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Brief Report
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Quantification of Flavorants and Nicotine in Waterpipe Tobacco and Mainstream Smoke and Comparison to E-cigarette Aerosol

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Abstract

Introduction: Waterpipe use remains popular among youth with the availability of flavored shisha tobacco being one of the main drivers of waterpipe use. Although waterpipe mainstream toxicant emissions are well understood, less is known about the carryover of flavorants such as vanillin, benzaldehyde, and eugenol. In this study, flavored waterpipe tobacco was analyzed for flavorants and nicotine, and subsequent carryover to mainstream smoke.

Methods: Flavorants vanillin, benzaldehyde, and eugenol, and nicotine were quantified in vanilla-, cherry-, and cinnamon-flavored shisha tobacco by gas chromatography/flame ionization detector and subsequently in waterpipe mainstream smoke generated by a smoking machine. The setup allowed for sampling before and after the water-filtration step.

Results: Flavorant and nicotine content in smoke was reduced 3- to 10-fold and 1.4- to 3.1-fold, respectively, due to water filtration. Per-puff content of filtered waterpipe mainstream smoke ranged from 13 to 46 µg/puff for nicotine and from 6 to 55 µg/puff for flavorants.

Conclusions: Although water filtration reduced flavor and nicotine content in waterpipe mainstream smoke, the detected flavorant concentrations were similar or higher to those previously reported in e-cigarette aerosol. Therefore, users could be drawn to waterpipes due to similar flavor appeal as popular e-cigarette products. Absolute nicotine content of waterpipe smoke was lower than in e-cigarette aerosol, but the differential use patterns of waterpipe (>100 puffs/session) and e-cigarette (mostly <10 puffs/session, multiple session throughout the day) probably result in higher flavorant and nicotine exposure during a waterpipe session. Strategies to reduce youth introduction and exposure to nicotine via waterpipe use may consider similar flavor restrictions as those for e-cigarettes.

Implications: Although waterpipe mainstream smoke is well characterized for toxicants content, little is known about carryover of molecules relevant for appeal and addiction: flavorants and nicotine. This study shows that flavorant content of waterpipe mainstream smoke is comparable or higher than e-cigarette aerosol flavorant content. Regulatory action to address tobacco use

behaviors targeting the availability of flavors should also include other tobacco products such as flavored shisha tobacco.

Introduction

Waterpipes are known by a variety of names including "huqqa"/"hookah" (Indian languages), "argileh"/"nargileh" (Arabic), and "shisha"/"sheesha" (Persian).[1] The most common type of waterpipe tobacco is "Moassel"/"Maassel"/"Mu'assel" or "shisha."[1] In this manuscript, the words "waterpipe" and "shisha tobacco" will be used for consistency. In brief, a waterpipe user draws ambient air past hot charcoal resting on top of the shisha tobacco in the ceramic head. Subsequently, the generated smoke passes through a water bath before reaching the user.[1]

Waterpipe use is popular among U.S. youth and young adults, with ~690 000 U.S. high and middle school students reporting past 30-day waterpipe use in 2019.[2] Common misbeliefs about waterpipe use include that it is safer and less addictive than cigarette use due to the water-filtration step,[3–5] which some users base on the "smoother" texture of the smoke compared with cigarettes and, interestingly, on the presence of flavors.[4] The availability of shisha tobacco in various flavors has further been reported as a main reason for waterpipe use among youth.[6,7] Although the content of tobacco- and charcoal-derived toxicants in waterpipe mainstream smoke is well characterized and includes carbon monoxide, volatile organic compounds, tobacco-specific nitrosamines, and heavy metals,[8–10] less is known about other components such as flavorants and nicotine and their delivery to mainstream smoke, which may contribute to the appeal and addiction potential of these products. The aims of this study were to (1) quantify selected flavorants and nicotine in mainstream waterpipe smoke to estimate user exposure and compare these concentrations to those in e-liquid-generated aerosol[11,12] for informing possible tobacco regulation and (2) determine the effect of water filtration on select flavorant and nicotine carryover by sampling before and after the water bath.

