Comparison of waterpipe smokers' exposures when using harm reduction components

Marielle C. Brinkman, B.S.;[1,2*+] Hyoshin Kim, Ph.D.;[3] Stephanie S. Buehler, Ph.D.;[4] Anna M. Adetona, Ph.D.;[4] Sydney M. Gordon, Ph.D.;[+] and Pamela I. Clark, Ph.D.[5]

[1] College of Public Health, The Ohio State University, Columbus, Ohio, USA.

[2] University of Maryland Tobacco Center of Regulatory Science, College Park, Maryland, USA.

[3] Battelle Public Health Center for Tobacco Research, Seattle, Washington, USA.

[4] Battelle Public Health Center for Tobacco Research, Columbus, Ohio, USA.

[5] University of Maryland Tobacco Center of Regulatory Science, School of Public Health, University of Maryland, College Park, Maryland, USA.

*Corresponding author, brinkman.224@osu.edu.

+ At the time that this work was performed, MCB and SMG were affiliated with Battelle

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY METHODS

Controlled Chamber and Environmental CO Monitoring

Participants smoked in a controlled chamber ventilated at a rate to simulate the higher range of typical U.S. residences (2.3 hr^{-1}). The air exchange rate of the chamber was determined using the standard sulfur hexafluoride tracer technique.[S1] To ensure consistent ventilation, the flow rate of the chamber air that was mechanically vented to waste was set and monitored using a hotwire anemometer and data acquisition hardware/software (National Instruments, LabView ver. 6, Texas, U.S.A.). To prevent carry-over in between smoking sessions, prior to each smoking session, chamber background levels were monitored using a compact proton transfer reaction mass spectrometer (PTR-MS; Ionicon, Innsbruck, Austria) in full scan mode. If ion count levels were >10% of baseline, the chamber walls were cleaned using an anionic soap solution, rinsed, and dried using lint-free wipes. This process was repeated until background levels were within normal background range. Details on the chamber characteristics and integrated glove box used to prepare and monitor the performance of the RWP are provided elsewhere.[S2] Environmental CO levels were continuously monitored in the breathing zone of the seated participant using a CO monitor (Model 48C, Thermal Environmental Instruments, Massachusetts, U.S.A.). Total mass of CO emitted during the smoking session was calculated from the area under the curve for the smoking period, the residual CO in the chamber at the end of the smoking session, and the ventilation rate of the chamber. One hour time weighted averages were calculated as the area under the curve multiplied by the time the participant spent smoking.

Exhaled Volatile HPHCs

Exhaled CO was collected before and immediately after the smoking session using a handheld electrochemical cell (Vitalograph, Kansas, U.S.A.). Exhaled benzene was measured by having participants breathe into a direct breath sampling interface[S3] attached to the PTR-MS, as described previously.[S4] Boost was calculated as the pre-exposure level subtracted from the post-exposure level.

Plasma Nicotine

Venous blood samples were collected into tubes (lavender top with ethylenediaminetetraacetic acid stabilizer, Becton & Dickinson, New Jersey, U.S.A.) two minutes before and immediately after the RWP smoking session. Whole blood was separated using centrifugation (3,000 rpm for 15 minutes) and the resulting plasma was frozen (-80°C) prior to shipment for analysis (using the liquid chromatography tandem mass spectrometry method TME-001, Labstat International, ULC, Ontario, Canada). Quality control (QC) samples, prepared by spiking non-smokers' blood with a known quantity of nicotine (30-50 ng/mL), were shipped to Labstat and analyzed alongside participant samples. An imputation method for log-normal data was used: values below the method detection limit (MDL; 1.2 ng/mL) or above the MDL and less than the limit of quantitation (LOQ; 4.1 ng/mL), were substituted with the MDL or LOQ (respectively) divided by the square root of two.[S5]

Heart Rate

To record the electrical activity of the heart, participants were fitted with a chestband (Ironman Midsize Race Trainer Heartrate Monitor with Data Xchanger, Timex) containing an electrode pad that was moistened with tap water prior to making contact with the participant's skin near his/her heart. Electric signals were transmitted to and continuously acquired by (2 Hz) a wristband; the resulting data files were downloaded and analyzed after the smoking session was completed.

