Figure II.1. The RWPs both operate with known precision and accuracy, and are well-accepted by established WP smokers in terms of satisfaction and reward.[94-96] More information on the RWP and RWP2 is provided in Appendix A to this protocol.

II.7. What will this study add?

Figure II.1. A research-grade waterpipe (RWP) equipped with human puffing topography data collection.



The study design is highly innovative in several ways in that it 1) includes human and machine smoking of a manipulated, ecologically valid WP tobacco product to isolate the effects of glycerol and sugar additives, 2) it draws on a comprehensive set of chemical measurement techniques to estimate ranges of volatile and semivolatile HPHCs delivered to the smoker, and 3) it uses a validated, research grade waterpipe (RWP) of known precision and accuracy. More detail on these study design aspects is given below.

Several studies[13,96-98] have used behavioral data and machine smoking to characterize the chemical composition, toxicity, and carcinogenicity of popular WP tobaccos. To the best of our knowledge, this is the first human study to use a precisely manipulated WP tobacco in order to systematically examine the effects of the additives of greatest interest with respect to evaluating direct and indirect harm associated with WP smoking: glycerol, fructose and glucose. As the initial raw material for the manipulated tobacco, we will use a popular unflavored WP tobacco with low glycerol content (see Table II.2). Our data on the sweet additives measured in a popular flavored and a popular unflavored WP tobacco were used to ensure the identities and concentrations of additives in the ITPs are ecologically valid.

The multi-analyte chemical characterization techniques for the measurement of established and proposed HPHCs have been developed and validated by MPI Brinkman for use in tobacco regulatory research on WPs.[13, 16, 95, 96] Although the chemical composition of WP tobacco and emissions are important, other factors also contribute to the potential health impacts of WP smoking. The subjective data collection techniques include the application of a food industry-established psychophysical measurement technique[100-104] to quantify the perceived intensities of tobacco flavors. Moreover, general WP harm perceptions (absolute, relative) and specific health risk perceptions (e.g., addictiveness, lung cancer, oral cancer) will be captured using survey items adapted from the US Population Assessment of Tobacco and Health Study[105] and being used in MPI Brinkman's current WP study (R01CA229306). We will also employ either the RWP or RWP2,[96] novel, standardized devices developed by MPI Brinkman that operate with high precision and accuracy for the measurement of WP puffing behavior for one (RWP) or two

people (RWP2) smoking from the same WP. The research-grade waterpipe will also be used for the generation of machine-smoked constituent data using both human puffing topography collected in the proposed study, and an accepted standardized, multi-staged puffing regimen derived from a previously examined cohort of experienced WP smokers smoking the RWP and a flavored WP tobacco.[95,96] The RWP2 was developed from the single hose research-grade waterpipe, the RWP, and is a tool with benchmarked performance metrics that was well-accepted by experienced WP smokers in a previously conducted clinical research.[94-96] The RWP2 is fabricated from inert materials that do not scavenge, via adsorption to surfaces, nor generate via thermal degradation or off-gassing, the HPHCs of interest being measured in mainstream smoke.

Currently there are no regulations governing the content of WP tobacco. Because mandated changes in tobacco content may lead to unintended consequences that ultimately result in public health declines, human use behaviors must be well understood prior to implementing regulatory product standards. Compensation, or the degree to which a smoker changes their intake of smoke to make up for the changes made to the tobacco product, is one possible unintended consequence. The proposed smoke HPHC yield measurements, made in smoke generated using both a standardized and a specific (to human-use of the tobacco) puffing regimen, along with the participants' plasma nicotine boost from using the product, will allow us to determine if compensation, and thus greater HPHC exposures, is associated with the presence/absence of sweet additives.

This study will be the first to combine powerful analytical chemistry techniques, CO and nicotine biomarkers, cutting edge psychophysical measurement tools, and risk perception instruments to map the relationship between sensory experiences and preferences of sweetness and flavor to specific additive content in WP tobacco that affect these experiences and preferences. The data from the proposed study will provide direct links between WP tobacco's primary additives, CO and nicotine biomarkers, smoker preferences, perceptions of harm and puffing behaviors, and the subsequent range of HPHC exposures associated with these additives and behaviors. This work will inform the FDA regarding its regulatory authorities surrounding the manufacture, distribution and marketing of WPs, and inform the evaluation of product applications and development of product standards, such as glycerol and sugar content, to reduce the resulting public health toll from WP tobacco product use in the US.

III. Procedures

III.1. Research Design Overview

The proposed study will include preparing and characterizing the content of 4 investigational tobacco products (ITPs) (Aim 1); characterizing the mainstream smoke toxicant emissions from machine smoking the 4 ITPs using a single, established puffing regimen (Aim 2); measuring human puffing behavior, general harm and specific health risk and flavor perceptions, lung function, and biomarkers of exposure in a group of established WP smokers smoking the 4 ITPs in the laboratory (Aim 3), and estimating toxicant exposure ranges using machine smoking and puffing regimens derived from the human laboratory testing (Aim 4).

AIM 1

III.A1. Aim 1 Research Design

Characterize the HPHC and sugar content of four WP tobaccos (one brand prepared four different ways to vary glycerol and sugars). Aim 1 will focus on the preparation and subsequent chemical characterization of WP tobacco with high and medium levels of sugars and glycerol. A commercial WP tobacco will be manipulated to prepare a 2 x 2 matrix of 4 tobaccos with medium and high levels of glycerol and sugars.

III.B1. Aim 1 Sample

There are no human participants planned for Aim 1. Sufficient quantities of the commercial WP tobacco for the grant will be purchased at the beginning of the study by a single vendor and stored in sealed containers at room temperature.

III.C1. Aim 1 Measurement/Instrumentation

Nicotine, humectants, sugars, carbonyls, and semivolatile furans, as detailed in Table III.3, will be determined twice: once at the beginning and once at the end of the human study. Nicotine content of the unburned tobaccos will be determined using gas chromatography with mass spectral detection (GC-MSD).[118] Humectant content will be determined using GC with flame ionization detection (FID),[22] or GC/MS. Sugar content, including sucrose, glucose, and fructose, will be determined using high performance liquid chromatography (HPLC) techniques.[155, 156] Carbonyls will be determined in the tobacco as their 2,4-dinitrophenylhydrazine (DNPH) derivatives using liquid chromatography tandem mass spectrometry.[22] Semivolatile furans will be determined in the tobacco using a liquid chromatography method previously used by our team.[13]

Total aerobic microbial and total yeast and mold counts (TAMC and TYMC) will be determined according to USP 61,[157] after the ITPs are prepared, midway through participant processing, and at the completion of participant processing.

III.D1. Aim 1 Detailed Study Procedures

Selection of WP Tobacco. As the starting material for the preparation of the four test tobaccos, we will use a popularly smoked unflavored tobacco (see Unflavored Tobacco in Table II.2) claimed by the manufacturer to "present a plain molasses flavor." This tobacco has a low glycerol content that is amenable to the proposed product manipulation.[16]

Preparation of WP Tobacco. A popular, commercially available WP tobacco brand (Moassel Saloum) will be prepared four different ways:

(1) Medium glycerol (60 mg/g), Medium Sugars (350 mg/g)
(2) High glycerol (120 mg/g), Medium Sugars (350 mg/g)
(3) Medium glycerol (60 mg/g), High sugars (510 mg/g)
(4) High glycerol (120 mg/g), High sugars (510 mg/g)

Several packages of the tobacco, purchased from the same source, will be combined, twigs and sticks removed, homogenized, and split into four equal batches. Food grade glycerol, glucose and fructose (>99% purity) will be added to the tobacco to obtain the concentrations shown for the ITPs in Table II.2.

Determination of Nicotine Content. To aid in determining if humectant levels affect the transfer of nicotine from the tobacco into the mainstream smoke, the nicotine content of Batches 1-4 will be individually determined using a method previously qualified by our team.[118] An aliquot (0.25 g) of each tobacco sample will be extracted individually for four hours in a mixture of isopropyl alcohol, methyl tert-butyl ether, and sodium hydroxide using an orbital shaker table at 160 rpm. The extraction solvent mixture will contain a known quantity of quinoline as the internal standard (IS). An aliquot of the extract will be quantitated for total nicotine using gas chromatography with mass spectral detection (GC-MSD).

Determination of Humectant Content. To aid in determining the fraction of humectants transferred to the mainstream smoke and verify the levels of added glycerol, the glycerol and propylene glycol content of each tobacco preparation will be determined using a Health Canada method adopted for use on WP tobacco by Schubert et al.[22]

Determination of Sugars in the Four Tobacco Preparations. The sugar content of the four prepared batches will be quantified. An aliquot (1 g, 3 replicates) of each tobacco preparation will be extracted into HPLC grade water and analyzed using validated methods based on standard liquid chromatography (LC) techniques.[155,156]

Determination of Carbonyl Content. To determine the fraction of carbonyls in the tobacco vs. those generated as a result of additives, carbonyls will be determined in the tobacco as their 2,4-dinitrophenylhydrazine (DNPH) derivatives using liquid chromatography tandem mass spectrometry.[22]

Determination of Semivolatile Furan Content. To determine the fraction of semivolatile furans in the tobacco vs. those generated as a result of additives, semivolatile furans will be determined in the tobacco using a liquid chromatography method previously used by our team.[13]

Determination of Aerobic Bacteria, Yeast and Mold. Because sugars and glycerol can function as preservatives in WP tobacco,[17] it is important to document the storage stability of the ITPs. Therefore, the total aerobic microbial and total yeast and mold counts (TAMC and TYMC) will be determined according to USP 61,[157] after the ITPs are prepared, midway through participant processing, and at the completion of participant processing.

AIM 2

III.A2. Aim 2 Research Design

Aim 2: **Characterize HPHC and sugars yield in mainstream smoke generated from the four WP tobaccos characterized under Aim 1 using machine smoking of the RWP2, and a standardized WP puffing regimen.** Aim 2 will focus on quantifying the yields of toxicants in the mainstream smoke from the four WP tobaccos.

III.B2. Aim 2 Sample

There are no human participants planned for Aim 2. Aliquots of the four tobacco preparations will be machine smoked and subsequent emissions will be collected/quantified for the toxicants of interest.

III.C2. Aim 2 Measurement/Instrumentation

Nicotine, sugars, glycerol, carbonyls, and semivolatile furans, as shown in Table III.3, will be quantified in the mainstream WP tobacco smoke for the four tobaccos. Additional sets of machine smoking will be individually conducted for each tobacco batch, the gases will be collected as their derivatives in impingers and bulk particles will be collected on glass fiber filters located at the mouth-end of the WP hose, and the resulting impinger solutions and filters will be extracted and analyzed according to the methods described in Aim 1.

III.D2. Aim 2 Detailed Study Procedures

Generation of WP Mainstream Smoke. The four tobacco preparations will be machine-smoked using a research-grade waterpipe, a calibrated smoking machine (Shisha Smoker, Borgwaldt), and an accepted standardized puffing regimen based on subjects smoking a popular flavored tobacco using the RWP in a laboratory environment.[13,95,96] Particles and organic chemicals will be characterized and collected at the mouth-end of the WP hose.

Determination of HPHCs in Mainstream WP Tobacco Smoke. Total particulate matter and the HPHC content of the volatile and semivolatile fractions of the mainstream WP tobacco smoke will be characterized. Selected HPHCs and other toxicants are shown, along with reported WP smoke yields and health effects, in Table II.3. Machine smoking will be conducted and total particulate matter will be collected at the mouth end of the WP hose. Particle samples will be chemically extracted and analyzed for the target semi-volatile HPHCs using GC/MS and LC-MS/MS. Gas phase HPHCs will be collected in impingers and quantified as their DNPH derivatives.

AIM 3

III.A3. Aim 3 Research Design

Aim 3: Determine how WP tobacco content impacts puffing behaviors, CO biomarker, pulmonary function, nicotine uptake, and perceived sensory attributes and appeal of WP smoking. Aim 3 will focus on using a group of established adult and young adult WP smokers, a cross-over study design, CO and nicotine biomarkers, spirometry, cutting edge psychophysical measurement tools, and risk perception instruments to map the relationship between sensory experiences and preferences of sweetness and flavor to specific additive content in WP tobacco that affect these experiences, preferences, acute health effects, and toxicant exposures.

Table III.3. Selected compounds for chemical characterization.

Compound	Mainstream Smoke Yield[12]		Reason for Selection
	Waterpipe	Cigarette	
Nicotine, TPM			
Nicotine (total), mg	0.01 – 9.29	0.1 – 3	Major addictive constituent
Total particulate matter, mg	242 – 2,350	1 – 27	Cardiovascular, pulmonary toxicant
Humectants, mg			
Glycerol	423	1.4 – 2.1[158]	Pulmonary toxicants, potential for carcinogenic degradation products
Propylene glycol	211	0.3 – 0.6[159]	
Semivolatile Furans, µg[160]			
5-(Hydroxymethyl)-2-furaldehyde (HMF)	2,420 – 62,300	1.3 – 7.4	Potential for genotoxic and mutagenic metabolites in the lung
Furfuryl alcohol (FFA)	55.7 – 552	18 – 65	Nasal, kidney carcinogen; potential for genotoxic and mutagenic metabolites in the lung
2-Furoic acid (2-FA)	32.0 – 401	na	Pulmonary toxicant
Furfural (2F)	29.6 – 206	0.71 – 27.5	Potential human carcinogen; data gap
2-Furyl methyl ketone (2-FMK)	4.77 – 12.5	na	Pulmonary toxicant; data gap
5-Methyl-2-furaldehyde (5-M2F)	4.62 – 215	na	Pulmonary toxicant; data gap
Carbonyl Compounds, µg			
Acetaldehyde	120 – 2,520	0.01	Nasal mucosa, laryngeal carcinomas; enhances nicotine's addictiveness
Acetone	20.2 – 118	506 – 773[161]	Pulmonary toxicant
Acrolein	10.1 – 892	60 – 240	Lung carcinomas; pulmonary toxicant
Benzaldehyde	<0.34	0.4 – 6.4[162]	Pulmonary toxicant
Butyraldehyde	10.9 – 70.6	5.5 – 51.2[163]	Respiratory and cardiovascular toxicant
Formaldehyde	36 – 360	0.02	Nasal sinus, nasopharyngeal carcinoma, leukemia
Propionaldehyde	5.71 – 403	48.4	Pulmonary and cardiovascular toxicant

na = data gap, data not available; compounds in blue shaded rows are classified as HPHCs; compounds in orange shaded rows are proposed as additions to the HPHC list.[11]

III.B3. Aim 3 Sample

We will recruit a total of 60 healthy adult men and women with the goal of having complete data sets for 50 participants. Participants will meet the eligibility criteria shown in Table III.4. Established waterpipe smokers will be recruited from advertisements

Table III.4. Participant inclusion and exclusion criteria.