Materials and Methods

Materials and Chemicals

A 34" single-hose hookah (Khalil-Mamoon type, stem 70 cm) was purchased online. The central stem was cut 18 cm from the top, and a three-way valve (McMaster-Carr, Elmhurst, IL) was soldered onto the stem. Clear PVC tubing was used to connect the side port of the three-way valve to the inlet of the smoke trap. Using this setup, smoke could be trapped from the side port without water filtration ("unfiltered") or with water filtration ("filtered"). The distance from the head to the smoke trap inlet was ~150 and ~275 cm, respectively. Carbopol Ring Charcoals (9.5 g, 38 mm OD, central hole ID 8 mm) and three flavors of shisha tobacco were purchased online: cherry, vanilla (both from Al-Fakher Premium), and cinnamon (Romman Premium). Five-gram samples of shisha tobacco was extracted with 12 mL of methanol (LCMS grade, J.T.-Baker, Center Valley, PA) for 7 days, and the extracts were filtered and further diluted for gas chromatography analysis. (−)-Nicotine, vanillin, benzaldehyde, and eugenol standards (≥99%) were purchased from Sigma–Aldrich (St. Louis, MO).

Filtered and unfiltered experiments were carried out separately (N = 3 for vanilla and cherry, N = 1 for cinnamon). The water bowl was filled with 1.2 L of RO water (resulting stem immersion ~3.5 cm), and ~10 g of shisha tobacco was added to the glazed ceramic head and covered with aluminum foil. The aluminum foil was punctured radially from the center outward to a diameter of 40 mm using a Pasteur-pipette, thereby extending beyond the charcoal ring.

Smoke Capture

To trap hookah-generated smoke, an in-house-built smoking machine was used as described previously,[13] modified with a stronger pump. In brief, an Arduino board (Somerville, MA) was used to control power input to a DC rocking piston pump (GTEK Automation, Lake Forest, CA). The airflow was monitored by a flow meter (Sierra Instruments, Monterey, CA), and the generated hookah smoke was pulled through two cold-finger traps chilled with liquid nitrogen (Airgas, Radnor, PA).

The puffing topography was 2.85-second puffs at a flow rate of 11.2 L/min to yield a puff volume of 532 mL, with 17-second breaks between puffs, adapted from the Beirut protocol.[14] The total number of puffs was recorded (40–50 puffs), and the trapped smoke was allowed to thaw and taken up in methanol (J.T.-Baker).

Gas Chromatography

Gas chromatography/flame ionization detector (Shimadzu GC-2010+, Kyoto, Japan) was employed to quantify flavorants and nicotine. Details on the gas chromatography method are provided in Supplementary Material.

Results

Vanilla-flavored shisha contained approximately 5× the amount of principal flavorant than cherry- or cinnamon-flavored shisha (Table 1). Similarly, the flavorant contents of the waterpipe-generated smoke contained 5–9× (filtered) or 2–4× (unfiltered) the amount of vanillin per puff, compared with benzaldehyde and eugenol. Comparing filtered versus unfiltered smoke content, a "water filtration reduction factor" was determined, ranging from 3.8 to 10 for the flavorants and from 1.4 to 3.1 for nicotine (Table 1).

Discussion

This study compared flavorant and nicotine delivery from shisha tobacco to smoke using a commercial waterpipe. The flavorants vanillin, benzaldehyde, and eugenol, as well as nicotine were effectively carried over to mainstream smoke, thereby creating the respective characterizing smoke odor. The flavorants were specifically chosen to facilitate comparison to e-cigarette aerosol contents that have been previously reported.[11,12] In addition, vanillin and eugenol have been identified as popular additives to shisha tobacco in a recent study from India.[15]

Although vanillin, benzaldehyde, and eugenol are commonly found in foods and used in cosmetics and are "generally recognized as safe" (GRAS) by the U.S. FDA, the GRAS status does not apply to inhalational safety.[16–18] In fact, the effects of regularly inhaling flavorants such as vanillin, benzaldehyde, or eugenol into the lungs *via* tobacco products are unclear although U.S. regulations for occupational exposure exist for vanillin and