Subjective Effects

Subjective effects were collected using four self-administered questionnaires: Minnesota Nicotine Withdrawal Scale (MNWS), Questionnaire for Urges to Smoke (QSU)-Brief, Direct Effect of Nicotine scale (DENS), and Direct Effects of Tobacco scale (DETS). For each questionnaire, the word "cigarette" was replaced with "waterpipe". The MNWS consists of 11 visual analog scale (VAS) items where each item is presented with a word or phrase at either side of a horizontal line from "Not at all" (0) to "Extremely" (100).[S6] The QSU-Brief consists of 10 items related to smoking urge/desire that participants rate on a 7-point scale ("Strongly disagree" to "Strongly agree"). Scores on the "intention to use waterpipe" and "relief from withdrawal" factors were calculated for each configuration and each participant.[S7] DENS consists of 10 VAS items developed to assess the incidence of nicotine-related side effects.[S8] DETS consists of 13 VAS items developed to assess commonly reported cigarette smoking effects.[S9] For MNWS, QSU-Brief, and DENS, participants responded to each item before and after smoking. For analysis, difference scores for each item or factor (post- minus pre-scores) were then calculated for each configuration/participant. For DETS, participants provided their ratings only after smoking.

Machine Smoking

To quantify mainstream smoke yields of semivolatile toxicants, and to measure tobacco and heat source temperatures, machine smoking of the RWP was conducted using a five-port cigarette smoking machine (FP2000, Borgwaldt KC, Virginia, U.S.A.) equipped with anodized aluminum manifolds as described previously.[S2] The temperature of the tobacco was measured using three thermocouples that were positioned in a straight line centered across the middle of the head at 10 mm from the bottom of the head; a fourth thermocouple was placed underneath the heat source. Tobacco and heat source temperature data were continuously acquired (0.1 Hz, Yokogawa MW100) using four thermocouples (Type J, 0.01" o.d.). The topography data from 34 participants smoking the Control configuration[S2] was averaged to produce one, two-stage human-derived puffing regimen, which is shown in supplementary Table S-1. The smoking machine was interfaced to an electrical low pressure impactor (ELPI, Dekati Ltd., Kangasala, Finland) as described previously.[S10] Size-resolved mainstream particulate for each machine smoking session was collected on foil and glass fiber filter substrates. Substrates were combined to produce one fine (0.093 μm < aerodynamic diameter ≤ 3.930 μm) and one ultrafine (aerodynamic diameter ≤ 0.093 μm) particulate fraction that were weighed and chemically extracted as composite samples. The fine and ultrafine samples were extracted with dichloromethane:acetonitrile (1:1) using sonication followed by an orbital shaker (150 rpm). Extracts were split, internal standards were added, and one aliquot was solvent exchanged into 100 mM ammonium acetate and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The other aliquot was diluted (fine samples only) and analyzed using gas chromatography/mass spectrometry (GC/MS) for the remaining semivolatile compounds (benzo[a]pyrene, nicotine, menthol, pyrene, and quinoline).

Comparison of Research Grade Waterpipe to Commercial Waterpipes in Laboratory Clinical Research

A comparison of our Control results with previously conducted studies with similar outcomes is shown in Supplementary Table S-2. Average plasma nicotine and heart rate boost, total puffing volume, and smoking time (when not controlled) reported in these studies were similar to the results of our study. Average exhaled CO boost varied much more widely, with a range of 20.6 – 93.2 ppmv across the five studies. Exhaled breath CO levels in our participants reflect not only mainstream waterpipe tobacco smoke exposure but also significant environmental tobacco smoke exposure; as our study simulated the scenario of a person smoking a waterpipe in a room with the window closed, such as might be done in very cold or hot climates when air conditioning or heating is in use. While average CO boost did not seem comparable across the studies, the average increase from baseline breath CO level for this study was similar (9-14 fold) to three of the other studies. Smokers participating in a group smoking study with natural ventilation (one open window) conducted by Bentur et al.,[31] showed the highest average post-smoking exhaled breath levels (measured using oximetry), but the greatest increase from baseline (26 fold) was reported by Jacob et al.,[33] (using electrochemical cell) in smokers smoking in a research hospital room with an unknown ventilation rate.

