Waterpipe tobacco smoke toxicity: the impact of waterpipe size

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ABSTRACT

Introduction Waterpipe tobacco smoking continues to show increasing popularity, especially among individuals between 18 and 22 years old. Waterpipe tobacco smoke (WTS) is a mixture of particulates and gases formed from the combustion of the charcoal and volatilisation and humidification of the tobacco+humectant+flavouring substrate known as shisha or mu'assel. As such, variation in the configuration of the waterpipe may affect the particles produced. Our study focuses on the effects of waterpipe size on the physical properties and cytotoxicity of the smoke produced.

Methods Shisha type and headspace volume were held constant and a modified Beirut puff protocol was followed while the size of the waterpipe was varied. Particle concentrations and size distributions were measured using a TSI Engine Exhaust Particle Sizer. Type II alveolar cells were exposed to smoke at the air-liquid interface and two metrics of cell health analysed.

Results In a 30 min session, we observed a decrease in total particle concentration (10^{14}–10^{13}) and mass (10 000–2800 mg/m^3) and an increase in particle size (125–170 nm) as pipe height increases from 22 to 55 cm and bowl size from 300 to 1250 mL. Smoke from all pipe sizes caused decreases in lysosomal function (>40%) and membrane integrity (>60%) 24 hours post 57 min exposure, and meet the National Institutes of Health definition of a cytotoxic agent (≥30% decrease in cell viability).

Conclusion Smoke from waterpipes of all sizes causes significant alveolar cellular harm, indicating that this device needs regulation as a hazard to human health.

INTRODUCTION

Waterpipe smoking has ancient origins but is an increasingly popular social activity in the USA, especially among 18 to 22 year olds.[1] Unlike cigarette smoke, waterpipe tobacco smoke (WTS) is generated by charcoal-heating a mixture of tobacco and flavourants and drawing the smoke through a bowl of liquid prior to inhalation. Even though waterpipe smoking has been classified as a 'burgeoning global epidemic' because it contributes to developing cardiovascular disease and chronic obstructive pulmonary disease and increases heart rate, systolic and diastolic blood pressure, respiratory rate and carbon monoxide levels, the public holds widespread misperceptions about the negative health effects of WTS.[2–4] Regulation around the purchase and use of waterpipes remains limited with a 2015 review of tobacco control legislation in 62 countries finding that most lack regulation specific to WTS and only one specified health warnings on waterpipes.[5] Significant hurdles to addressing limitations in current regulation include the proliferation of producers, importers and manufacturers of waterpipe components, exemptions and inconsistencies in current policy and gaps in our understanding of the unique features of WTS.[5] Our work advances current understanding by reporting connections between a waterpipe's size and the physical properties and cellular harm caused by the smoke produced.

Research investigating the health impacts of combustion particulate and nanoparticles converges to indicate that exposure to smaller particles increases damage and toxicity, even if the particles have the same chemical composition.[5 6] WTS is, fundamentally, an aerosol with particles suspended in a gas. To date, studies have focused more on overlap between the chemical composition of WTS particulate and cigarette tobacco smoke particulate than on the physical properties of WTS particulate. WTS research can draw on studies of atmospheric particulates for both experimental direction and potential regulation. Aerosol properties depend on the configuration of the apparatus generating them. Therefore, it seems logical, but is previously untested, that the dimensions of the waterpipe itself will alter the WTS properties and some waterpipe sizes may produce more of the harmful, smaller particles.

To reduce public exposure to smaller particles, regulation of atmospheric particulate (ie, outdoor air quality) has focused on the mass of particulate with diameters below 2.5 µm ($PM_{2.5}$) and set 24 hours mean exposure guidelines to 25 µg/m^3.[7] Waterpipe cafes are venues where smoking affects indoor air quality and have been shown to expose patrons and workers to $PM_{2.5}$ at 374 µg/m^3, levels that are significantly above the WHO guidelines and approximately three times higher than levels measured in facilities where cigarette smoking was occurring.[8 9] However, these $PM_{2.5}$ concentrations represent environmental tobacco smoke (ETS), not the concentration of $PM_{2.5}$ that a waterpipe user inhales. Given that aerosols will likely agglomerate as they pass through the humidified lung environment and age, the initially inhaled WTS is likely to contain higher concentrations of ultrafine particles (UFPs) than are found in ETS.