Table 1. Mean (SEM) of Quantified Compounds in Vanilla- (Van.), Cherry- (Ch.), and Cinnamon-Flavored (Cin.) Shisha, Water-Filtered Mainstream Smoke, and Unfiltered Mainstream Smoke

Compound	Shisha tobacco content; *N* = 5 (mg/g shisha)	In water-filtered mainstream smoke; *N* = 3 (µg/puff)	In unfiltered mainstream smoke; *N* = 3 (µg/puff)	"Water filtration reduction factor"
Vanillin (Van.)	4.9 (0.43)	55 (24)	209 (18)	3.8
Benzaldehyde (Ch.)	1.1 (0.11)	9.8 (0.86)	101 (5.8)	10
Eugenol (Cin.)	1.3 (0.18)[a]	6.1[b]	25[b]	4.1
Nicotine (Van.)	1.4 (0.43)	14 (5.7)	44 (3.7)	3.1
Nicotine (Ch.)	0.65 (0.14)	12 (2.1)	23 (3.8)	1.8
Nicotine (Cin.)	1.8 (0.12)[a]	43[b]	61[b]	1.4

[a]*N* = 2.
[b]*N* = 1.

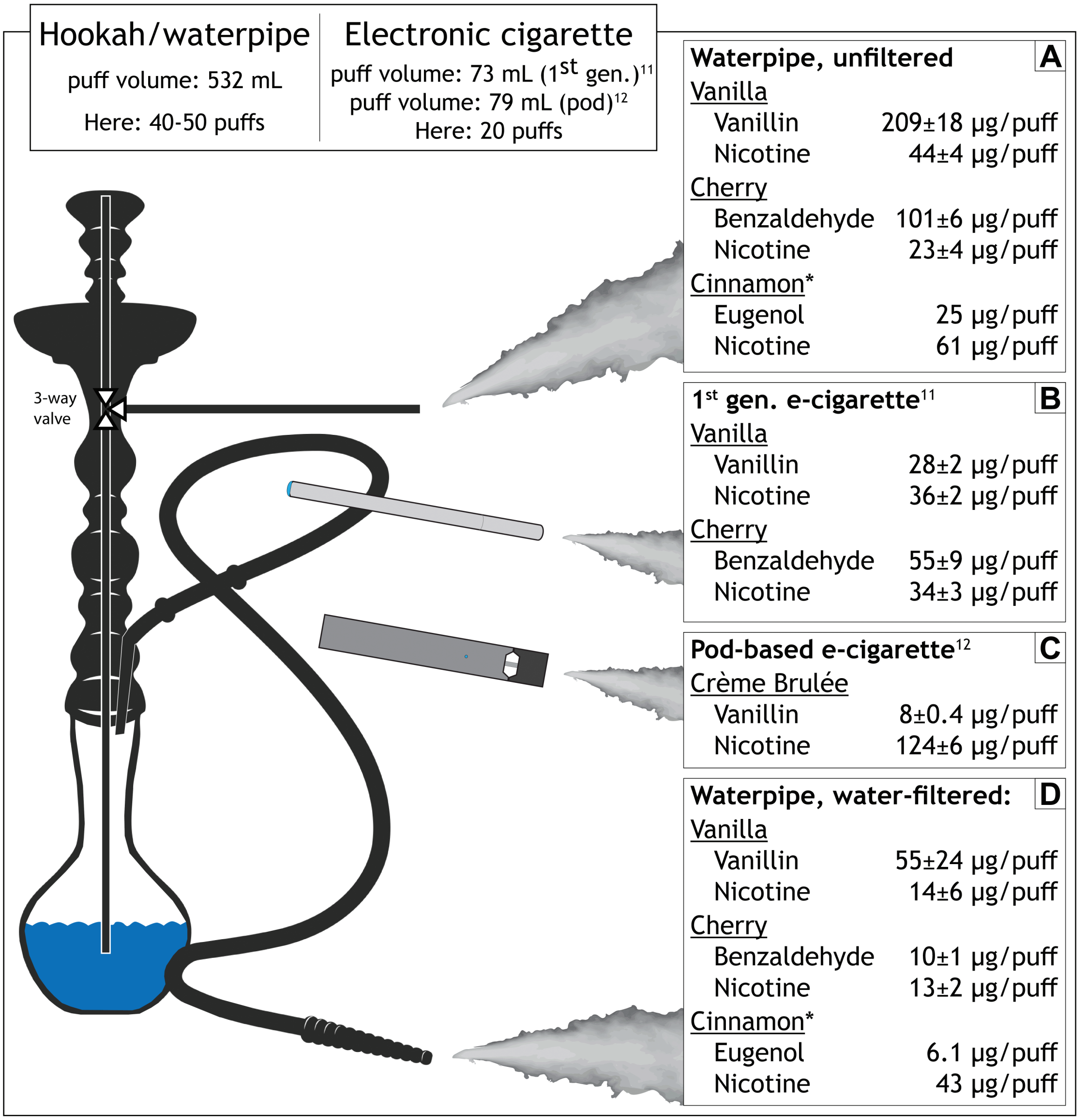

Figure 1. Mean ± SEM (*N* = 3) of flavorant (benzaldehyde, vanillin, eugenol) and nicotine per-puff content measured in mainstream waterpipe smoke pre-water (A) and post-water filtration (D), aerosol generated by a "cig-a-like" e-cigarette (B),[11] and a pod-based Juul e-cigarette (C).[12] *$N = 1$.

benzaldehyde.[12] Although benzaldehyde and vanillin are mild to moderate airway irritants, eugenol is known to be a potent skin sensitizer, and all three compounds have been linked to asthma and could therefore be specifically problematic for some populations, such as asthmatics.[11,16–18] Beyond toxicological questions, flavorants play other roles including increasing the attractiveness of waterpipe use and partially masking the aversive taste of nicotine, thereby facilitating nicotine inhalation.[6,19] As flavorants have recently come under scrutiny for their role in U.S. youth "vaping" uptake, per-puff flavorant contents of hookah smoke and e-cigarette aerosol generated by two different e-cigarettes (V2 cartridge with vanilla- or cherry-flavored e-liquid,[11] U.S. Juul "Crème Brulée"[12]) using the same smoke/aerosol trapping system were compared (Figure 1): The per-puff mainstream smoke content of benzaldehyde was lower than in aerosol generated with a first-generation "cig-a-like," yet vanillin content was higher than in aerosols from either e-cigarette (Figure 1B–D). This suggests that the flavor intensity of waterpipe smoke depends on the "flavor strength" of the shisha tobacco, but this study showed specific flavorants concentrations in waterpipe smoke to be on the same or one order of magnitude higher than in e-cigarette aerosol. This result supports earlier findings that one of the main reasons for young adult waterpipe use is the availability of flavors.