Inclusion	**Justification for Inclusion**
Sufficient understanding of consent form and study procedures	Necessary to ensure that participants are adequately informed about the study
Age 21-50 years old	Legally of age to purchase, use, and possess tobacco and provide informed consent
Experienced WP smokers defined as having smoked a WP at least 3 times per month in the last month	To assure that participants are experienced WP smokers, and increase the probability of subjects being able to comfortably smoke
Willing/able to abstain from nicotine product use for at least 12 hours prior to the laboratory visits	Necessary to normalize participants' willingness to smoke WP across visits
Willing to attend four laboratory sessions at the same time of day lasting approximately 3 h each	Necessary to complete all testing sessions and provide adequate power for data analysis
Read and speak English.	To ensure that participants are adequately informed about the study and can follow the
Exclusion	**Justification for Exclusion**
Evident intoxication on any visit	Intoxication would interfere with informed consent and study procedures
Exhaled breath CO > 10 ppm	Indicates recent combustible tobacco use.
Pregnancy (known or suspected), trying to become pregnant, breastfeeding, unwillingness to use contraception for the duration of the study.	Unethical to enroll pregnant or breastfeeding women for smoking research
Significant smoking-related disease (by history)	Unethical to enroll subjects with tobacco-related diseases
Any of the following in the past 30 days (self-report): • Uncontrolled asthma or asthma that is worse than usual. • Severe respiratory allergies, such as wheezing, coughing, shortness of breath when exposed to known allergens. • Acute upper or lower respiratory illnesses like the flu, common cold, or pneumonia. • Any other serious lung infection or disease, such as tuberculosis, cystic fibrosis, or lung cancer. • Hospitalization for difficulty breathing	Unethical to enroll participants with significant pulmonary dysfunction or communicable disease
Currently engaging in a WP tobacco smoking quit attempt	Unethical to interfere with tobacco cessation

in college and community newspapers and through a variety of media outlets and the internet, including Study Search, as well as community events. Additionally, we will place flyers in WP cafés, and stores that cater to WP smokers, such as head shops and ethnic grocery stores. Participants from other studies who have agreed to be contacted regarding other study opportunities will also be contacted. Staff from those studies will prepare contact letters/emails and call participants on behalf of this study. Participants interested will be referred to this study for screening. Advertisements will direct interested potential participants to complete a secure, online screener. Participants will access the screening questionnaire using a public survey link generated by REDCap. Information regarding eligibility and the participants' email addresses and phone numbers will be uploaded into a secure database. Potential participants will then be further screened by phone. Further assessment will be completed in person in a private setting. We will facilitate visits by offering weekend appointments and using additional retention strategies (e.g., reminder calls/texts/emails) that have been successfully used in the past for WP studies of similar size, crossover design, and subject burden.

III.C3. Aim 3 Measurement/Instrumentation

Exhaled CO levels will be used to determine eligibility and CO boost (post- minus pre-smoking levels) from WP smoking using a handheld electrochemical sensor. The primary quantitative outcomes will be exhaled breath CO and plasma nicotine boost[172] (post- subtracted from pre-smoking levels), puffing topography, spirometry measures [e.g., forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), FEV1/FVC, peak expiratory flow (PEF) and forced expiratory flow at 25-75% of FVC; and height and weight will also be assessed to calculate expected spirometry measures for participants.[169] In addition, data will be collected electronically on participants' demographic characteristics, tobacco use history, nicotine dependence (Hooked on Nicotine Checklist,[164] and Lebanese WP Dependence Scale[165]), general harm and specific health risk perceptions, and subjective effects, including Direct Effects of Tobacco scale,[166] Direct Effects of Nicotine scale,[167] and Questionnaire for Urges to Smoke.[168]

The primary qualitative outcomes will be verbal and non-verbal communication regarding the WP smoking experience and flavors perception. To determine the relative intensity of specific flavors, e.g., sweetness, we will use the general version of the Labeled Magnitude Scale (gLMS),[102-104] to obtain data that would permit ratio comparisons of perceived sensation magnitudes. To assess the degree of liking or disliking of flavors, we will use the Labeled Hedonic Scale (LHS) to collect ratio-level data on the magnitude of liking and disliking of sensation.[134] Other themes that emerge from the discussions will also be coded.

IV. Detailed Study Procedures for the Human Study (Aim 3)

Study subjects will participate in a series of 4 laboratory sessions, each visit separated by at least a week, in which they smoke one of 4 tobacco preparations (blinded) using the RWP2, shown in Figure II.1, in a randomly assigned sequence, using the block randomization method.[174] Smoking sessions will be carefully standardized: they will take place in a controlled setting, individual roomsdesignated for smoking research with sufficient ventilation rate at the Center for Tobacco

Research) within one hour of the same time-of-day after overnight nicotine abstinence (≥8 hours, exhaled CO ≤ 10 ppm[173]).

Research staff will light and add the charcoal and record tobacco and charcoal weights. The research-grade waterpipes will be prepared by the laboratory staff, and participants will smoke ad lib to satiation. Participants will be limited to one bowl (15 g) of tobacco and one quick-light charcoal (~10 g).

Prior to the start of the first laboratory visit, a research assistant will explain the study to the participant and answer any questions they may have. The participants will give an exhaled breath sample into the handheld CO monitor to determine if they are eligible to participate in the laboratory smoking session. If the participant's exhaled CO concentration is less than or equal to 10 ppm) they are deemed eligible to participate further, and will be asked to read the informed consent form. Once all participant questions have been answered, the participants will each be asked to sign their consent to participate in the study. Each female participant of child-bearing age will be asked to provide a urine sample for use in conjunction with a "dip-stick" pregnancy test to determine participation eligibility. Female participants with a negative urine pregnancy will be eligible to participate if they provide written agreement (via signing the consent form) to use reliable contraception throughout their participation in the study.

Each participant will then be asked to provide a pre-smoking blood sample by venipuncture. If the phlebotomist is unable to take a sufficient blood sample (10 mL) from the participant, the participant will be deemed ineligible to participate further in the study. Participants will then answer demographic and tobacco use questions.

After a successful blood sample collection, all participants will provide responses electronically to a series of questionnaires gathering information on nicotine dependence, and other subjective ratings. Participants will complete a practice session to learn how to record flavor perceptions. Height and weight will be measured for each participant.

Smoking will take place in a negative pressure room with a stable ventilation rate sufficient to keep ambient CO levels < 30 ppmv. Participants will be seated in comfortable chairs designed for ease of blood collection by venipuncture. Using warm bottled water (body temperature), participants will clean their mouth and spit into a cup three times. Then, for 20 seconds, they will smell the unburned tobacco that they are assigned to smoke during that visit, and complete the questionnaire on their flavor perceptions. Participant lung function data will be collect using a handheld spirometry device. Exhaled breath CO levels will be collected for each participant just prior to the smoking session.

Participants will be encouraged to go to the bathroom prior to the start of the smoking session. The participant will be provided with a research-grade waterpipe that has been prepared with 15 g of one of the four tobacco preparations and one lit, quick-light charcoal. The participants will be instructed to smoke as they normally would or at a rate that is natural for them, through the hose/mouthpiece for up to 60 minutes. Puff topography parameters, including puff volume, duration, average and peak flow rate, and interpuff interval, will be measured continuously during the smoking session using the research-grade waterpipe.[96] After the first 10 minutes of

smoking, and at the end of each smoking session, participants will be asked to rate the perceived intensities of relevant sensory attributes, including "sweetness," "harshness," "bitterness," "smoothness," and overall intensity, using the general version of the Labeled Magnitude Scale (gLMS). We will also collect data on liking/disliking by using the Labeled Hedonic Scale (LHS). If participants choose to smoke for 45 minutes, they will be asked to provide exhaled breath CO levels. If any participants breath CO levels exceed 50 ppmv, the smoking session will be stopped. Otherwise, participants may smoke *ad libitum* for as long as they choose, up to 60 total minutes. At the end of the smoking session, a second exhaled breath sample will be collected, and participants will then provide answers to questions regarding flavor perception. They will give a second blood sample and perform another lung function test, as before. Afterwards they will answer questions regarding nicotine dependence and their waterpipe smoking experience.

At the end of each smoking session, participants will respond to all subjective questionnaires including the gLMS and LHS. For each test tobacco, participants will be asked to rate perceived intensities of all relevant flavor qualities (i.e., sweetness, harshness, bitterness, smoothness, overall intensity, and specific flavor) as well as their liking/disliking on gLMS and LHS.

Sensory Intensity and Hedonic Scales. To determine the relative intensity including sweetness, harshness, and bitterness, and specific attributes of added flavors (e.g., apple) as well as the degree of liking/disliking for the four tobacco preparations, a psychophysical measurement technique will be employed to quantify the relationship between the tobacco flavors and their perceived intensity. Specifically, we will use the general version of the Labeled Magnitude Scale (gLMS),[102-104] to obtain data that would permit ratio comparisons of perceived sensation magnitudes. The gLMS is a category-ratio scale bounded by "no sensation" at the bottom and "strongest imaginable sensation of any kind" at the top, with its intermediate intensity labels (weak, moderate, strong, and very strong) spaced logarithmically according to the empirically determined semantic magnitude.[102,103] To assess the degree of liking or disliking of flavors, we will use the Labeled Hedonic Scale (LHS) to collect ratio-level data on the magnitude of liking and disliking of sensation.[134] The properties of LHS are similar to the properties of the gLMS where the bipolar gLMS has ''neutral'' at its midpoint with negative descriptors to the left bounded by "strongest imaginable displeasure of any kind,'' and positive descriptors to the right bounded by ''strongest imaginable pleasure of any kind."

Biological Sample Collection. Exhaled breath CO (via handheld electrochemical cell) and whole blood will be collected before and immediately after the WP smoking sessions according to previously developed protocols. Exhaled CO will also be measured after 45 minutes of *ad libitum* smoking, should participants choose to smoke that long. If after smoking for 45 minutes, any participant's exhaled breath is greater than 50 ppm, the smoking session will be stopped. Within one hour of collection, the blood will be separated using centrifugation (1200 xg for 15 minutes), and the plasma layer will be transferred into a cryotube and stored at -80 °C until analysis for nicotine according to validated methods using LC-MS/MS.[172]

Spirometry. Before and after smoking at each clinic visit, subjects will be asked to take a deep breath and then instructed to exhale forcefully into a handheld spirometer [NDD EasyOne Air Spirometer (NDD Medical Technologies, Andover, MA),[169] or similar spirometer] according to American Thoracic Society Guidelines. This maneuver will be repeated up to 5 times in order to

ensure reproducible results. This non-invasive procedure will take up to 15 minutes to perform. Participants' height and weight will also be measured to calculate their expected spirometry measures as thresholds to use for determining potential impairment in lung function.

Safe Departure and Remuneration. At the end of each clinic session, research assistants will assess each of the participants for safe departure using a standardized checklist. An example checklist is provided in Appendix C. Visits 2-4 will be similar to the first visit, but the tobacco and use history data collection and the urine pregnancy test (women only) will not be repeated. Participants will receive $50 for each laboratory visit and a bonus payment of $50 for completing all four visits, for a total of $250 at the conclusion of the last laboratory visit.

V. Risks, Benefits, Safety Plans

V.1. Risks associated with the human study

Potential risks are minimal for the human participant study. Potential risks associated with the procedures and equipment that will be used to collect the exhaled breath CO, topography, and pulmonary data are minimal. Potential risks resulting from collecting blood samples include being unable to provide blood samples because forearm veins are not adequate, feeling light-headed or faint, nausea, dizziness, hematoma or bruising at or near the needle site. We minimize this risk by using certified technicians with extensive experience in collecting blood samples. However, if the participant does not feel comfortable for any reason and wants to end their participation in the study, he/she can do so at any time without penalty. Some of the survey questions may upset individuals; however, we have used these questions and procedures in many previous studies without upsetting participants.

There are no potential risks involved in the collection of waterpipe smoking topography, as the device does not change the resistance to draw or toxicity of the smoke inhaled. The data are acquired continuously to an electronic file, and may be displayed on a laptop screen that is not viewable by either participant. Therefore the possibility of the participant amplifying their puffing rate in order to see the on-screen response is eliminated.

Participants may feel hungry because they will not be permitted to eat until after their post-smoking lung function test is completed. Pulmonary function studies involve forceful respiratory maneuvers and occasionally people develop light-headedness during the procedures or soreness of the chest for a few days. Subjects will be seated and closely monitored by trained/certified personnel during those procedures.

The RWP that participants will smoke at the clinic is specially designed for investigational research only, and as such may have unknown health risks. Both the single- and dual-hose versions of the RWP have been used by participants in several previous studies.

A potential risk is loss of confidentiality. We have rigid procedures in place to protect against loss of confidentiality. Participant identifiers will be kept in a locked file cabinet. This study is not unlike our previous work investigating the efficacy of WP warning labels; we collect the

information and keep it confidential and anonymous through the use of IDs only and not personally identifying information.

De-identified, coded participant data from the general version of the Labeled Magnitude Scale (gLMS) and the Labeled Hedonic Scale (LHS) will be analyzed by Dr. Juyun Lim, Associate Professor, Department of Food Science and Technology, Oregon State University. Dr. Lim will receive all scale ratings, matched with participant identification codes, date of participant laboratory visit, period data was collected (e.g., pre-smoking, post-smoking, etc.) and select demographic and tobacco use history data such as sex, age, and how many cigarettes smoked per day. All data sent to Dr. Lim will be stripped of participant identifying information.

During an ongoing virus pandemic (such as COVID-19), we will follow strict safety procedures to reduce risk of spread of the disease among the participants and staff. Depending on currently recommended guidelines, these procedures may entail:

- Collecting data on forehead temperature and assessing symptoms of viral infection at each visit
- Wearing personal protective equipment and sanitizing hands
- Routine cleaning of surfaces and equipment
- Implementing physical distancing

V.2. Benefits associated with the study

There are no direct benefits to be gained by participants. However, the anticipated societal benefit resulting from this study is considerable, given the increasing prevalence of WP tobacco smoking. Societal benefits include increased public health resulting from this study providing evidence that directly informs and strengthens FDAs tobacco control efforts. Few studies have been conducted on WP tobacco additives and at the same time, there is mounting evidence that young adults, a vulnerable population from a tobacco control perspective, are largely misinformed of the risks of this form of tobacco use.

VI. Data Storage

VI.1. Paper materials

Informed consent forms, receipts, and paper surveys will be stored in locked file cabinets only accessible by study staff. Paper surveys may be shredded after they have been entered in an electronic database.

VI.2. Electronic data and subject identifying data

REDCap will be used for entry of paper surveys and collection of survey data. 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. Only study research assistants and the PIs 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.