SUPPLEMENTARY REFERENCES

S1. ASTM. Standard Test Method for Determining Air Leakage Rate by Tracer Dilution. E741-83. Philadelphia, PA: American Society for Testing and Materials, 1983.

S2. Brinkman MC, Kim H, Gordon SM, Kroeger RR, Reyes IL, Deojay DM, et al. Design and validation of a research-grade waterpipe equipped with puff topography analyzer. Nicotine & Tobacco Research. 2015;18(5):785-93.

S3. Gordon, S. M., Wallace, L. A., Brinkman, M. C., Callahan, P. J., & Kenny, D. V. (2002). Volatile organic compounds as breath biomarkers for active and passive smoking. Environmental health perspectives, 110(7), 689.

S4. Gordon S, Brinkman M, Meng R, Anderson G, Chuang J, Kroeger R, et al. Effect of cigarette menthol content on mainstream smoke emissions. Chemical research in toxicology. 2011;24(10):1744-53.

S5. Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Applied occupational and environmental hygiene. 1990;5(1):46-51.

S6. Hughes JR, Hatsukami D. Signs and symptoms of tobacco withdrawal. Arch Gen Psychiatry. 1986;43(3):289-94.

S7. Cox LS, Tiffany ST, Christen AG. Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. Nicotine & Tobacco Research. 2001;3(1):7-16.

S8. Evans SE, Blank M, Sams C, Weaver MF, Eissenberg T. Transdermal nicotine-induced tobacco abstinence symptom suppression: nicotine dose and smokers' gender. Experimental and clinical psychopharmacology. 2006;14(2):121.

S9. 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. 1992;106(3):421-7.

S10. Brinkman MC, Chuang JC, Gordon SM, Kim H, Kroeger RR, Polzin GM, et al. Exposure to and deposition of fine and ultrafine particles in smokers of menthol and nonmenthol cigarettes. Inhalation toxicology. 2012;24(5):255-69.

SUPPLEMENTARY TABLES AND FIGURES

Table S-1. Two-stage puffing regimen derived from human smoking of the Research-Grade Waterpipe in the Control configuration.

Stage	Time (min)	Number of Puffs	Puff Duration (s)	Puff Volume (L)	Interpuff Interval (s)
1	11.2	32	4.6	0.720	16.4
2	22.6	42	3.6	0.455	28.7
Total	33.3	74	298.4	42.15	1702

Table S-2. Comparison of reported puffing topography, physiological responses, and exhaled breath and blood biomarkers levels from laboratory waterpipe tobacco smoking; data presented as means ± standard deviations (when provided).

Study Details	This Study (Control configuration)	Bentur et al., 2014[a] (Active)	Blank et al., 2011[b]	Jacob et al., 2011[c]	Maziak et al., 2011[d]
Location	Columbus, OH, U.S.A.	Haifa, Israel	Richmond, VA, U.S.A.	San Francisco, CA, U.S.A.	Aleppo, Syria
Sample size	n = 34	n = 47	n = 37	n = 16	n = 56
Age	24.4 ± 5.5	24.9 ± 6.2[e]	20.5 ± 2.1	22.9	30.9 ± 9.5
WP use (times/month)	3.1 ± 3.0	NS	3.8 ± 1.0	2.5	33.4[f]
Baseline Exhaled CO (ppmv)	5.3 ± 6.4	8.7 ± 14.1[g]	2.2 ± 0.2	1.5	4 ± 1.7
Exhaled CO boost (ppmv)	51.7 ± 19.9	93.2[e]	20.6	38.2	31.5
CO fold increase from baseline	11x	12x	14x	26x	9x
Plasma nicotine boost (ng/mL)	12.2 ± 12.7	18.0	3.6	11.7	12.6
Heart Rate Boost (bpm)	13.9 ± 10.6	15.5	8.6	--	--
Total puffing volume (L)	41.7 ± 25.8	--	57.0 ± 45.6	--	79.1 ± 54.5[h]
Smoking Time (min)	31.6 ± 14.5	30 (required)	≥45 (truncated)	39	33 ± 13.1
Waterpipe type (commercial, RWP)	RWP	Commercial	Commercial	Commercial	Commercial
Heating method	Quick-light charcoal	Quick-light charcoal	Quick-light charcoal	Quick-light charcoal	Traditional lump charcoal
Hose material	Plastic	NS	Leather	NS	Leather
Ventilation (hr^{-1})	2.3, mechanical	NS, natural (open window)	NS, mechanical	NS	NS