[6] Further studies on the topic are warranted to test a wider range of shisha tobaccos and waterpipe setups as well as to better understand the inhalational toxicology of flavorants such vanillin and benzaldehyde.[18]

The differential nicotine contents of the tested shisha tobacco were not indicated on the packaging, nor were the terms "unwashed" or "washed," which are sometimes used to indicate "high" or "low" nicotine concentrations, respectively.[20] As a result, users cannot determine the nicotine strength of the tested shisha tobaccos. However, waterpipe smoke only contained ~10%–35% (vs. Juul) and ~40%–120% (V2) the amount of nicotine (Figure 1D), but ~700% (vs. Juul) and ~200% (vs. V2) the amount of vanillin (vanilla). Comparing only the vanillin-containing products (Figure 1B–D) and taking the average puff numbers into account (e.g., 171 puffs as per the Beirut protocol[14]), an e-cigarette user would need 19 puffs (Juul) or 67 puffs (V2) to match nicotine delivery, but 1176 puffs (Juul) or 336 puffs (V2) to match vanillin delivery. These results need to be considered in light of the differential ways waterpipes and e-cigarettes are used: waterpipes must be used in one session due to the setup, whereas e-cigarettes are generally used in smaller sessions throughout the day. As a result, waterpipe users are probably exposed to higher absolute amounts of flavorants and similar or higher amounts of nicotine during a single session, as most e-cigarette sessions only last for 2–10 puffs.[21] However, the question of youth exposure to nicotine facilitated by sweet-flavored waterpipe shisha is not attracting nearly the same amount of attention as flavored e-cigarettes despite the U.S. National Youth Tobacco Survey (NYTS) data showing relatively constant rates of 3%–5% of U.S. high school students reporting current waterpipe use (past 30 days) since 2011,[22] and the latest 2019 NYTS data showing approximately 690 000 U.S. middle and high school students currently use waterpipes (past 30-day use).[2]