VII. Data Analysis

VII.1 Analysis Plan

The main research questions from the four Aims in the proposed study have the same structure for data analysis. That is, there are differences attributable to the four tobaccos in the means of the outcome measures of interest. All relevant outcome variables will be first examined with exploratory/descriptive statistical analyses that focus on describing and understanding patterns and distributions for all relevant measures (e.g., tobacco chemical content, mainstream smoke constituents, spirometry measures, topography measures, psychophysical ratings) by the four tobaccos. We will identify any potential outliers, assess distributional assumptions, and apply appropriate transformations to the outcome variables (e.g., log-transformation) if the normality assumption is violated.

For each of the four aims, the following pairwise comparisons will be performed:

$$H0i\ j: \mu i = \mu j \text{ vs. } Hai\ j : \mu i \neq \mu j$$

where μi = mean target outcome for tobacco type i, and μj = mean target outcome for tobacco type j. In Aims 1, 2, and 4, the data will be analyzed using one-way ANOVA models for independent outcome data and Tukey's method will be used to control type-I error rate across the six multiple comparisons. A different analysis strategy will be used in Aim 3 due to the clustered study design: participants will complete four different smoking sessions. Thus, in order to account for the hierarchical design, the data in Aim 3 will be analyzed using linear mixed models containing random subject effects. Since linear mixed models allow for incomplete data (e.g.,

due to dropout), data from all completed smoking sessions will be used in the analysis. As in the other aims, Tukey's method will be used to adjust for multiple comparisons of the tobacco treatments in Aim 3. In addition, for Aim 3, we will use linear mixed models to examine the relationships between the perceived sensory intensity ratings and the liking/disliking ratings for a given tobacco preparation.

Integrating results from Aims 1, 2 and 4 with Aim 3 of the study, the absence or presence, or exposure level of certain HPHCs and other chemical compounds across the different tobacco preparations will be mapped with the data on perceived sensory intensity such as sweetness and liking/disliking ratings.

VII.2. Sample Size Justification.

Since Aims 1, 2, and 4 do not involve human subjects, we expect low variability across replicates. Thus, assuming a somewhat conservative coefficient of variation (CV) of 20% and log-normal outcomes, a total of **4 replicates per tobacco preparation** will provide 80% power to detect a two-fold change in the geometric means of two groups at a conservative two-sided Bonferroni-corrected type-I error rate of 0.0083.

Our sample size for Aim 3 was selected to ensure that we have adequate power to detect moderate effect sizes in our comparisons of topography outcomes. We made the following assumptions for the power analysis:

- Based on the intravariability data analysis for the RWP,[94] correlation coefficients within subjects (r) were assumed to be 0.6, 0.7 or 0.8.
- Small, medium, and large effect sizes (d) of 0.2, 0.5, and 0.8 standard deviations.
- A two-sided, Bonferroni corrected type-I error rate of 0.0083.

Our power analysis, summarized in Table VII-5, revealed that a sample size of 43 subjects will provide over 80% power to detect a moderate effect size unless the within pair correlation is high (0.2 or 0.3) and the within participant correlation is modest (0.6). However, since this power analysis assumes complete follow-up, we will recruit **60 participants** to account for a possible attrition rate of 20%., and ensure the Latin square ordiering is balanced across the four tobacco types.

Table VII.5. Estimated % power to for pairwise comparisons of tobacco preparations based N = 25 pairs (50 subjects).

		Power		
r	**ρ**	**d=0.2**	**d=0.5**	**d=0.8**
0.6	0.1	0.108	0.803	0.998
	0.2	0.097	0.759	0.996
	0.3	0.088	0.717	0.994
0.7	0.1	0.153	0.918	> 0.999
	0.2	0.138	0.889	> 0.999
	0.3	0.125	0.857	0.999
0.8	0.1	0.255	0.989	>0.999
	0.2	0.228	0.981	>0.999
	0.3	0.207	0.971	>0.999

VIII. Gender/Minority/Pediatric Inclusion for Research

VIII.1 Inclusion of Women and Minorities

Inclusion of Women and Minorities: According to U.S. Census Data, 51.3% of Columbus, OH residents are female. In our previous studies with smokers, 55-62% of participants were female. According to U.S. Census data, the racial composition of individuals living in Columbus is 61% White, 28% Black or African American, 5% Asian, 0.2% American Indian/Alaska Native, 0% Native Hawaiian/Other Pacific Islander, and 4% two or more races. The ethnic composition of individuals living in Columbus is 6% Hispanic/Latino. We expect that our distributions will be similar to these but we may potentially have a larger distribution of ethnic and racial minorities, based on our previous studies. However, we will continuously monitor enrollment in order to ensure that we are meeting recruitment goals to avoid under-recruiting minorities. If the targeted enrollment for minorities is not met because they do not respond to the advertisements, we will make special efforts to solicit their participation by advertising in community newspapers, local church organizations, and community centers.

VIII.2 Inclusion of Children

This study will be restricted to individuals 21 years of age and older, which is the legal age to purchase tobacco products.

IX. Data and Safety Monitoring Plan

The Data and Safety Monitoring Plan can be found in Appendix D to this Research Protocol.

All data and safety monitoring will adhere to the policies and procedures of the OSU Comprehensive Cancer Center's Data and Safety Monitoring Plan which has been approved by the National Cancer Institute

X. References

[1] Maziak W, Ward KD, Soweid RA, Eissenberg T. Tobacco smoking using a waterpipe: a re-emerging strain in a global epidemic. Tob Control. 2004;13:327-333. doi:10.1136/tc.2004.008169.

[2] Jamal A, Gentzke A, Hu SS, Cullen KA, Apelberg BJ, Homa DM, King BA. Tobacco use among middle and high school students—United States, 2011–2016. MMWR. Morbidity and mortality weekly report. 2017 Jun 16;66(23):597.

[3] Gathuru, I. M., Tarter, R. E., & Klein-Fedyshin, M. (2015). Review of hookah tobacco smoking among college students: policy implications and research recommendations. The American journal of drug and alcohol abuse, 41(4), 272-280.

[4] Kassem NO, Jackson SR, Kassem NO, Liles S, Posis AI, Hovell MF. College student beliefs and behavior regarding sharing when smoking hookahs. American journal of health behavior. 2019 Jan 1;43(1):133-44.

[5] Heinz AJ, Giedgowd GE, Crane NA, Veilleux JC, Conrad M, Braun AR, et al. A comprehensive examination of hookah smoking in college students: use patterns and contexts, social norms and attitudes, harm perception, psychological correlates and co-occurring substance use. Addict Behav. 2013 Nov;38(11):2751-60.

[6] Villanti, A. C., Cobb, C. O., Cohn, A. M., Williams, V. F., & Rath, J. M. (2015). Correlates of Hookah Use and Predictors of Hookah Trial in US Young Adults. American journal of preventive medicine, 48(6), 742-746.

[7] Akl EA, Jawad M, Lam WY, Co CN, Obeid R, Irani J. Motives, beliefs and attitudes towards waterpipe tobacco smoking: a systematic review. Harm Reduction. 2013;10:12. doi:10.1186/1477-7517-10-12

[8] Minaker, L. M., Shuh, A., Burkhalter, R. J., & Manske, S. R. (2015). Hookah use prevalence, predictors, and perceptions among Canadian youth: findings from the 2012/2013 Youth Smoking Survey. Cancer Causes & Control, 26(6), 831-838.

[9] Aboaziza E, Eissenberg T. Waterpipe tobacco smoking: what is the evidence that it supports nicotine/tobacco dependence? Tob Control. 2014. doi:10.1136/tobaccocontrol-2014-051910

[10] US Food and Drug Administration. Harmful and potentially harmful constituents in tobacco products and tobacco smoke; established list. Fed Regist. 2012;77:20034-37.

[11] DOCKET NO. FDA-2012-N-0-0143, “Harmful and Potentially Harmful Constituents in Tobacco Products; Established List; Proposed Additions; Request for Comments. Fed Reg Vol 84, No 150, August 5, 2019, 38032-35.

[12] Shihadeh A, Schubert J, Klaiany J, Sabban ME, Luch A, Saliba NA. Toxicant content, physical properties and biological activity of waterpipe tobacco smoke and its tobacco-free alternatives. Tob Control. 2015. doi:10.1136/tobaccocontrol-2014-051907

[13] Brinkman MC, Teferra AA, Kassem NO, Kassem NO. Effect of electric heating and ice added to the bowl on mainstream waterpipe semivolatile furan and other toxicant yields. Tobacco control. 2019 Sep 21.

[14] Watkins SL, Glantz SA, Chaffee BW. Association of noncigarette tobacco product use with future cigarette smoking among youth in the population assessment of tobacco and Health (path) study, 2013-2015. JAMA Pediatr 2018;172:181–7.

[15] van Nierop LE, Pennings JL, Schenk E, Kienhuis AS, Talhout R. Analysis of Manufacturer's Information on Tobacco Product Additive Use. Tobacco Regulatory Science. 2019 Mar 1;5(2):182-205.

[16] Adetona AM, Brinkman MC, Strozier ED, Karam HS, Butler JM, Kim H, Lim J, McCauley M, Clark PI. Unflavored vs. flavored waterpipe tobacco additives and potential harms. Under review.

[17] Brinkman, M.C., Adetona, A.M., Buehler, S.S., Strozier, H.K. (2018). *The Effect of Humectant and Flavorant Content on Harmful and Potentially Harmful Constituent Emissions in Mainstream Waterpipe Tobacco Smoke*. Presented as an Oral Presentation at the Society for Research on Nicotine and Tobacco, February 23, 2018.

[18] Elson LA, Betts TE, Passey RD. The sugar content and the pH of the smoke of cigarette, cigar and pipe tobaccos in relation to lung cancer. *Int J Cancer* 1972 May 15;9(3):666-75.

[19] Liu C. Glycerol transfer in cigarette mainstream smoke. Beiträge zur Tabakforschung International/Contributions to Tobacco Research. 2004 Jul 1;21(2):111-6.

[20] van Nierop LE, Pennings JL, Schenk E, Kienhuis AS, Talhout R. Analysis of Manufacturer's Information on Tobacco Product Additive Use. Tobacco Regulatory Science. 2019 Mar 1;5(2):182-205.

[21] Adetona AM, Brinkman MC, Strozier ED, Karam HS, Butler JM, Kim H, Lim J, McCauley M, Clark PI. Unflavored vs. flavored waterpipe tobacco additives and potential harms. Under review.

[22] Schubert J, Heinke V, Bewersdorff J, Luch A, Schulz TG. Waterpipe smoking: the role of humectants in the release of toxic carbonyls. Archives of toxicology. 2012 Aug 1;86(8):1309-16.

[23] Glycerol (21 CFR 182.1320); High fructose corn syrup (21 CFR 184.1866).

[24] Al Rashidi M, Shihadeh A, Saliba NA. Volatile aldehydes in the mainstream smoke of the narghile waterpipe. Food and Chemical Toxicology. 2008 Nov 1;46(11):3546-9.

[25] Brinkman MC, Teferra AA, Kassem NO, Kassem NO. Effect of electric heating and ice added to the bowl on mainstream waterpipe semivolatile furan and other toxicant yields. Tobacco control. 2019 Sep 21.

[26] Schubert J, Bewersdorff J, Luch A, et al. Waterpipe smoke: a considerable source of human exposure against furanic compounds. Anal Chim Acta 2012;709:105–12.

[27] Wagener TL, Leavens ELS, Mehta T, Hale JJ, Shihadeh A, Eissenberg T, Halquist MS, Brinkman MC, Johnson AL, Floyd EL, Ding K. Impact of Flavors and Sweeteners on Waterpipe Tobacco Smoking Topography, Abuse Liability, Toxicant Exposure, and Intentions for Continued Use. Under review, Tobacco Control.

[28] Leavens EL, Driskill LM, Molina N, Eissenberg T, Shihadeh A, Brett EI, Floyd E, Wagener TL. Comparison of a preferred versus non-preferred waterpipe tobacco flavour: subjective experience, smoking behaviour and toxicant exposure. Tobacco control. 2018 May 1;27(3):319-24.

[29] Maziak W, Taleb ZB, Bahelah R, et al. The global epidemiology of waterpipe smoking. Tob Control. 2014. doi:10.1136/tobaccocontrol-2014-051903.

[30] Arrazola RA, Singh T, Corey CG, et al; Centers for Disease Control and Prevention (CDC). Tobacco use among middle and high school students—United States, 2011-2014. MMWR Morb Mortal Wkly Rep 2015;64 (14):381-385.

[31] Villanti, A. C., Cobb, C. O., Cohn, A. M., Williams, V. F., & Rath, J. M. (2015). Correlates of Hookah Use and Predictors of Hookah Trial in US Young Adults. American journal of preventive medicine, 48(6), 742-746.

[32] Fielder, R. L., Carey, K. B., & Carey, M. P. (2012). Prevalence, frequency, and initiation of hookah tobacco smoking among first-year female college students: a one-year longitudinal study. Addictive behaviors, 37(2), 221-224.

[33] Shepardson, R. L., & Hustad, J. T. (2015). Hookah Tobacco Smoking during the Transition to College: Prevalence of Other Substance Use and Predictors of Initiation. Nicotine & Tobacco Research, ntv170.

[34] Martinasek, M. P., Gibson-Young, L. M., Davis, J. N., & McDermott, R. J. (2015). Waterpipe tobacco smoking impact on public health: implications for policy. Risk management and healthcare policy, 8, 121.

[35] Kassem NO, Jackson SR, Boman-Davis M, Kassem NO, Liles S, Daffa RM, Yasmin R, Madanat H, Hovell MF. Hookah smoking and facilitators/barriers to lounge use among students at a US university. American journal of health behavior. 2015 Nov 1;39(6):832-48.

[36] Kates FR, Salloum RG, Thrasher JF, Islam F, Fleischer NL, Maziak W. Geographic proximity of waterpipe smoking establishments to colleges in the US. American journal of preventive medicine. 2016 Jan 1;50(1):e9-14.

[37] Rastam, S., Ward, K. D., Eissenberg, T., & Maziak, W. (2004). Estimating the beginning of the waterpipe epidemic in Syria. BMC public Health, 4(1), 32.

[38] Meier, E. M., Tackett, A. P., Miller, M. B., Grant, D. M., & Wagener, T. L. (2015). Which nicotine products are gateways to regular use?: First-Tried tobacco and current use in college students. American journal of preventive medicine, 48(1), S86-S93.

[39] The Family Smoking Prevention and Tobacco Control Act, Pub.L. 111–31, H.R. 1256, June 22, 2009.

[40] Food and Drug Administration, HHS. Deeming tobacco products to be subject to the federal food, drug, and cosmetic Act, as amended by the family smoking prevention and tobacco control act; restrictions on the sale and distribution of tobacco products and required warning statements for tobacco products. final rule. Fed Regist 2016;81.