Abbreviations: RWP = research-grade waterpipe, NS = not specified, WP = waterpipe, -- = data not collected.

[a] Active refers to 47 active waterpipe smokers; 15 passive smokers were also monitored but their data are not compared. Bentur L, Hellou E, Goldbart A, Pillar G, Monovich E, Salameh M, et al. Laboratory and clinical acute effects of active and passive indoor group water-pipe (narghile) smoking. *Chest*. 2014;145(4):803-9.

[b] Participant data for smoking nicotine-containing WP tobacco; placebo condition is not compared. Blank MD, Cobb CO, Kilgalen B, Austin J, Weaver MF, Shihadeh A, Eissenberg T. (2011). Acute effects of waterpipe tobacco smoking: a double-blind, placebo-control study. *Drug Alcohol Depen*. 2011;116:102-109.

[c] Jacob P, Raddaha AHA, Dempsey D, Havel C, Peng M, Yu L, Benowitz NL. Nicotine, carbon monoxide, and carcinogen exposure after a single use of a water pipe. *Cancer Epidem Biomar*, 2011;20:2345-2353.

[d] Maziak W, Rastam S, Shihadeh AL, Bazzi A, Ibrahim I, Zaatari GS, ... & Eissenberg T. Nicotine exposure in daily waterpipe smokers and its relation to puff topography. *Addict Behav*. 2011;36:397-399.

[e] Average age of active + passive smokers; average age of active smoker (only) not specified.

[f] Reported mean number of weekly waterpipe smoking episodes was 7.8±5.7; times per month was calculated by 7.8/7 * 30 days.

[g] Exhaled breath concentration estimated from carboxyhemoglobin in blood using regression equation:
Exhaled CO pm = (COHb % - 0.63) / 0.16; provided in Jarvis M, Belcher M, Vesey C, Hutchison D. Low cost carbon monoxide monitors in smoking assessment. *Thorax*. 1986;41:886.

[h] Reported in Maziak W, Rastam S, Ibrahim I, Ward KD, Shihadeh A, Eissenberg T. CO exposure, puff topography, and subjective effects in waterpipe tobacco smokers. *Nicotine Tob Res*, 2009;11:806-811.