This study allowed for a content comparison between unfiltered and water-filtered waterpipe-generated smoke (Table 1): smoke flavorant content was reduced ~4- to 10-fold in the filtered experiments and smoke nicotine content was reduced on a similar scale (~1.5–3) to previous reports (~2–6),[10] thereby validating the experimental setup. As a result, users could be exposed to significantly higher amounts of flavorants and nicotine when smoking a waterpipe that lacks the filtration step. Notably, although nicotine is completely miscible with water, vanillin (~10 g/L), benzaldehyde (~7 g/L), and eugenol (~2.5 g/L) have limited water solubilities,[23] suggesting that water solubility probably only plays a minor role for determining the "efficiency" of the filtration step (e.g., the amount of flavorant and nicotine removed due to the filtration step). Other criteria have to be considered, such as shisha tobacco composition, user puffing profile, and the waterpipe build, which influence parameters such as path length and absorption along its path, water bath immersion-depth, bubble size and resulting mass transfer across the smoke-liquid interface, as well as smoke pH.[20] Some reports suggest that users are adding ice and/or alcoholic beverages to the water bath to further modify the flavor and inhalation experience,[24] thereby further complicating the question of filtration. Additional studies are warranted in this area to better understand the impact of (1) the various user-controlled parameters and (2) the nature and concentration of flavorants present in shisha tobacco on the filtration step.

As U.S. legislators consider how to effectively regulate flavors and flavorants in e-cigarettes to render these less attractive to youth and thereby reduce youth exposure to nicotine, other flavored tobacco products such as shisha tobacco and cigarillos should also be included in the strategy.[25] Messaging strategies should be targeted specifically to the demographic drawn to sweet and flavored shisha tobacco (i.e., youth and young adults[4,26,27]) and one approach could be to include equivalencies of toxicant, flavorant, and nicotine contents of other flavored tobacco products such as e-cigarettes or little cigars, while simultaneously addressing the lack of nicotine content labeling on shisha tobacco packaging.

Supplementary Material

A Contributorship Form detailing each author's specific involvement with this content, as well as any supplementary data, is available online at https://academic.oup.com/ntr.

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Contributors

H.C.E. and J.B.Z. designed the study with input from T.M.d.W. and M.M.F. H.C.E., D.S.G.T., and J.G.W. collected the data. H.C.E. analyzed and visualized the data and drafted the manuscript with input from P.T.A. and J.B.Z. All authors critically reviewed, edited, and approved the final manuscript. J.B.Z. is the guarantor of the paper. J.B.Z. attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Declaration of Interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author). H.C.E., D.S.G.T., J.G.W., T.M.d.W., M.M.F., P.T.A., and J.B.Z. declare no competing interest. Unrelated to the current study, over the past 3 years SSO reports: having been a consultant or an advisory board member for Alkermes, Amygdala, Indivior, Mitsubishi Tanabe, and Opiant; a NIDA Clinical Trials Network DSMB member with honorarium from the Emmes Corporation; having received donated study medications from Astra-Zeneca, Pfizer, and Novartis; and being a member of the American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative (ACTIVE Group) supported in the past 3 years by Alkermes, Amygdala Neurosciences, Arbor Pharmceuticals, Dicerna, Ethypharm, Indivior, Lundbeck, Mitsubishi, and Otsuka. Unrelated to the current study, over the past 3 years SKS reports receiving donated study medications from Novartis and Astra-Zeneca.

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