[41] Mays, D., Arrazola, R. A., Tworek, C., Rolle, I. V., Neff, L. J., & Portnoy, D. B. (2016). Openness to using non cigarette tobacco products among US young adults. American Journal of Preventive Medicine, 50(4), 528–534

[42] Johnston LD, Miech RA, O'Maley PM, Bachman JG, Schulenber JE, Patrick ME. Monitoring the future national survey results ondrug use, 1975-2018. Institute for Social Research, The University of Michigan, sponsored by National Institute on Drug Abuse, The National Institutes of Health. Jan. 2019.

[43] Watkins SL, Glantz SA, Chaffee BW. Association of noncigarette tobacco product use with future cigarette smoking among youth in the population assessment of tobacco and Health (path) study, 2013-2015. JAMA Pediatr 2018;172:181–7.

[44] Case KR, Creamer MR, Cooper MR, Loukas A, Perry CL. Hookah use as a predictor of other tobacco product use: A longitudinal analysis of Texas college students. Addictive behaviors. 2018 Dec 1;87:131-7.

[45] Salloum RG, Haider MR, Barnett TE, Guo Y, Getz KR, Thrasher JF, et al. Peer Reviewed: Waterpipe Tobacco Smoking and Susceptibility to Cigarette Smoking Among Young Adults in the United States, 2012–2013. Prev Chronic Dis. 2016;13.

[46] Jaber R, Madhivanan P, Veledar E, Khader Y, Mzayek F, Maziak W. Waterpipe a gateway to cigarette smoking initiation among adolescents in Irbid, Jordan: a longitudinal study. Int J Tuberc Lung D. 2015;19:481-7.

[47] Jensen PD, Cortes R, Engholm G, Kremers S, Gislum M. Waterpipe use predicts progression to regular cigarette smoking among Danish youth. Subst Use Misuse. 2010;45:1245-1261.Monitoring the Future, National Survey Results on Drug Use, 1975-2017. 2017: Vol. II.

[48] Asfar T, Weg MV, Maziak W, Hammal F, Eissenberg T, Ward KD. Outcomes and adherence in Syria's first smoking cessation trial. Am J Health Behav. 2008;32:146-56.

[49] Hammal F, Mock J, Ward KD, Eissenberg T, Maziak W. A pleasure among friends: how narghile (waterpipe) smoking differs from cigarette smoking in Syria. Tob Control. 2008;17:e3.

[50] Soneji S, Sargent J, Tanski S. Multiple tobacco product use among US adolescents and young adults. Tobacco control. 2016 Mar 1;25(2):174-80.

[51] Mamtani R, Cheema S, Sheikh J, Al Mulla A, Lowenfels A, Maisonneuve P. Cancer risk in waterpipe smokers: a meta-analysis. Int J Public Health. 2017 Jan 1;62(1):73-83.

[52] Waziry R, Jawad M, Ballout RA, et al. The effects of waterpipe tobacco smoking on health outcomes: an updated systematic review and meta-analysis. Int J Epidemiol 2017; 46(1): 32-43.

[53] El-Zaatari ZM, Chami HA, Zaatari GS. Health effects associated with waterpipe smoking. Tob Control 2015; 24 Suppl 1: i31-i43

[54] Rezk-Hanna M, Benowitz NL. Cardiovascular effects of hookah smoking: potential implications for cardiovascular risk. Nicotine Tob Res 2018; 1-11.

[55] Elson LA, Betts TE, Passey RD. The sugar content and the pH of the smoke of cigarette, cigar and pipe tobaccos in relation to lung cancer. *Int J Cancer* 1972 May 15;9(3):666-75.

[56] Liu C. Glycerol transfer in cigarette mainstream smoke. Beiträge zur Tabakforschung International/Contributions to Tobacco Research. 2004 Jul 1;21(2):111-6.

[57] Hoffmann, D., and Hoffmann, I. 1997. The changing cigarette 1950–1995. J. Toxicol. Environ. Health 50:307–364.

[58] Shelar GR, Bernasek PF, Furin OP. Sugar/Nicotine Study, Report # ATP 92-210, ATP-R&D, July 29, 1992. Bates 510697389-510697410.. Available: https://www.industrydocuments. ucsf. edu/ docs/ fxbl0037

[59] Baker RR. The generation of formaldehyde in cigarettes-overview and recent experiments. Food Chem. Toxicol 2006a;44:1799–1822.

[60] Nimlos MR, Blanksby SJ, Qian X, Himmel ME, Johnson DK (2006) Mechanisms of glycerol dehydration. J Phys Chem A 110:6145– 6156.

[61] Stein YS, Antal MJ (1983) A study of the gas-phase pyrolysis of glycerol. J Anal Appl Pyrolysis 4:283–296

[62] Moghe, A., Ghare, S., Lamoreau, B., Mohammad, M., Barve, S., McClain, C., and Joshi-Barve, S. (2015) Molecular mechanisms of acrolein toxicity: relevance to human disease. Toxicol. Sci. 143, 242−255.

[63] Lambert C, Li J, Jonscher K, Yang T, Reigan P, Quintana M, Harvey J, Freed BM. Acrolein inhibits cytokine gene expression by alkylating cysteine and arginine residues in the NF-κB1 DNA binding domain. J. Biol. Chem 2007;282:19666–19675.

[64] Bekki K, Uchiyama S, Ohta K, Inaba Y, Nakagome H, Kunugita N. Carbonyl compounds generated from electronic cigarettes. International journal of environmental research and public health. 2014 Nov;11(11):11192-200.

[65] Paine III JB, Pithawalla YB, Naworal JD, Thomas Jr CE. Carbohydrate pyrolysis mechanisms from isotopic labeling: Part 1: The pyrolysis of glycerin: Discovery of competing fragmentation mechanisms affording acetaldehyde and formaldehyde and the implications for carbohydrate pyrolysis. Journal of Analytical and Applied Pyrolysis. 2007 Oct 1;80(2):297-311.

[66] Hoffmann D, Djordjevic MV, Hoffmann I. The changing cigarette. Prev. Med 1997;26:427–434.

[67] Talhout, R., Opperhuizen, A., and van Amsterdam, J. G. (2007) Role of acetaldehyde in tobacco smoke addiction. Eur. Neuropsychopharmacol. 17, 627−636.

[68] Belluzzi, J. D., Wang, R., and Leslie, F. M. (2005) Acetaldehyde enhances acquisition of nicotine self-administration in adolescent rats. Neuropsychopharmacology 30, 705−712.

[69] Fujioka, K., and Shibamoto, T. (2006) Determination of toxic carbonyl compounds in cigarette smoke. Environ. Toxicol. 21, 47−54

[70] Yao, H., and Rahman, I. (2011) Current concepts on oxidative/carbonyl stress, inflammation and epigenetics in pathogenesis of chronic obstructive pulmonary disease. Toxicol. Appl. Pharmacol. 254, 72−85.

[71] Corma A, Iborra S, Velty A. Chemical routes for the transformation of biomass into chemicals. Chem Rev 2007 Jun 13;107(6):2411-502.

[72] Pynnonen B. Analysis of sucrose, raffinose, betaine, and invert in molasses and in the extract and raffinate obtained from ion exclusion. 26th General Meeting American Society of Sugar Beet Technologists, Monterey, CA, February 25, 1991.

[73] Toxicology and carcinogenesis studies of furfuryl alcohol (CAS No. 98-00-0) in F344/N rates and B6C3f1 mice (inhalation studies). February 1999. National Toxicology Program, NTP TR 482, Publ. No. 99-3972.

[74] IARC Monograph, Vol. 63, 1995.

[75] Abraham K, Gürtler R, Berg K, et al. Toxicology and risk assessment of 5-hydroxymethyl-furfural in food. Mol Nutr Food Res 2011;55:667–78.

[76] Glatt H, Schneider H, Murkovic M, et al. Hydroxymethyl-substituted furans: mutagenicity in Salmonella typhimurium strains engineered for expression of various human and rodent sulphotransferases. Mutagenesis 2012;27:41–8.

[77] Coughtrie MWH. Function and organization of the human cytosolic sulfotransferase (SULT) family. Chem Biol Interact 2016;259:2–7.

[78] IARC Monographs on the evaluation of carcinogenic risks to humans. Lyon, France IARC; 2014.

[79] Ley JP. Masking bitter taste by molecules. Chemosens Percept 2008;1:58–77.

[80] Villanti AC, Johnson AL, Ambrose BK, et al. Flavored tobacco product use in youth and adults: findings from the first wave of the path study (2013-2014). Am J Prev Med 2017;53:139–51.

[81] Chen L. pH of Smoke, a Review. 1976. Lorillard Research Center Greensboro, Bates Number USX0951046-USX0951063

[82] Mosser LO. Effects of varying smoke pH on Kool Ks/244, March 19, 1984. Brown & Williamson Tobacco Corporation Research, Development & Engineering File Note, Bates Number 682094559-682094563

[83] Bahelah R, DiFranza JR, Fouad FM, et al. Early symptoms of nicotine dependence among adolescent waterpipe smokers. Tob Control 2016;25:e127–e134.

[84] Doran N, Godfrey KM, Myers MG. Hookah use predicts cigarette smoking progression among college smokers. Nicotine Tob Res 2015;17:1347–53.

[85] Ben Taleb Z, Breland A, Bahelah R, Kalan ME, Vargas-Rivera M, Jaber R, Eissenberg T, Maziak W. Flavored versus nonflavored waterpipe tobacco: a comparison of toxicant exposure, puff topography, subjective experiences, and harm perceptions. Nicotine and Tobacco Research. 2019 Sep;21(9):1213-9.

[86] Khater EM, Amr M, Chaouachi K. Elemental characterization of shisha moassel smoking mixtures using ICP-MS and comparison with other tobacco products. Wulfenia J 2014;21:428-49.

[87] El Sohaimy SA, Masry SH, Shehata MG. Physicochemical characteristics of honey from different origins. Annals of Agricultural Sciences. 2015 Dec 1;60(2):279-87.

[88] Strayer SE, Everstine K, Kennedy S. Economically motivated adulteration of honey: Quality control vulnerabilities in the international honey market. Food Prot Trends 2014 Jan 1;34(1):8-14.

[89] Hookah (Shisha) Tobacco Market 2018 Global Industry Demand, Sales, Suppliers, Analysis & Forecasts to 2023, Press release, ABNewswire.com, Sept. 27, 2018.

[90] Chen L, Xue X, Ye Z, Zhou J, Chen F, Zhao J. Determination of Chinese honey adulterated with high fructose corn syrup by near infrared spectroscopy. Food Chemistry. 2011 Oct 15;128(4):1110-4.

[91] Biester A, Wood MW, Wahlin CS. Carbohydrate studies: I. the relative sweetness of pure sugars. American Journal of Physiology-Legacy Content. 1925 Jul 1;73(2):387-96.

[92] Moskowitz HR. Ratio scales of sugar sweetness. Atten Percept Psychophys. 1970;7(5):315-320.

[93] WHO STUDY GROUP ON TOBACCO PRODUCT REGULATION. Report on the Scientific Basis of Tobacco Product Regulation: World Health Organization; 2017. Report No.: 6.

[94] Kim H, Brinkman MC, Sharma E, Gordon SM, Clark PI. Variability in Puff Topography and Exhaled CO in Waterpipe Tobacc...: Ingenta Connect. Tobacco Regulatory Science. 2016;2(4):301.

[95] Brinkman MC, Kim H, Buehler SS, Adetona AM, Gordon SM, Clark PI. Evidence of compensation among waterpipe smokers using harm reduction components. Tobacco control. 2018 Oct 30:tobaccocontrol-2018.

[96] Brinkman MC, Kim H, Gordon SM, Kroeger RR, Reyes IL, Deojay DM, Chitwood C, Lane TE, Clark PI. Design and validation of a research-grade waterpipe equipped with puff topography analyzer. Nicotine & Tobacco Research. 2016 May 1;18(5):785-93.

[97] Shihadeh A, Salman R, Jaroudi E et al. Does switching to a tobacco-free waterpipe product reduce toxicant intake? A crossover study comparing CO, NO, PAH, volatile aldehydes,“tar” and nicotine yields. *Food Chem Toxicol*. 2012;50:1494-1498. doi:10.1016/j.fct.2012.02.04

[98] Shihadeh A, Eissenberg T, Rammah M, Salman R, Jaroudi E, El-Sabban M. Comparison of tobacco-containing and tobacco-free waterpipe products: effects on human alveolar cells. *Nicotine Tob Res.* 2014;16:496-499.

[99] Analysis of Chemical Constituents and HPHCs in Waterpipe Tobacco, Charcoal and Wastewater, FDA standalone contract CON00025809/HHSF223201600607A, 9/2016 – 4/2018.

[100] Bartoshuk LM. Comparing sensory experiences across individuals: Recent psychophysical advances illuminate genetic variation in taste perception. Chem. Senses 25(4):447-460 (2000).

[101] Krut LH, Perrin MJ, Bronte-Stewart B. Taste perception in smokers and non-smokers. British Med. J. 1(5223):384 (1961).

[102] Green BG, Shaffer GS, Gilmore MM. Derivation and evaluation of a semantic scale of oral sensation magnitude with apparent ratio properties. Chem. Senses, 18:683–702 (1993).

[103] Green BG, Dalton P, Cowart B, Shaffer G, Rankin K, Higgins J. Evaluating the 'Labeled Magnitude Scale' for measuring sensations of taste and smell. Chem. Senses. 21(3):323-34 (1996).

[104] Bartoshuk LM, Duffy VB, Green BG, Hoffman HJ, Ko CW, Lucchina LA, Marks LE, Snyder DJ, Weiffenbach JM. Valid across-group comparisons with labeled scales: The gLMS versus magnitude matching. Physiol. Behav. 82(1):109-14 (2004).

[105] United States Department of Health and Human Services. National Institutes of Health. National Institute on Drug Abuse, and United States Department of Health and Human Services. Food and Drug Administration. Center for Tobacco Products. Population Assessment of Tobacco and Health (PATH) Study [United States] Restricted-Use Files. Inter-university Consortium for Political and Social Research [distributor], 2019-11-21.

[106] WHO TobReg: report on the scientific basis of tobacco product regulation: 6th report of a WHO study group (2018): Brinkman M and Shihadeh A. Section 5: Waterpipe Toxicant Content and Emissions; Brinkman M, Klerx W, Shihadeh A, Talhout R and Zaatari G. Section 6: Applicability and adaptability of the WHO Tobacco Laboratory Network standard operating procedures for cigarettes to waterpipe tobacco.

[107] Kumar PS, Clark P, Brinkman MC, Saxena D. Novel nicotine delivery systems. *Advances in Dental Research*. 2019 Oct;30(1):11-5.