With diameter ≤0.1 µm, UFPs have a higher potential to cause harm because they are more likely to be deposited within alveolae, the deep compartments of the lung.[10] Although UFPs are deposited in alveolae and these sacks play critical roles in gas

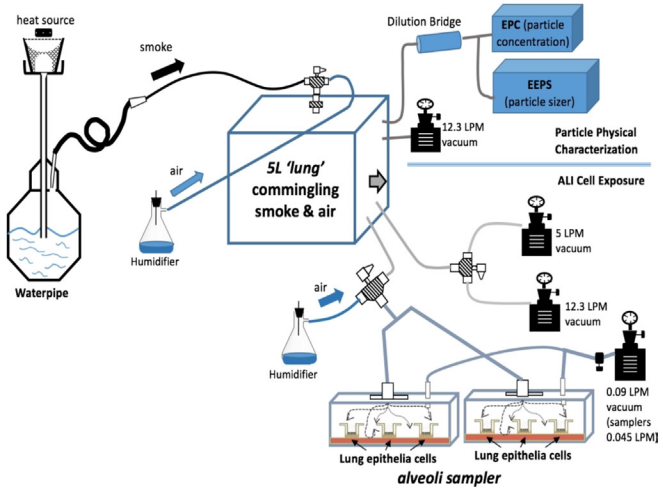

Figure 1 Experimental apparatus.[18] ALI, air-liquid interface. EEPS, Engine Exhaust Particle Sizer. EPC, Environmental Particle Counter.

exchange, the effect of WTS on alveolar cells is understudied. Particles would damage alveolar cells through a combination of physically impacting cells, covering the surface and reacting with cell membrane components. Per unit mass, smaller particles can penetrate more deeply into the lung and have more surface area available for particle–cell interactions, making the lung-deposited surface area (LDSA) an important metric for predicting relative toxicity.[11–13] By similar reasoning, the harm from smoking the same waterpipe tobacco product could, therefore, be increased if the individual is using a waterpipe apparatus that generates higher numbers of UFPs.

The US 2009 Family Smoking Prevention and Tobacco Control Act requires evidence-based product warning labels and provides the Food and Drug Administration (FDA) jurisdiction over regulating waterpipe design features that impact the toxicity of PM.[14] The type of charcoal and shisha used, pipe height, filtration media volume and the material and length of the hose used to inhale the smoke may affect smoke's physical characteristics of size, concentration, mass and LDSA. Thus, clear evidence of the impact of waterpipe size on the size and concentration of PM and WTS toxicity is required to allow meaningful regulation for protecting public health. In this work, we focus on the effect of the size of the waterpipe on the physical properties and cytotoxicity of the smoke produced.

METHODS

Smoking session

Based on commonly marketed styles, waterpipes with three heights, small (22 cm), medium (36 cm) and large (55 cm), were selected. During a session, the bowl was filled with dH_2O, maintaining 100 mL in head space to provide a constant flow resistance, and the waterpipe was assembled according to manufacturer instructions. As previous work has shown varying infiltration and leakage rates as a function of hose type, Tygon 2375 (ID ½", 153 cm length) was used with all pipes.[15 16] The head was prepared with 10 g Starbuzz Exotic Apple Americano shisha (Hookah-Shisha.com) and wrapped with aluminium foil containing 18 holes.[17] To begin a session, a lit, ash covered 35 mm Starbuzz Coconut Shell Instant Light was placed on top of the foil layer.

Characterising the physical properties of WTS particles

Additional experimental details can be found in Bernd *et al*.[18] Briefly, smoke was drawn into a 5 L Plexiglass box, following a puff pattern variant of the commonly used Beirut method, before sampling by a TSI Engine Exhaust Particle Sizer (EEPS, Model 3090, TSI, Shoreview, Minnesota, USA) over a 30 min session (figure 1).[19] An adjustable, calibrated dilution bridge containing three filters (HEPA Capsule Filter #1602051, TSI, Stillwater, Minnesota, USA) in series and filtered nitrogen was placed in line between the 5 L 'lung' and the instruments to maintain concentrations below instrument saturation. The 3 s concentration maxima corresponding to the 3 s hookah smoke puffs were identified throughout each trial, added to produce a total puff concentration for each bin size during the session and multiplied by the appropriate dilution factor (online supplementary information 1). T-tests were used to evaluate differences in particle concentrations and sizes. $P \leq 0.05$ was considered significant.