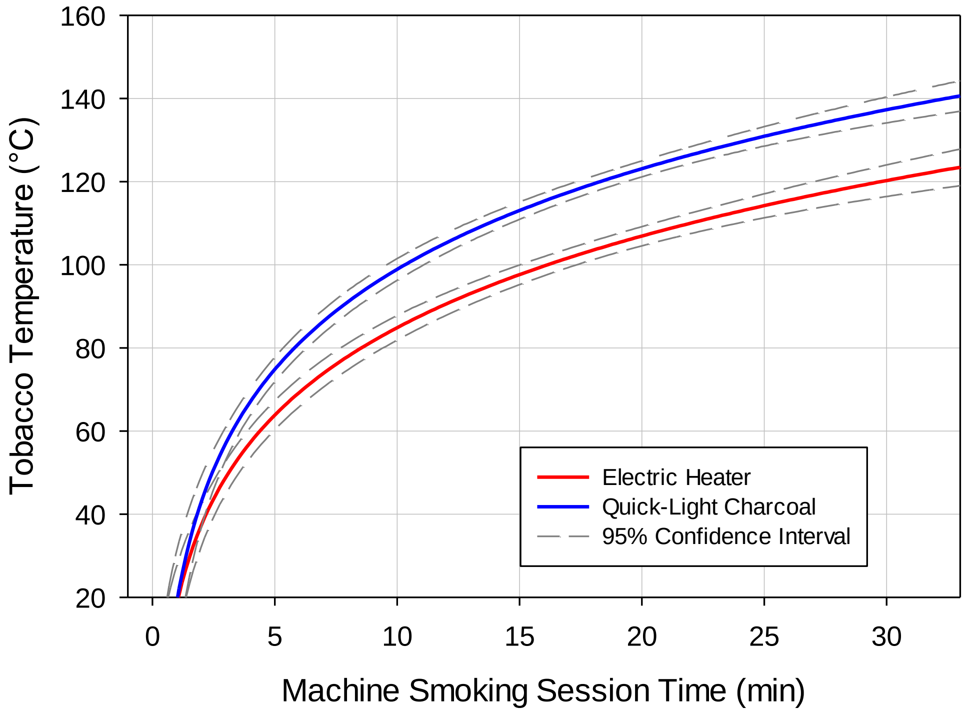

Figure S-1. Tobacco temperature curves fitted to averaged thermocouple data collected during machine smoking of the Research-Grade Waterpipe using electric and charcoal heating.

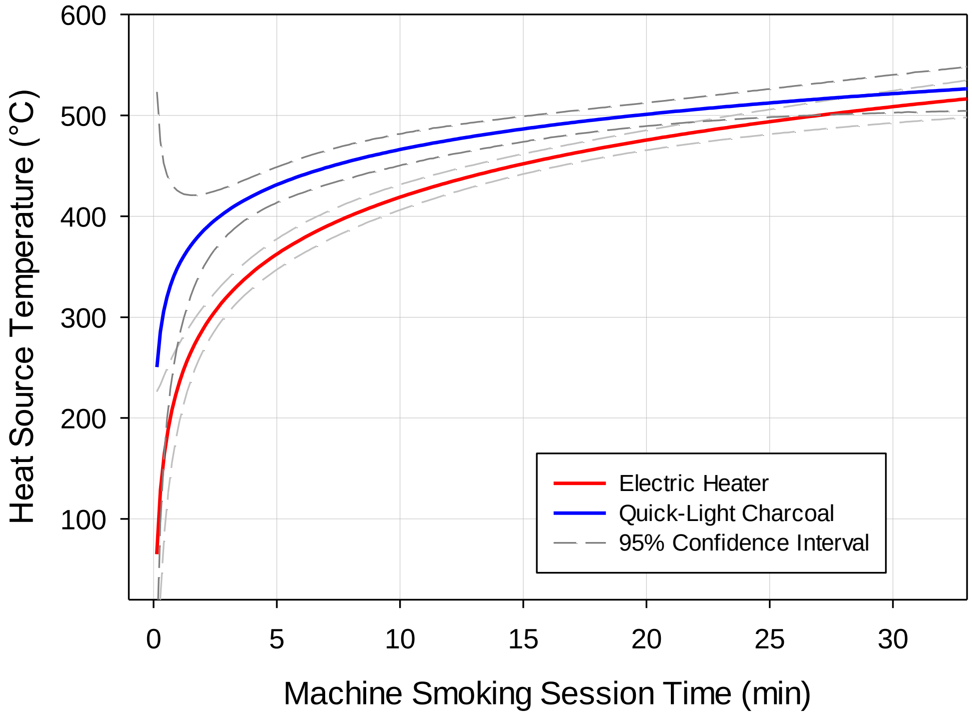

Figure S-2. Heat source temperature curves fitted to averaged thermocouple data collected during machine smoking of the Research-Grade Waterpipe using electric and charcoal heating.