[108] Pullicin AJ, Kim H, Brinkman MC, Buehler SS, Clark PI, Lim J. Impacts of Nicotine and Flavoring on the Sensory Perception of E-Cigarette Aerosol. *Nicotine & Tobacco Research*. 2019 Apr 17.

[109] Mikheev VB, Buehler SS, Brinkman MC, Granville CA, Lane TE, Ivanov A, Cross KM, Clark PI. The application of commercially available mobile cigarette topography devices for e-cigarette vaping behavior measurements. *Nicotine & Tobacco Research*. 2018 Sep 12.

[110] Kim H, Lim J, Buehler SS, Brinkman MC, Johnson NM, Wilson, L, & Cross KM. Role of Sweet and Other Flavors in Liking and Disliking of Electronic Cigarettes. *Tobacco Control*. 2016 Nov;25(Suppl 2):ii55-ii61. PubMed PMID: 27708124.

[111]Crenshaw MD, Tefft ME, Buehler SS, Brinkman MC, Clark PI, Gordon SM. Determination of Nicotine, Glycerol, Propylene Glycol and Water in Electronic Cigarette Fluids Using Quantitative 1H NMR. *Magnetic Resonance in Chemistry*, 2016; 54(11), 901-904.

[112] Mikheev VB, Brinkman MC, Granville CA, Gordon SM, Clark PI. (2016). Real-time measurement of electronic cigarette aerosol size distribution and metals content analysis. *Nicotine & Tobacco Research*, ntw128.

[113] Hamad SH, Johnson NM, Tefft ME, Brinkman MC, Gordon SM, Clark PI, Buehler SS. Comparison of Little Cigars to 3R4F Reference Cigarettes: Physical Properties and

Semivolatile HPHCs. *Tobacco Regulatory Science*, 2017; 3(4):459-78. DOI: 10.18001/TRS.3.4.7.

[114] Klupinski TP, Strozier ED, Friedenberg DA, Brinkman MC, Gordon SM, Clark PI. Identification of new and distinctive exposures from little cigars. Chemical research in toxicology. 2016 Feb 15;29(2):162-8.

[115] Cruz-Cano R, Rangel-Gomez M, Van Wagoner C, Kidanu A, Brinkman MC, Clark PI. The acceptability of smokeless tobacco products depends on nicotine levels. *Addictive Behaviors Reports*. 2019 Dec 1;10:100217.

[116] Brinkman MC, Stepanov I. WHO Technical Report Series No. 1015; WHO study group on tobacco product regulation. Report on the scientific basis of tobacco product regulation: Seventh report of a WHO study group; Chapter 8, Updated priority list of toxicants in combusted tobacco products. ISBN 978-92-4-121024-9, World Health Organization, 2019.

[117] Brinkman MC, Kim H, Chuang JC, Kroeger RR, Deojay D, Clark PI, and Gordon SM. Comparison of true and smoothed puff profile replication on smoking behavior and mainstream smoke emissions. *Chemical Research in Toxicology*. 2015;28:182-190.

[118] MacGregor IC, Stanfill SB, Gordon SM, Turner DJ, Butler JM, Hanft EA, Kim H, Kroeger RR, Brinkman MC, Tefft ME, Clark PI, Buehler SS. Custom Mentholation of Commercial Cigarettes for Research Purposes. *Toxicology Reports*. 2014.

[119] Brinkman MC, Chuang JC, Gordon SM, Kim H, Kroeger RR, and Richter PA. Exposure to and deposition of fine and ultrafine particles in smokers of menthol and nonmenthol cigarettes. *Inhalation Toxicology*. 2012;24(5):255–269.

[120] Gordon SM, Brinkman MC, Meng RQ, Anderson GM, Chuang JC, Kroeger RR, Reyes IL, and Clark PI. Effect of cigarette menthol content on mainstream smoke emissions. *Chemical Research in Toxicology*. 2011;24(10):1744–1753.

[121] Bernert JT, Gordon SM, Jain RB, Brinkman MC, Sosnoff CS, et al. Increases in tobacco exposure biomarkers measured in non-smokers exposed to sidestream cigarette smoke under controlled conditions. *Biomarkers*. 2009;14(2):82–93.

[122] Gordon SM, Wallace LA, Brinkman MC, Callahan PJ, and Kenny DV. Volatile organic compounds as breath biomarkers for active and passive smoking. *Environmental Health Perspectives*. July 2002;110:689–698.

[123] Tackett AP, Lechner WV, Meier E, et al. Biochemically verified smoking cessation and vaping beliefs among vape store customers. Addiction. 2015;110(5):868-874.

[124] Lechner WV, Tackett AP, Grant DM, Tahirkheli NN, Driskill LM, Wagener TL. Effects of duration of electronic cigarette use. Nicotine & Tobacco Research. 2014;17(2):180-185.

[125] Lechner WV, Meier E, Wiener JL, et al. The comparative efficacy of first-versus second-generation electronic cigarettes in reducing symptoms of nicotine withdrawal. Addiction. 2015;110(5):862-867.

[126] James S, Meier E, Wagener T, Smith K, Neas B, Beebe L. E-cigarettes for immediate smoking substitution in women diagnosed with cervical dysplasia and associated disorders. International journal of environmental research and public health. 2016;13(3):288.

[127] Busch AM, Leavens EL, Wagener TL, Buckley ML, Tooley EM. Prevalence, reasons for use, and risk perception of electronic cigarettes among post-acute coronary syndrome smokers. Journal of cardiopulmonary rehabilitation and prevention. 2016;36(5):352.

[128] Wagener TL, Floyd EL, Stepanov I, et al. Have combustible cigarettes met their match? The nicotine delivery profiles and harmful constituent exposures of second-generation and third-generation electronic cigarette users. Tobacco control. 2017;26(e1):e23-e28.

[129] Leavens EL, Brett EI, Morgan TL, Lopez SV, Shaikh RA, Leffingwell TR, Wagener TL. Descriptive and injunctive norms of waterpipe smoking among college students. Addictive behaviors. 2018 Feb 1;77:59-62.

[130] Stevens EM, Kim S, Leshner G, Leavens ELS, Vidrine JI, Frank-Pearce SG, Hale JJ, Wagener TL. The Attitudinal and Motivational Effects of Anti-waterpipe Messages. American Journal of Health Behavior. 2019;43: 478-489.

[131] Leavens EL, Morgan TL, Brett EI, Patzkowsky K, Son J, Molina N, Eissenberg T, Shihadeh A, Leffingwell TR, Wagener TL. Concurrent alcohol use and waterpipe tobacco smoking: smoking topography, toxicant exposure, and abuse liability. Nicotine & Tobacco Research. 2019 Mar 1.

[132] Leavens EL, Meier E, Tackett AP, Miller MB, Tahirkheli NN, Brett EI, Carroll DM, Driskill LM, Anderson MP, Wagener TL. The impact of a brief cessation induction intervention for waterpipe tobacco smoking: a pilot randomized clinical trial. Addictive behaviors. 2018 Mar 1;78:94-100.

[133] Leavens EL, Brett EI, Frank S, Shaikh RA, Leffingwell TR, Croff JM, Wagener TL. Association between breath alcohol concentration and waterpipe lounge patrons' carbon monoxide exposure: A field investigation. Drug and alcohol dependence. 2017 Jan 1;170:152-5.

[134] Lim J, Wood A, Green BG. Derivation and evaluation of a labeled hedonic scale. Chemical Senses. 34: 739-51, 2009. PMCID: PMC2762053.

[135] Lim J. Hedonic scaling: A review of methods and theory. Food Quality and Preference. 22: 733-747, 2011.

[136] Lim J, Fujimaru T. Evaluation of the Labeled Hedonic Scale under different experimental conditions. Food Quality and Preference. 21: 521-530, 2010.

[137] Roberts, M.E., Ferketich, A.K. (2019). Hookah susceptibility and transitions over the first year of college. In press at Journal of Studies on Alcohol and Drugs.

[138] Roberts, M.E., Klein, E.G., Berman, M.L., Berhane, B., & Ferketich, A.K. Young adult perceptions surrounding hookah use (2017). Health Behavior Policy and Review, 4(6), 593-600.

[139] Evans, A.T., Peters, E., Keller-Hamilton B., Loiewski, C., Slater, M.D., Lu B., Roberts, M.E., & Ferketich, A.K. (2018). Warning Size Affects What Adolescents Recall from Tobacco Advertisements. Tobacco Regulatory Science, 4(3), 79-87.

[140] Keller-Hamilton, B., Roberts, M.E., Slater, M.D., Berman, M., & Ferketich, A.K. (2019). Adolescent males' responses to blu's fake warnings. Tobacco Control.

[141] Keller-Hamilton, B., Roberts, M.E., Slater, M.D., & Ferketich, A.K. (2019). Memorability of cigarette advertisements making "natural" claims among adolescents. Tobacco Regulatory Science, 5(4), 326-331.

[142] Paravisini, L. and Peterson, D.G. 2019. Reactive Carbonyl Species as a Control Point of Reaction Flavors. Food Chem. 274: 71-78.

[143] Paravisini, L. and Peterson, D.G. 2018. Role of Reactive Carbonyl Species on Non-Enzymatic Browning of Apple Juice During Storage. Food Chem. 245: 1010-1017.

[144] Kokkinidou, S. Peterson, D.G. 2016. Identification of compounds that contribute to trigeminal burn in aqueous ethanol solutions. Food Chem. 211: 757-762.

[145] Zhang, L., Xia, Y. and Peterson, D.G. 2014. Identification of bitter modulating Maillard-catechin reaction products. J. Agric. Food Chem. 62, 10092−10100.

[146] Brinkman MC, Keller-Hamilton B, Carney-Knisely G, Somogyi A, Roberts ME, Ferketich A, Stepanov I, Wagener TL, Shields PG, Halquist MS, Clark PI. Effect of pH of the nicotine liquid on JUUL puffing behavior and nicotine uptake. Scheduled for podium presentation, March 14, 2020, Society for Research on Nicotine and Tobacco annual meeting.

[147] Knobloch, T.J.; Ryan, N.M.; Brushweiler, L.; Wang, C.; Bernier, M.C.; Somogyi, Á.; Yan, S.P.; Copperstone, J.; Mo, X.; Bruschweiler, R.P.; Weghorst, C.M.; Qghumu, S. "Metabolomic Regulation of Glycolysis and AMP Activated Protein Kinase Pathways during Black Raspberry-Mediated Oral Cancer Chemoprevention" Metabolites 2019, 9, 140.

[148] Maltz, R.M.; Keirsey, J.; Kim, S.C.; Mackos, A.R.; Gharaibeh, R.Z.; Morre, C.C.; Somogyi, Á.; Bailey, M.T. Social Stress Affects Colonic Inflammation, the Gut Microbiome, and Short Chain Fatty Acid Levels and Receptors J. Pediatr Gastroenterol Nutr. 2019, 68, 533-540.

[149] Leggett, A.; Wang, C.; Li, D.W.; Somogyi, Á.; Bruschweiler-Li, L.; Bruschweiler, R.P. "Identification of Unknown Metabolomics Mixture Compounds by Combining NMR, MS and Chemoinformatics" Methods in Enzymology 2019, 615, 407-422.

[150] Somogyi, Á." Probing Peptide Fragment Ion Structures by Combining Sustained Off-Resonance Collision-Induced Dissociation and Gas-Phase H/D Exchange (SORI-HDX) in Fourier Transform Ion-Cyclotron Resonance (FT-ICR) Instruments", J. Am. Soc. Mass Spectrom. 2008, 19, 1771-1775.

[151] Russomanno J, Patterson, JG, Jabson JM. Food Security and Transgender and Gender Non-conforming Individuals in the Southeast United States: A Qualitative Study. Transgender Health 2019; 4(1), 1-11. doi: 10.1089/trgh.2018.0024.

[152] Jabson Tree JM, Patterson JG (2019). A Test of Feasibility and Acceptability of Online Mindfulness-Based Stress Reduction for Lesbian, Gay, and Bisexual Women and Men at Risk for High Stress: Pilot Study. JMIR Mental Health, 6(8), e15048. doi: 10.2196/15048

[153] Patterson JG, Jabson Tree JM, Kamen C. 2019. Cultural competency and microaggressions in the provision of care to LGBT patients in rural and Appalachian Tennessee. Patient Education Counseling 2019; doi: 10.1016/j.pec.2019.06.003

[154] Macisco JM, Patterson JG, Nemeth JM. Lapse to Relapse: Homeless Youth Return to Smoking When Supportive Structures and Self-Regulation Are Weakened. (accepted) Society for Behavioral Medicine, San Francisco, CA, April 1-4, 2020.

[155] Shaw, P. E. Handbook of Sugar Separations in Foods by HPLC; CRC Press: Boca Raton, FL, 1988.

[156] Yang D-J, Chen B. Simultaneous determination of nonnutritive sweeteners in foods by HPLC/ESI-MS. J. Agric. Food. Chem 57:3022-3027 (2009).

[157] United States Pharmacopeia (USP). <61> Microbiological examination of nonsterile products: microbial enumeration tests. Available from https://hmc.usp.org/sites/default/files/documents/HMC/GCs-Pdfs/c61.pdf

[158] Liu C. Glycerol transfer in cigarette mainstream smoke. Beiträge zur Tabakforschung International/Contributions to Tobacco Research. 2004 Jul 1;21(2):111-6.

[159] Laurene AH, Cundiff RH, Greene GH. Determination of glycerol and propylene glycol in cigarette smoke. Research Department, R.J. Reynolds Tobacco Company, 1965. Bates number 510552130-510552133.

[160] Brinkman MC, Wagener TL, Kassem NOF. COMMENTS REGARDING DOCKET NO. FDA-2012-N-0-0143, “Harmful and Potentially Harmful Constituents in Tobacco Products; Established List; Proposed Additions; Request for Comments. Comment Tracking Number: 1k3-9cj9-o3vi.

[161] Ding YS, Yan X, Wong J, Chan M, Watson CH. In situ derivatization and quantification of seven carbonyls in cigarette mainstream smoke. Chemical research in toxicology. 2016 Jan 19;29(1):125-31.

[162] Pang X, Lewis AC. Carbonyl compounds in gas and particle phases of mainstream cigarette smoke. Science of the total environment. 2011 Nov 1;409(23):5000-9.

[163] Gregg E, Hill C, Hollywood M, Kearney M, McAdam K, McLaughlin D, Purkis S, Williams M: The UK Smoke Constituents Testing Study. Summary of Results and Comparison with Other Studies. Beitr zur Tabakforsch Int. 2004, 21: 117-138.

[164] DiFranza JR, Savageau JA, Fletcher K, et al. Measuring the loss of autonomy over nicotine use in adolescents: the DANDY (Development and Assessment of Nicotine Dependence in Youths) study. Arch Pediatr Adolesc Med. 2002;156(4):397-403.