Cell maintenance and exposure

Rat alveolar type II (L2) cells (ATCC#: CCL-149) were maintained and exposed as per Bernd *et al*.[20] Briefly, air-liquid interface (ALI) cultures included 1×10^6 cells seeded apically in 12-well Corning Transwell plates with 1.5 mL basolateral low glucose media (LGM: (1 g/L) Dulbecco's Modified Eagle's Media, 10% Fetal Bovine Serum, 1× Antibiotic-Antimycotic (Gibco #15240062)). Mock-exposed and exposed conditions were processed in parallel for each treatment condition. Mock-exposed cells remained in 37°C, 5% CO_2 with 1 mL basolateral LGM and exposed cells were placed in the alveoli sampler with ALI maintained (figure 1).[20] Exposed cells were subjected to ambient air (non-smoking control) or smoke plus air drawn from the 5 L aluminium 'lung' immediately after each puff; 0.045 L/min humidified ambient air was drawn into the alveoli sampler during the intervening 17 s intervals. After a session, filters were returned to 37°C, 5% CO_2 for 24 hours with ALI maintained. Each exposure included six technical replicates. Data from three biological replicates per exposure condition are presented. Ambient air drawn through a medium pipe was defined as the 'non-smoking' referent condition.

Measuring cellular health and viability metrics

Membrane and lysosomal integrity were monitored via cleavage of fluorescein diacetate by cytoplasmic esterases and neutral red dye uptake (NRU), respectively.[20] Values were background adjusted and normalised to mock-exposed samples within that exposure. One-way Analysis of Variance and Tukey hsd post hoc testing were performed to reveal differences between treatments. T-tests were used to determine whether a treatment conditions' damage satisfied the ISO/National Institutes of Health (NIH) standard for cytotoxicity (≤70% of viability seen in non-smoking exposure). Statistical analyses were performed in RStudio statistical software. $P \leq 0.05$ was considered significant.

RESULTS

As the size of the waterpipe decreased from 55 to 22 cm, there was a significant increase in the total number concentration of particles generated in a smoking session (large: 1.7×10^{13}, medium: 6.0×10^{13}, small: 1.3×10^{14}, $p \leq 0.03$) and a significant decrease in the mean particle diameter (large: 170 nm, medium and small: 125 nm, $p \leq 0.01$) (figure 2A). The corresponding mass increased from 2800 to 10 000 mg/m^3 (figure 2B) and the LDSA from 7.7×10^6 to 3.6×10^7 µm^2/cm^3 (figure 2C).