[165] Salameh P, Waked M, Aoun Z. Waterpipe smoking: construction and validation of the Lebanon Waterpipe Dependence Scale (LWDS-11). Nicotine Tob Res. 2008;10(1):149-158.

[166] Foulds J Stapleton J Feyerabend C Vesey C Jarvis M Russell MA . Effect of transdermal nicotine patches on cigarette smoking: a double blind crossover study . Psychopharmacology (Berl) . 1992 ; 106 : 421 – 427.

[167] Evans SE Blank M Sams C Weaver MF Eissenberg T . Transdermal nicotine-induced tobacco abstinence symptom suppression: nicotine dose and smokers' gender . Exp Clin Psychopharmacol. 2006;14:121-135 .

[168] Tiffany ST, Drobes DJ. The development and initial validation of a questionnaire on smoking urges. Br J Addict. 1991;86(11):1467-1476.

[169] Pérez-Padilla R, Vázquez-García JC, Márquez MN, Jardim JR, Pertuzé J, Lisboa C, Muino A, López MV, Tálamo C, De Oca MM, Valdivia G. The long-term stability of portable spirometers used in a multinational study of the prevalence of chronic obstructive pulmonary disease. Respiratory care. 2006 Oct 1;51(10):1167-71.

[170] Pals SL, Murray DM, Alfano CM, Shadish WR, Hannan PJ, Baker WL. Individually randomized group treatment trials: a critical appraisal of frequently used design and analytic approaches. Am J Public Health. 2008 Aug;98(8):1418-24

[171] Donner A, Klar N. Design and Analysis of Cluster Randomization Trials in Health Research. New York, NY: Wiley; 2000.

[172] Breland, A. B., Kleykamp, B. A., & Eissenberg, T. (2006). Clinical laboratory evaluation of potential reduced exposure products for smokers. Nicotine & Tobacco Research, 8,727–738.

[173] Cobb CO, Blank MD, Morlett A, Shihadeh A, Jaroudi E, Karaoghlanian N, et al. Comparison of puff topography, toxicant exposure, and subjective effects in low- and high-frequency waterpipe users: a double-blind, placebo-contro... - PubMed - NCBI. Nicotine Tob Res. 2015;17(6):667.

[174] Altman DG, Bland JM. How to randomise. BMJ. 1999 Sep 11;319(7211):703-4.

Appendix A: Construction and Verification of the Dual-Hose Research-Grade Waterpipe (RWP2) and Single-Hose Research-Grade Waterpipe (RWP)

Dual-Hose Research-Grade Waterpipe (RWP2)

RWP2 Construction

The RWP2, pictured in Figure A-1, is constructed from the same materials as the single-hose research-grade waterpipe (RWP) we developed and validated previously.[1-3] Briefly, all wetted materials have inert surfaces to reduce contamination, guard against memory effects and degradation over time, and facilitate easy cleaning between smoking sessions.[2]

Modifications that were made to the RWP to create the dual-hose RWP2 included changes to the cap design and the addition of a second hose, as shown in Figure 1. Both hoses are equipped with inline topography systems consisting of polycarbonate pneumotachometers (2 total) that are connected to an amplifier as described previously.[2] Two sets of pressure transducers inside the amplifier convert the pressure drop across the restriction inside each pneumotachometer to an electrical response. These data are now recorded electronically for each hose using LabView 2017. Data files are processed manually or automatically in batches using the same algorithm for peak recognition and analysis (MatLab R2018a). The result is a spreadsheet report that describes the volume, duration, average and peak flow rates of each puff, and interpuff intervals for each of the two participants smoking the RWP2.

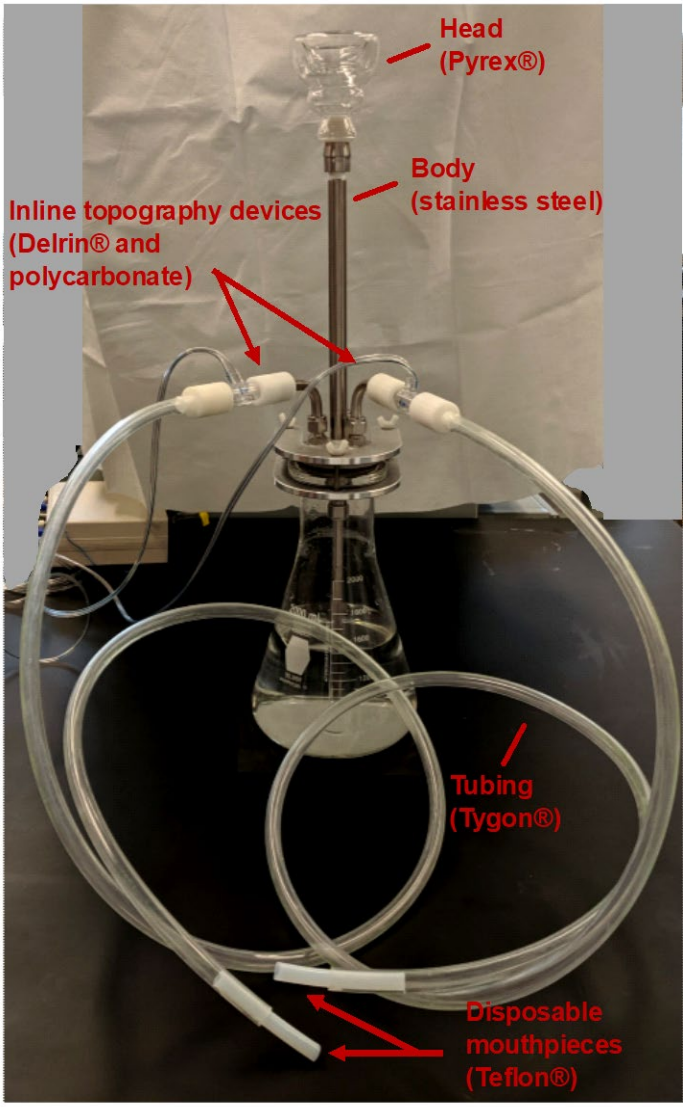


Figure A-1. Photo showing the main components of the dual-hose research-grade waterpipe (RWP2); data acquisition system not shown.

To increase ruggedness, the design of the neck of the glass flask was changed to eliminate the force associated with the cap's o-ring seal against the inside of the neck. Because the flasks are now being manufactured (by Kimax) with

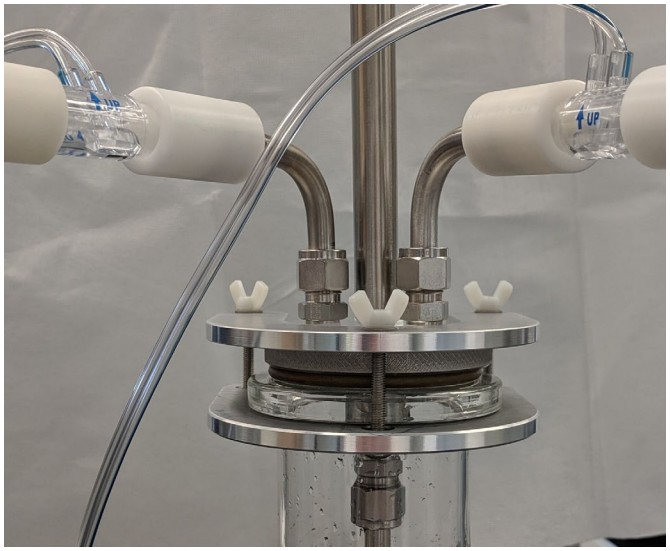


Figure A-2. The neck of the RWP2 was modified with a thicker upper lip and clamp to create a safer seal with the cap, given the manufacturer has reduced the flask's wall thickness.

thinner walls, the neck could no longer safely support the old design. The new design includes a thicker mouth on the flask which supports the weight of the cap. The cap is now sealed to the top of the mouth of the flask using an o-ring and a clamp, as shown in Figure A-2. The dimensions and materials of the head, body, and one-way valve of the RWP2 are unchanged from the original RWP.

Topography Data Collection and Analysis

The RWP2 has an inline topography system that allows real-time measurement and recording of the flow rate of smoke drawn through each of the two hoses. This allows the separate measurement and reporting of two individual's puffing behaviors (puff volume, duration, flow rate, and interpuff interval) when smoking a single, shared waterpipe in our controlled clinical research facility. The pneumotachometers introduce minimal draw resistance to the waterpipe (<5% at 27 L/min), and perform reliably, with known precision and accuracy, when measuring flows of waterpipe tobacco smoke.[1-3]

As with the RWP, the relationship between pneumotachometer response and flow through hoses 1 and 2 of the RWP2 is best described using a quadratic curve fit, as shown in Figure A-3. These data agree well with what was previously reported for the RWP.[2] To simulate normal waterpipe "smoking etiquette," these data were collected in series, first for Hose #1 while Hose #2 was plugged, then for Hose #2 while Hose #1 was plugged. As with all commercial dual-hose waterpipes, smokers of the RWP2 must put their thumb over their mouthpiece when the other person is puffing, otherwise the person will only be sucking in room air. Study participants will be reminded to follow normal waterpipe "smoking etiquette," during the clinic smoking sessions:

- Do not puff at the same time as your partner.
- Unless you are in the act of puffing, plug your hose end with your thumb so that your partner isn't just sucking air from their hose.
- Do not blow or exhale into your hose.

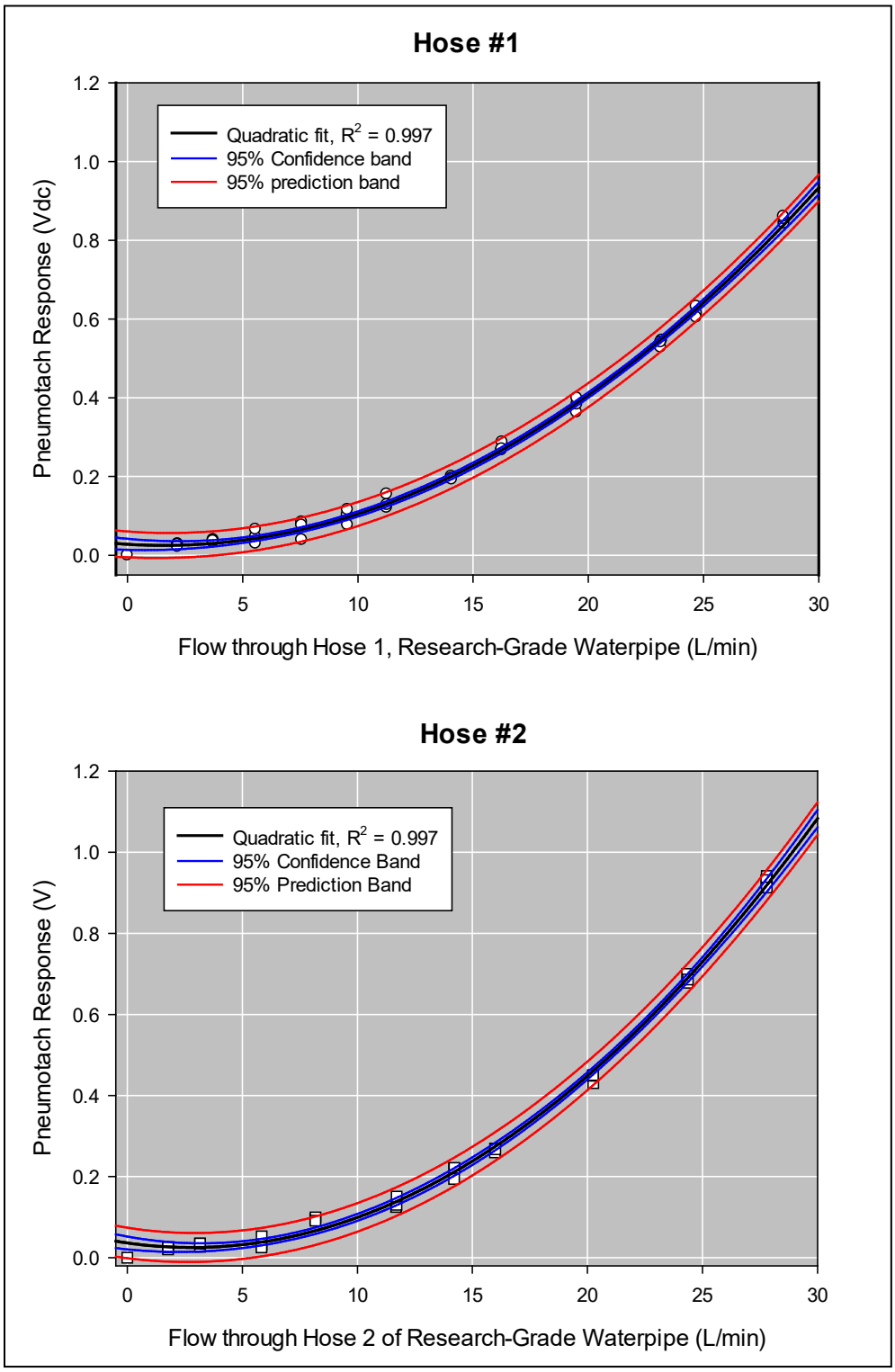


Figure A-3. Relationship between flow rate through each hose of the dual-hose research-grade waterpipe and the topography measurement device response.

Single-Hose Research-Grade Waterpipe (RWP)

RWP Construction

The RWP, pictured in Figure A-4, was developed and validated previously.[1-3] Briefly, all wetted materials have inert surfaces to reduce contamination, guard against memory effects and degradation over time, and facilitate easy cleaning between smoking sessions.[2]

For this study, modifications were made to the RWP including the use of new data acquisition hardware and software, and a change to how the cap seals to the flask, as shown in Figures A-4 and A-5. The hose is equipped with an inline topography system consisting of a polycarbonate pneumotachometer that is connected to an amplifier as described previously.[2] The amplifier can take inputs from two separate RWPs. Two sets of pressure transducers inside the amplifier convert the pressure drop across the restriction inside the pneumotachometer to an electrical response. These data are now recorded electronically for each RWP using LabView 2017. Data files are processed manually or automatically in batches using the same algorithm for peak recognition and analysis (MatLab R2018a). The result is a spreadsheet report that describes the volume, duration, average and peak flow rates of each puff, and interpuff intervals for each of the two participants separately smoking their own RWP.

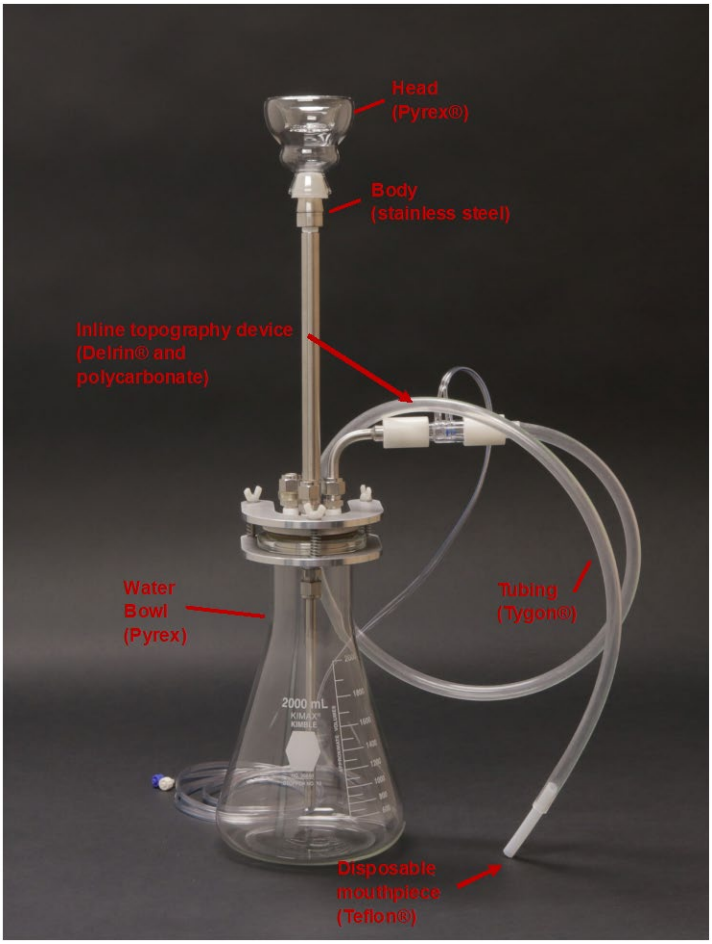


Figure A-4. Photo showing the main components of the dual-hose research-grade waterpipe (RWP); data acquisition system not shown.

To increase ruggedness, the design of the neck of the glass flask was changed to eliminate the force associated with the cap's o-ring seal against the inside of the neck. Because the flasks are now

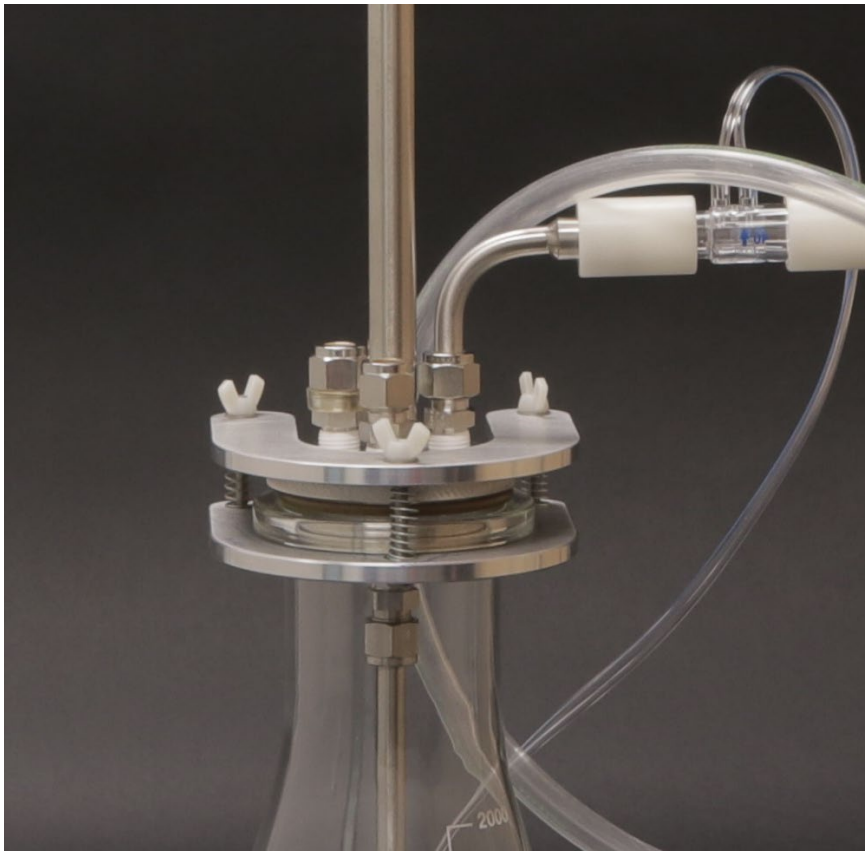


Figure A-6. The neck of the RWP was modified with a thicker upper lip and clamp to create a safer seal with the cap, given the manufacturer has reduced the flask's wall thickness.

being manufactured (by Kimax) with thinner walls, the neck could no longer safely support the old design. The new design includes a thicker mouth on the flask which supports the weight of the cap. The cap is now sealed to the top of the mouth of the flask using an o-ring and a clamp, as shown in Figure A-6. The dimensions and materials of the head, body, and one-way valve are unchanged from the original RWP.

Topography Data Collection and Analysis

Each RWP has an inline topography system that allows real-time measurement and recording of the flow rate of smoke drawn through the hose. The two RWPs are connected to the same signal amplifier. This allows the separate measurement and reporting of two individual's puffing behaviors (puff volume, duration, flow rate, and interpuff interval) when each are smoking separate RWPs in separate, well-ventilated rooms in our controlled clinical research facility. The pneumotachometer introduces minimal draw resistance to the RWP (<5% at 27 L/min), and performs reliably, with known precision and accuracy, when measuring flows of waterpipe tobacco smoke.[1-3] The relationship between pneumotachometer response and flow through the hose of each RWP is best described using a quadratic curve fit, as shown previously in Figure A-3.[2]

References

1. Brinkman MC, Kim H, Buehler SS, Adetona AM, Gordon SM, Clark PI. Evidence of compensation among waterpipe smokers using harm reduction components. Tob Control. 2018 Oct 30:tobaccocontrol-2018.
2. Brinkman MC, Kim H, Gordon SM, Kroeger RR, Reyes IL, ... & Clark PI. Design and Validation of a Research-Grade Waterpipe Equipped with Puff Topography Analyzer. Nicotine Tob Res. 2015;18(5):785-93.
3. Kim H, Brinkman MC, Sharma E, Gordon SM, Clark PI. 2016. Variability in puff topography and exhaled CO in waterpipe tobacco smoking. Tobacco Regulatory Science, 2(4), pp.301-8.

Appendix B: COVID-19 Related Procedures

Due to the COVID-19 pandemic, processes and procedures have been implemented to help protect participants and research staff. These processes and procedures will be followed as long as social distancing requirements are necessary for conducting study visits.. All study participants will be provided with a facemask upon entry. The research assistant will meet the participant outside the building for a temperature check**,** direct the participant into the building, and the two of them will ride the elevator to the 4th floor physically distanced at least 6 feet apart, both wearing masks. No more than 2 persons may ride the elevator at any given time. The participant will immediately be escorted to a private exam/draw room. Therefore, there will be no waiting in open lobby/waiting areas.

When in the exam room, the research assistant will stand at least 6 feet away from the study participant to give instructions. Afterwards, the study coordinator will leave the exam room to allow the study participant to conduct the instructed procedures. The research assistant and study participant will be at least 6 feet away from one another and wearing protective masks at all times during each visit.

Each research assistant will have a designated exam/draw room and smoking room in which to conduct their designated research study. Each smoking room is separated from the staff control station in the hallway by its own door and contains a large window for the research assistant to be able to see in and monitor study participant activity within the room. There is also a speaker and microphone system within each smoking room along with the Genetec software system on the outside of each room at the computer stations. Therefore, the research assistant and study participant can communicate with one another without being in the room together.

For study measures which cannot be physically distanced, appropriate PPE will be worn at all times by research staff during these procedures including goggles, face masks, gloves, and isolation gowns or lab coats.

After each participant visit is complete, there will be at least a 45-minute period for cleaning and air exchange in the negative pressure rooms, and for cleaning exam rooms and equipment before the next participant visit. All smoking rooms are under negative pressure with a ventilation rate of 36.8 – 44.1 air changes per hour (ACH).

Appendix C: Example Safe Departure Checklist

Brinkman/Ferketich, IRB 2018C0064, Evaluating Waterpipe Puffing Behaviors

Participant ID: ☐☐☐ **DYAD ID:** ☐☐ _ ☐☐☐ **Date:** ☐☐ / ☐☐ / ☐☐
Month Day Year

Visit #: _____

Visit Start: ☐☐ : ☐☐ ☐☐ **Visit End:** ☐☐ : ☐☐ ☐☐
Hour Minute AM or PM Hour Minute AM or PM

Participant Height: ☐☐☐ . ☐ cm **Participant Weight:** ☐☐☐ . ☐☐ kilograms

Safe Departure Checklist

Questions to assess safe departure from laboratory

At the end of the laboratory session, ask participant these questions and record the answers:

	Yes	No
1. Do you have a headache?		
2. Are you feeling any dizziness?		
3. Do you feel overly tired or fatigued?		
4. Are you feeling nauseous?		
5. Are you feeling light-headed?		
6. Do you feel mentally confused, disoriented, or like you are having trouble ordering your thoughts?		

If the answer to any of these questions is yes, ask the participant to remain seated. Offer the participant a snack and water, but do not force them to eat or drink. If you must leave the laboratory, make sure a study team member sits in the room with the participant, and notify the study PI, or the PI's designee if the PI is not available, as soon as safely possible.
If the participant requests to go to the bathroom, ask the participant to please remain seated until further assessment can be made prior to allowing them to stand unassisted.

Notes:

Study PI Contact information:

Office phone: 614-688-3226

Mobile phone: 614-354-3851

Form Completed by: _____ Date: _____

PI/Designee Signature: _____ Date: _____

Instructions:
The Safe Departure Checklist is a standard set of data entered for every subject that engaged in laboratory smoking prior to their departure from the lab.

Version 3, Date: 02/28/2019 Page 1 of 1

Appendix D: Data and Safety Monitoring Plan

Responsibility of DSM

Because a Data and Safety Monitoring Board (DSMB) 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 DSMB. However, the Center for Tobacco Research in conjunction with the OSU Comprehensive Cancer Center does have a standing DSMB that we can utilize if the funding agency or the reviewers request one.

Co-PD/PIs Brinkman and Wagener will be responsible for monitoring the safety and integrity of the research project, executing the DSM plan for the projects, and complying with the reporting requirements. They will also provide annual and interim progress reports to NCI and FDA.

With this project, the DSM will begin by reviewing the revised protocol and establishing guidelines for data and safety monitoring. This will include reviewing the standard procedures for day-to-day monitoring by the internal monitors, principal and co-investigators and study staff. DSM will include evaluating the progress of the trial; reviewing data quality, participant recruitment, and study retention; and examining other factors that may affect study outcome. The PIs will review major proposed modifications to the study prior to their implementation. They will also review the participants' ability to achieve the study requirements, and the rates of adverse events to determine whether there has been any change in participant risk. Their review will ensure that subject risk does not outweigh the study benefits. Following each DSMB meeting, a written report of their findings will be generated for the study record and provided to NCI and each site's Institutional Review Boards (IRB).

The specific DSM responsibilities will include the following:

- Review the research protocol, informed consent documents, and plans for data and safety monitoring;
- Evaluate the progress of the trial, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the trial sites, and other factors that can affect study outcome;
- Consider factors external to the study that may be relevant, such as scientific or therapeutic developments, which could have an impact on the safety of the participants or the ethics of the study;
- Protect the safety of the study participants and report on the safety and progress of the trial;
- Make recommendations to the research team and sponsor, as required, concerning continuation, termination or other modification(s) to the trial.
- Ensure the confidentiality of the trial data and the results of monitoring;
- Assist on any problems with study conduct, enrollment, sample size, and/or data collection.
- Review data for accuracy and quality

Study progress reports will be prepared by the research team. These reports will include updates on the protocol, including revisions, enrollment progress and projections, retention, and safety data (description, severity, attribution, response, reporting and resolution of those events as well as if any events are unanticipated. If more than 10% of the participants experience an

adverse event during the course of the study then the PIs will consider suspension or discontinuation of the study.

Adverse Events

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 OSU IRB. We will monitor for risk of smoking waterpipe by screening participants for general medical precautions (pregnancy, respiratory and cardiovascular disease). Participants will be given contact numbers of study personnel and Co-PD/PIs Brinkman and Wagener. Any adverse events, breaks of confidentiality, or any other data or safety issues that arise will be discussed immediately between study personnel and the Co-PD/PIs.

Co-PD/PI Brinkman will be responsible for completing an Adverse Events Form should an event occur. She will report Serious Adverse Events to the OSU IRB within 24 hours of having received notice of the event. She will gather any information needed to investigate the event and to determine subsequent action. Any subsequent action will be documented and reported to the OSU IRB and the Program Officer at NIH. Adverse event reports will be reviewed annually with the OSU IRB to ensure participant safety.

Protocol Amendments/Changes

Any changes or amendments to the protocol made in response to adverse events/SAEs (or independent of AEs/SAEs) will be requested in writing to the IRB, which will then grant or deny permission to make the requested change in protocol. Approved changes and amendments will be reported to the NIH.

Frequency of DSM

On a regular basis (e.g., weekly), investigators and key personnel will review data quality, recruitment, retention, and examine other factors that may affect outcomes. They will also review any adverse events to determine if there are any significant or unanticipated participant risks. The Project Leaders (M. Brinkman & T. Wagener) and the Project Manager will be available to meet outside of the scheduled meetings if concerns regarding any adverse trends or other major problems should arise.

Content of DSM Report

The DSM annual report will include enrollment information, demographics and characteristics of the participants, the expected versus actual recruitment rates, quality assurance or regulatory issues that may have occurred during the year, a summary of adverse events and SAEs, protocol violations, and any actions or changes to the protocol. Also included will be any and all actions by the IRB as they occur.

The Ohio State University Consent to Participate in Research

Study Title: Evaluating waterpipe tobacco additives

Principal Investigator: Marielle C. Brinkman and Theodore L. Wagener

Sponsor: National Cancer Institute, National Institutes of Health; Food and Drug Administration Center for Tobacco Research

- **This is a consent form for research participation.** It contains important information about this study and what to expect if you decide to participate. Please consider the information carefully. Feel free to discuss the study with your friends and family and to ask questions before making your decision whether or not to participate.
- **Your participation is voluntary.** You may refuse to participate in this study. If you decide to take part in the study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your usual benefits. Your decision will not affect your future relationship with The Ohio State University. If you are a student or employee at Ohio State, your decision will not affect your grades or employment status.
- **You may or may not benefit as a result of participating in this study.** Also, as explained below, your participation may result in unintended or harmful effects for you that may be minor or may be serious depending on the nature of the research.
- **You will be provided with any new information that develops during the study that may affect your decision whether or not to continue to participate.** If you decide to participate, you will be asked to sign this form and will receive a copy of the form. You are being asked to consider participating in this study for the reasons explained below.