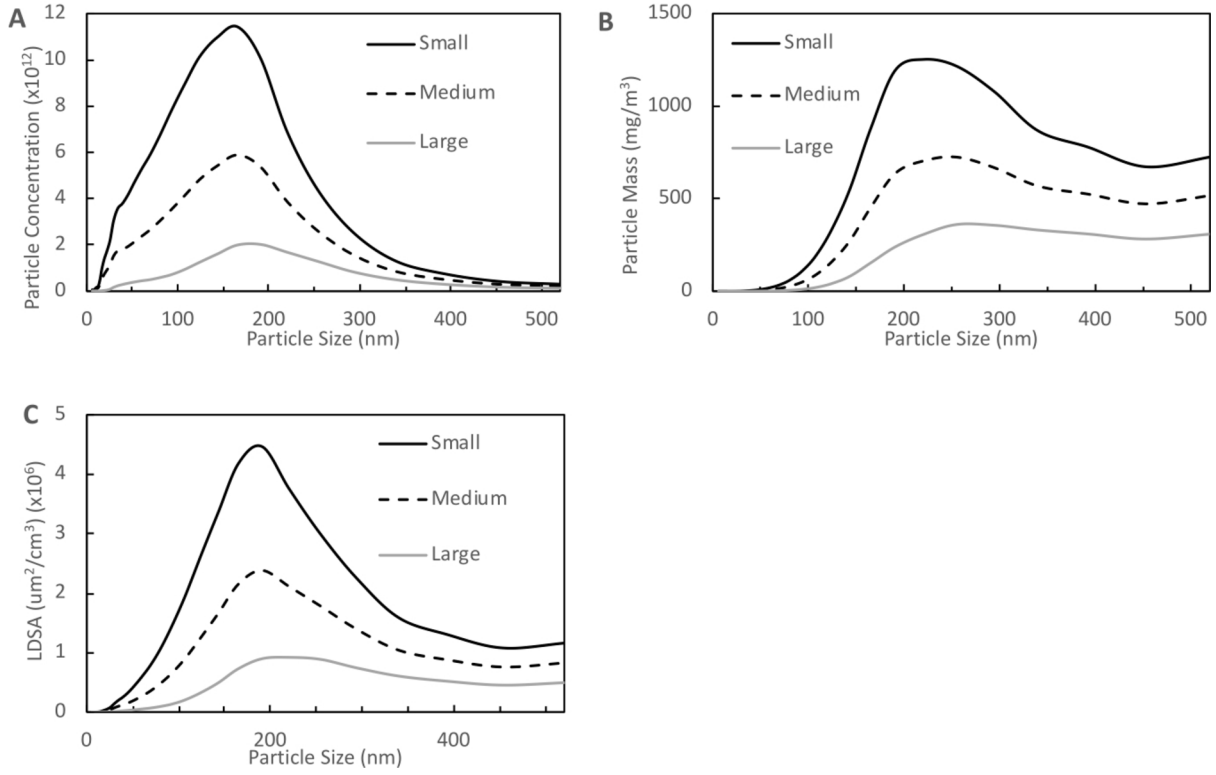

Figure 2 Effect of waterpipe size on (A) total particle size distribution, (B) mass distribution and (C) LDSA of waterpipe tobacco smoke measured during a 30 min smoking session. LDSA, lung-deposited surface area.

WTS from all size pipes significantly decreased both metrics of cell survival tested (figure 3A lysosomal function, p≤0.01; figure 3B cytoplasmic esterase, p≤0.002). Exposure to smoke from the small and medium pipes decreased lysosomal function to 40% and 35% of non-smoking control treatment and smoke from all pipes decreased cytoplasmic esterase activity to less than 40% of non-smoking treatment. Thus, smoke from all pipes fulfils the ISO/NIH definition of cytotoxic substances, decreasing cell viability metrics below 70% of non-smoking controls (puffed ambient air).

DISCUSSION

WTS is formed as heat from burning charcoal is drawn through shisha, volatilizing components from the syrup and the tobacco. These semivolatile constituents condense as they leave the head and enter the cooler region of the pipe stem, forming particles that are inhaled by the user. When considering appropriate policies to reduce public harm, waterpipe smoking includes dynamic aspects that cannot be regulated, like individual variations in puff topography, and more static aspects that can come under regulatory purview, like waterpipe size. Compared with cigarettes that are produced in sizes that vary by millimetres, waterpipes can vary by 20 or more centimetres in height and hundreds of millilitres in bowl volume. While multiple research groups are investigating the relationship between head components and the range of toxicants produced, this work characterises the effect that the size of the waterpipe apparatus has on WTS particle profile and toxicity.

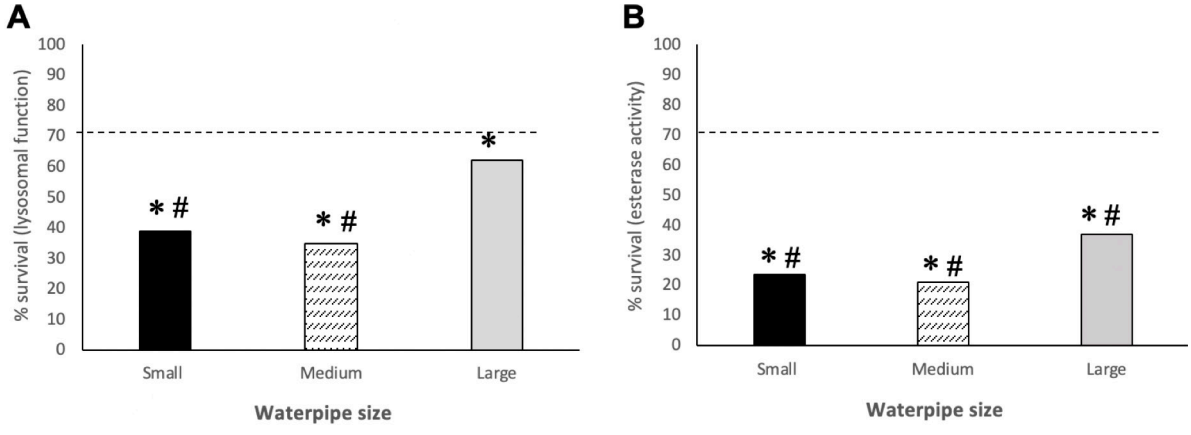

Figure 3 Effect of waterpipe size on waterpipe tobacco smoke cytotoxicity as measured by changes in alveolar cell (A) % lysosomal function (NRU) and (B) % membrane integrity (esterase activity). NIH cytotoxic threshold=dashed line. *p≤0.01 versus non-smoking; #p≤0.05 versus NIH cytotoxic threshold. NIH, National Institutes of Health; NRU, neutral red dye uptake.