Key Information About This Study

The following is a short summary to help you decide whether or not to be a part of this study. More detailed information is listed later in this form.

The purpose of this study is to evaluate hookah (waterpipe) tobacco additives and their effect on puffing behaviors, lung function, and the appeal of hookah smoking. Eligible participants will attend four clinic visits to smoke hookah, give exhaled breath and blood samples, and answer questions about your smoking experience at the clinic and your tobacco use history. Clinic visits will be roughly one week apart and take place at roughly the same time of day. The clinic is near Riverside Hospital and has ample free parking. Each clinic visit will take 2.5-3 hours, so participants can expect to spend up to 12 hours over the course of 4 weeks participating in the study. The biggest risk and discomfort may be dizziness or nausea from

giving two blood samples at every clinic visit, for a total of eight blood samples. Another risk is loss of confidentiality if other people find out about your participation in the study. All efforts are made to keep your information confidential, but confidentiality is not absolute. There are no personal benefits from being in the study; however, society stands to benefit from your providing valuable information for public health research on tobacco products.

1. Why is this study being done?

The goal of this study is to evaluate hookah (waterpipe) tobacco additives and their effect on puffing behaviors, lung function, and the appeal of hookah smoking.

2. How many people will take part in this study?

Up to 60 people will participate in this study.

3. What will happen if I take part in this study?

If you decide to take part in the study, you will make a series of 4 visits to our clinic, at about the same time each day, each visit separated by at least a week. Each visit will last about 2.5-3 hours, and include the activities listed in Table 1. For each visit, you must come to the clinic after not using any tobacco- or nicotine-containing products after 10 pm the previous day. We also encourage you to eat two hours before coming to your scheduled appointment because we will be drawing your blood. In addition, we suggest that you drink plenty of water before your scheduled appointment and refrain from drinking alcohol after 10 pm the previous day. Doing these things will make it easier to draw your blood. During your session, you will be instructed to smoke a hookah as you normally would, and we will explain everything in detail to you. On arrival at the laboratory you will be asked briefly about your tobacco and hookah use history and patterns.

Please note: you will be asked not to eat or drink anything other than water during your clinic visits. For this reason, please make sure to have eaten regularly prior to attending your clinic visits.

In addition, at each visit, we will require you to follow safety procedures in place to enter the clinic and engage as a participant to reduce your risk of acquiring or spreading COVID-19, or a similar pandemic disease. You will be asked to do the following things if you agree to participate in this study:

Table 1. Sequence of experimental procedures and durations for laboratory visits.

Experimental Procedures	Length of Time Needed	
	Visit 1	Visits 2-4
Informed Consent and Pre-Smoking Questionnaires	**30 min**	**10 min**
Complete or review informed consent + consent addendum	X	X
Confirm eligibility (exhaled breath sample and questionnaires)	X	X
Confirm eligibility (urine pregnancy test, women only)	X	
Give blood sample	X	X
Eligibility status will be communicated to you	X	
Complete pre-smoking subjective effects questionnaires	X	X
Complete practice session for flavor perceptions questionnaires	X	
Measure height and weight	X	
Pre-Smoking Sample Collection and Smelling of the Hookah Tobacco	**30 min**	**30 min**
Cleanse mouth with bottled water 3 times	X	X
Smell assigned tobacco type for 20 seconds, then give flavor perceptions	X	X
Complete lung function test	X	X
Give exhaled breath sample	X	X
Hookah Smoking	**30-90 min**	**30-90 min**
Smoke hookah with assigned tobacco type as you normally would for 10 minutes, then give flavor perceptions	X	X
After smoking for 45 minutes give exhaled breath sample	X	X
Continue to smoke hookah until satiated	X	X
Puffing behavior is recorded automatically	X	X
Post-Smoking Sample Collection	**20 min**	**20 min**
Give exhaled breath sample	X	X
Give flavor perceptions	X	X
Give blood sample	X	X
Complete lung function test	X	X
Give answers to other post-smoking questionnaires	X	X
Complete demographic/tobacco product use questionnaires	X	

First clinic visit

You will arrive at the clinic at your scheduled time. You will be asked to conduct all of the study activities. First you will breathe into a handheld carbon monoxide monitor to confirm that you have not smoked tobacco in the last 12 hours. The study design will be explained to you, and you will have the opportunity to ask questions before making the decision to participate further. If you decide to participate further, you will sign your name giving your informed consent to participate. If you are biologically able to become

pregnant, you will also be asked to take a urine pregnancy test to confirm that you are not pregnant. If the result of the test is positive, this will be communicated to you privately and you will not be eligible to participate in the study.

A blood sample will be collected by a trained and certified phlebotomist technician using a needle and syringe to draw up to 2 teaspoons or 10 mL on each stick. A second sample, for a total of 20 mL per needle stick, may be collected for quality control (QC) purposes if the subject agrees. Only one QC sample will be collected for any subject at any given clinic visit. If we have difficulty drawing your blood, there will be a maximum of 3 needle sticks per draw. If you are unable to give a sufficient blood sample, you will be ineligible to participate further.

You will be asked to answer some general questions about yourself, like your age, gender, and your history of tobacco product use. If your survey responses and/or pregnancy test (if applicable) and/or ability to give a sufficient blood sample indicate that you are eligible to continue participation you will proceed with participation; otherwise you will not participate further.

We will collect data on your height and weight. Prior to smoking hookah, you will participate in a training session to learn how to complete liking/disliking and flavor and answer some questions about hookah smoking. If there is an active pandemic, such as COVID-19, you will be put into a ventilated room designed to vent tobacco smoke from the room. You will be provided with warm (body temperature) bottled water and asked to rinse out your mouth and spit three separate times into a cup. You will be provided with a small container of the waterpipe tobacco that you will smoke that day. You will be asked to smell the tobacco and then provide your responses to the questionnaires describing your liking/disliking and flavor perceptions.

To measure lung function before and after smoking, you will be asked to take a deep breath and then breathe out forcefully into a handheld spirometer to measure your lung function. You may be asked to repeat this procedure up to five times before and after smoking. You will then be provided with a prepared research-grade hookah and asked to smoke as you normally would until you feel satiated.

The hookah will be connected to a device that measures how you are smoking it. You will be restricted to one bowl of tobacco and one charcoal.

After 10 minutes, we will ask you to stop smoking to provide your responses to the questionnaires describing your liking/disliking and flavor perceptions. After smoking for 45 minutes you will again be asked to exhale into the same handheld carbon monoxide monitor as before; at that time, we may stop the smoking session. If we do not stop the smoking session, you will then proceed to smoke the hookah as you normally would until you feel satiated.

When you feel you are done smoking, you will notify us and we will end the smoking session. Immediately after your smoking session, you will be asked to exhale into the carbon monoxide monitor and provide your responses about liking/disliking and flavor perceptions on questionnaires. We will collect blood samples from you and repeat the lung function measurement. You will be asked to answer some more questions about your hookah smoking experience. You will schedule a second clinic visit to take place in one week at approximately the same time of day.

Clinic visits 2-4

If you are female and able to get pregnant, you will be asked to use a birth control method for the duration of your participation in the study (e.g., birth control pills, implants, IUD, Depo-Provera, or condoms) to prevent pregnancy. All participants will be asked to abstain from smoking hookah or other tobacco products for at least 12 hours prior to their next clinic visit.

It will take you up to 3 hours from the time you check in with the research staff until the time you complete all the procedures for the first visit. The remaining 3 visits will take about 2.5 hours each.

4. How long will I be in the study?

Each clinic visit will last up to three hours and be separated by at least a week. Therefore participants can expect to be in the study for about four weeks.

5. Can I stop being in the study?

You may leave the study at any time. If you decide to stop participating in the study, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with The Ohio State University.

6. What risks, side effects or discomforts can I expect from being in the study?

While completing questionnaires, there is a small risk of mental discomfort. You can refuse to answer any question(s) if you feel uncomfortable.

Although many hookah smokers think it is less harmful, hookah smoking has many of the same health risks as cigarette smoking. Carbon monoxide is produced through hookah smoking, therefore sufficient ventilation is used to ensure safe levels in the room. However, if at any time you feel faint, light-headed, dizzy, headache, or shortness of breath please notify us immediately and the smoking session will be stopped at that time.

You will give 20 mL of blood before and at the end of each smoking session, for a total of 40 mL for each laboratory visit. The amount of blood you give during each laboratory visit is approximately 8 teaspoons, which is about ten times *less* than would be taken in a routine blood donation at the American Red Cross (about 450 mL). You may feel some pain, like a slight sting or "pinch" in your arm, when the blood is drawn. You may also get a small bruise as a result of the blood draw. Some people get dizzy, feel nauseous, or faint when their blood is drawn, but this is somewhat rare. There is a very small chance that you might get an infection as a result of giving the blood sample. Our professional staff will follow a standardized procedure and take any necessary steps to ensure your safety. Each needle used in the procedure is sterile and is disposed of after a single use. If you have a history of problems resulting from blood donations or blood draws, you should not take part in this study.

The hookah you will be smoking is specially designed for investigational research only, and as such may have unknown health risks. This hookah has been used by participants in three previous studies.

You may feel hungry during the clinic visit because you will not be permitted to eat until after your last lung function test is completed.

Lung function tests involve forceful breathing out. Sometimes, people become light-headed during the tests or have chest soreness for a few days. You will be seated and closely monitored during these tests.

There is a risk of breach of confidentiality or a loss of privacy if other people find out about your participation. All efforts are made to keep your information confidential, but confidentiality is not absolute.

7. What benefits can I expect from being in the study?

You will not experience any personal benefits from being in the study. However, you will be providing valuable information for the researchers to use in future phases of the study.

8. What other choices do I have if I do not take part in the study?

You may choose not to participate without penalty or loss of benefits to which you are otherwise entitled.

9. What are the costs of taking part in this study?

There are no costs to you for participating in this study.

10. Will I be paid for taking part in this study?

By law, payments to participants are considered taxable income. You will be paid up to $250 for taking part in the study clinic visits, as outlined below. You will receive your payments at the end of your last clinic visit. Payments will be made using the Greenphire ClinCard to increase accountability and facilitate ease of payment. If you withdraw from the study before your last clinic visit, you may receive payment for the visit(s) you attended.

- Visit 1: $50
- Visit 2: $50
- Visit 3: $50
- Visit 4: $50
- Bonus payment for completing all four visits: $50

11. What happens if I am injured because I took part in this study?

If you suffer an injury from participating in this study, you should notify the researcher or study doctor immediately, who will determine if you should obtain medical treatment at The Ohio State University Wexner Medical Center.

The cost for this treatment will be billed to you or your medical or hospital insurance. The Ohio State University has no funds set aside for the payment of health care expenses for this study.

12. What are my rights if I take part in this study?

If you choose to participate in the study, you may discontinue participation at any time without penalty or loss of benefits. By signing this form, you do not give up any personal legal rights you may have as a participant in this study.

You will be provided with any new information that develops during the course of the research that may affect your decision whether or not to continue participation in the study.

You may refuse to participate in this study without penalty or loss of benefits to which you are otherwise entitled.

An Institutional Review Board responsible for human subjects research at The Ohio State University reviewed this research project and found it to be acceptable, according to applicable state and federal regulations and University policies designed to protect the rights and welfare of research participants.

13. Will my de-identified information (and bio-specimens) be used or shared for future research?

No.

14. Will my study-related information be kept confidential?

Efforts will be made to keep your study-related information confidential. However, there may be circumstances where this information must be released. For example, personal information regarding your participation in this study may be disclosed if required by state law.

Also, your records may be reviewed by the following groups (as applicable to the research):

- Office for Human Research Protections or other federal, state, or international regulatory agencies;
- U.S. Food and Drug Administration;
- The Ohio State University Institutional Review Board or Office of Responsible Research Practices;
- The sponsor supporting the study, their agents or study monitors; and
- Your insurance company (if charges are billed to insurance).

If this study is related to your medical care, your study-related information may be placed in your permanent hospital, clinic, or physician's office records. Authorized Ohio State University staff not involved in the study may be aware that you are participating in a research study and have access to your information.

The NIH has issued a Certificate of Confidentiality for this study. This Certificate provides extra protection for you and your study information, documents, or samples (cheek cells, etc.). The Certificates are issued so that we cannot be required to disclose any identifiable, sensitive information collected about you as a part of this study in a lawsuit or legal proceeding. We are also prevented from releasing your study information without your consent. This is a layer of protection over and above the already existing protections in place for you and your information, documents, or samples.

However, these protections do not apply in some situations. For example, we may have to release your information if a law requires us to do so, the Agency that is funding this study requests the information, or if the FDA tells us to release this information. We may also use your information to conduct other scientific research as allowed by federal regulations.

Study information that has health implications may be placed in your medical record where authorized employees may see the information. Further, authorized requests for your records (medical record release for continuity of care) may result in research-related information being released.

Please talk to your study team, or contact the Office of Responsible Research Practices at 614-688-8641, if you have questions. You may also visit the NIH website at https://humansubjects.nih.gov/coc/faqs to learn more.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search the website at any time.

You may also be asked to sign a separate Health Insurance Portability and Accountability Act (HIPAA) research authorization form if the study involves the use of your protected health information.

15. Who can answer my questions about the study?

For questions, concerns, or complaints about the study you may contact Marielle Brinkman at 614-688-3226, or Additive Study staff by email at Additive-Study@osumc.edu.

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact the Office of Responsible Research Practices at 1-800-678-6251.

If you are injured as a result of participating in this study or for questions about a study-related injury, you may contact Marielle Brinkman at 614-688-3226. If, after you leave the clinic, you are unsure if you are having an adverse or negative reaction, please call Marielle Brinkman as soon as possible.

Signing the consent form

Future contact

We would like your permission for study staff to contact you if we need to discuss or clarify your questionnaire responses or to participate in future studies. Only individuals authorized by the principal investigator will contact you.

Please sign and date below if you agree to be contacted in the future.

Signature:_____ Date: _____

I have read (or someone has read to me) this form and I am aware that I am being asked to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to participate in this study.

I am not giving up any legal rights by signing this form. I will be given a copy of this form.

Printed name of participant	**Signature of participant**
	AM/PM
	Date and time
Printed name of person authorized to consent for participant (when applicable)	**Signature of person authorized to consent for participant (when applicable)**
	AM/PM
Relationship to the participant	**Date and time**

Investigator/Research Staff

I have explained the research to the participant or his/her representative before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

Printed name of person obtaining consent	**Signature of person obtaining consent**

_____ **AM/PM**
Date and time

Witness(es) - *May be left blank if not required by the IRB*

Printed name of witness	**Signature of witness**
	_____ **AM/PM**
	Date and time
Printed name of witness	**Signature of witness**
	_____ **AM/PM**
	Date and time