Exposing alveolar cells to commingled whole smoke at the ALI using commercially available waterpipes with heights ranging from 22 to 55 cm, we found that whole smoke from all size waterpipes decreased cell health to levels that deem the mixtures cytotoxic. WTS generated by the large pipe meets the criterion of a cytotoxic agent by one measure (membrane integrity), while WTS generated by medium and small pipes are cytotoxic by two (lysosomal function and membrane integrity). The fact that the two metrics reported differentiate between relative levels of harm underscores the importance of including multiple cellular health metrics within a study, as using only a large pipe and the NIH-recommended NRU assay may have resulted in different conclusions.[21]

Analysis of the physical properties of smoke provides insight into the pipes' different toxicity profiles. We found that although pipes of different sizes consumed equivalent amounts of shisha during a smoking session, smoke from the large pipe showed lower particle mass and number concentration and larger mean particle diameter than the smoke generated by the medium and small pipes (figure 2). Stem length and insertion depth may play a role in our observed differences in particle number and size between pipes. The lower number of smaller particles emitted by the large pipe, for example, could be due to the longer pipe stem providing the particles with increased time to evaporate and agglomerate prior to reaching the water in the bowl. Additionally, the longer pathlength through the bowl water in the large and medium pipes relative to the small pipe may result in an increased filtration of particles similar to the role of impingers used in aerosol sampling.[22–24] However, the medium pipe had a greater insertion depth and still resulted in a higher particle concentration relative to the large pipe. Therefore, in this system, insertion depth is not independent of pipe size. The observed decrease in the mass of the particulate measured is similarly due to the combination of evaporation and filtration within the pipe during the smoking session. Although increasing the smoke's pathlength through the water significantly decreases the total particle concentration that the cells were exposed to, the public perception of waterpipe as a safer alternative to cigarette smoking is incorrect. Liquid filtration does not result in a mixture that can be considered 'safe' to inhale.

In addition to a higher total particulate mass, the smoke generated by the small pipe contains almost 10 times more particles, with a smaller mean diameter than smoke from the large pipe. Application of LDSA modelling predicts that WTS particles generated from any size waterpipe would travel past the upper respiratory defences and accumulate in lung alveolae.[10] With 30%–50% of its particles falling in the UFP range, WTS' LDSA values are predicted to be three orders of magnitude greater than those measured for a traditional cigarette by Geiss *et al*.[11] According to the LDSA model, 30% of the particles generated by the small pipe would accumulate within alveolae in comparison with 22% generated by the large pipe. Furthermore, the total mass dose per waterpipe smoking session (2.8×10^{6}–1.0×10^{7} µg/m^3) exceeds current WHO air quality guidelines (25 µg/m^3) 24 hours mean exposure.[7] The physical properties of the particles support the hypothesis that the size of the waterpipe impacts the particle dose.

Based on the data from the physical property studies, the estimated particle dose in the alveolae sampler for a session is $2.0\times10^{13}-1.5\times10^{14}$. However, the fraction of particles cleared from the 'lung' is higher in the physical properties studies than in the exposure studies where the 'lung' did not purge between puffs and particles accumulate during a 57 min smoking session. Therefore, the actual particle dose delivered to cells during a session using any size waterpipe is likely be greater than the estimates provided above.

This study combines analysis of the physical properties of WTS with measurement of the impact of whole smoke exposure at the ALI on alveolar cells. We found that as waterpipe size decreases, the particulate number concentration and mass increase and the mean particle diameter decreases. The importance of using multiple metrics in evaluating toxicity is underscored by data showing that smoke generated by medium or small pipes decreased lysosomal function but smoke generated by all pipes decreased membrane integrity. Our results support the conclusions that generating smoke using different sized commercial waterpipes impacts smoke dose and cellular health outcomes. WTS from all pipe sizes tested damaged lung cells. Therefore, the manufacture and sale of waterpipes as a device to smoke tobacco should be regulated as a risk factor to human health.

What this paper adds

What is already known on this subject

- Waterpipe smoking has increased in popularity worldwide, with social aspects and misperceptions regarding health risks feeding the trend among young adults.

What important gaps in knowledge exist on this topic

- The practice of waterpipe smoking can use waterpipes that vary from under 25 cm to over 100 cm. Gaps in our understanding of the impact of waterpipe configurations on the toxicity of the smoke hinder effective regulation of the industry and public education. The generalisability of data obtained for a given waterpipe configuration has not been explored.

What this paper adds

- Systematic characterisation of the physical properties of waterpipe tobacco smoke (WTS).
- Waterpipe size impacts the physical properties of the particles generated in the WTS.
- WTS from all size pipes tested is toxic.
- WTS from all size pipes tested is significantly higher in concentration than outdoor air exposure regulations and exceed measurements found in polluted environments.

Contributors CDFH and KB are the guarantors. CDFH and KB conceived the experimental design and drafted the manuscript. RM and CDFH performed the physical characteristic studies, data analysis and figure preparation. HS, JR, SC, EU and KB performed the cell exposure studies, data analysis and figure preparation.

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