



Waterpipe tobacco smoke: Characterization of toxicants and exposure biomarkers in a cross-sectional study of waterpipe employees



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ABSTRACT

Introduction: Few studies have comprehensively characterized toxic chemicals related to waterpipe use and secondhand waterpipe exposure. This cross-sectional study investigated biomarkers of toxicants associated with waterpipe use and passive waterpipe exposure among employees at waterpipe venues.

Method: We collected urine specimens from employees in waterpipe venues from Istanbul, Turkey and Moscow, Russia, and identified waterpipe and cigarette smoking status based on self-report. The final sample included 110 employees. Biomarkers of exposure to sixty chemicals (metals, volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), nicotine, and heterocyclic aromatic amines (HCAAs)) were quantified in the participants' urine.

Results: Participants who reported using waterpipe had higher urinary manganese (geometric mean ratio (GMR): 2.42, 95% confidence interval (CI): 1.16, 5.07) than never/former waterpipe or cigarette smokers. Being exposed to more hours of secondhand smoke from waterpipes was associated with higher concentrations of cobalt (GMR: 1.38, 95% CI: 1.10, 1.75). Participants involved in lighting waterpipes had higher urinary cobalt (GMR: 1.43, 95% CI: 1.10, 1.86), cesium (GMR: 1.21, 95% CI: 1.00, 1.48), molybdenum (GMR: 1.45, 95% CI: 1.08, 1.93), 1-hydroxypyrene (GMR: 1.36, 95% CI: 1.03, 1.80), and several VOC metabolites.

Conclusion: Waterpipe tobacco users and nonsmoking employees of waterpipe venues had higher urinary concentrations of several toxic metals including manganese and cobalt as well as of VOCs, in a distinct signature compared to cigarette smoke. Employees involved in lighting waterpipes may have higher exposure to multiple toxic chemicals compared to other employees.

1. Introduction

Waterpipes (also known as hookahs, narghile, shisha) have been used to smoke tobacco in the Eastern Mediterranean region and parts of Asia and Africa for centuries (World Health Organization, 2005). In the US and other western countries, the popularity of waterpipe smoking

has surged dramatically in recent years particularly among youth (Maziak et al., 2015). The prevalence of waterpipe use among high school students nearly doubled between 2011 and 2014 (Jamal et al., 2017). This increase in prevalence has been related to the perception that waterpipe smoking is less harmful than cigarettes, social culture of waterpipe use, and aggressive marketing (Maziak et al., 2015). Most

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indoor smoke-free policies are exempt or are not enforced in waterpipe venues (Jawad et al., 2015). Although limited relative to cigarettes, studies indicate that waterpipe smoking is associated with acute and chronic health effects similar to cigarette smoking (El-Zaatari et al., 2015). For instance, acute exposure to waterpipe tobacco smoke induces short-term increases in systolic blood pressure and heart rate in humans (Azar et al., 2016). In mice, waterpipe tobacco smoke induces changes in oxidative and inflammatory markers in the lungs (Khabour et al., 2012).

Toxicants in waterpipe smoke originate both from the charcoal and tobacco used in the waterpipe (Jukema et al., 2014). Most studies examining waterpipe secondhand smoke (SHS) constituents have measured markers of indoor air quality (Moon et al., 2015, Al Mulla et al., 2015, Fiala et al., 2012). In a chamber experiment study, the sidestream smoke of a single waterpipe session had four times higher carcinogenic polycyclic aromatic hydrocarbons (PAHs), four times higher volatile organic compounds (VOCs) and 30 times higher carbon monoxide (CO) than a single cigarette (Daher et al., 2010). A case control study reported that, relative to never-smokers ($n = 40$), concentrations of cadmium, lead, and zinc were higher in blood and saliva samples of waterpipe users ($n = 88$) (Khabour et al., 2018). Only a small number of cross-sectional studies have measured biomarkers of exposure among non-smokers (Kumar et al., 2015). In a human subject study measuring VOC metabolites in urine samples of waterpipe users in hookah bars from San Francisco, USA ($n = 55$), 2-cyanoethylmercapturic acid, a metabolite of acrylonitrile, increased 71% and phenylmercapturic acid, a benzene metabolite, increased 91% in urine samples of waterpipe users immediately after a single session of waterpipe smoking (Helen et al., 2014).

No comprehensive studies have determined the chemical profile of waterpipe tobacco smoke in humans compared to cigarette tobacco smoke. In a cross-sectional study in Turkey, Russia and Egypt, we collected urine specimens from workers in waterpipe venues, as part of a multi-site study to characterize exposure to waterpipe smoke exposure (Moon et al., 2018). In an environmental assessment, we found relatively high concentrations of particulate matter (PM_{2.5}), CO, particle bound PAHs, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and nicotine, indicating the presence of relatively high SHS concentrations that could affect the health of venue employees and customers (Moon et al., 2015). In a limited biomarker assessment, we found that nonsmoking employees of waterpipe tobacco venues in Istanbul, Moscow, and Cairo had relatively high concentrations of SHS biomarkers, including two biomarkers of smoke carcinogens (urine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and urine 1-hydroxypyrene glucuronide (1-OHPG)) (Moon et al., 2018).

For the current study, we have comprehensively characterized internal dose and metabolic biomarkers of active and secondhand waterpipe use and exposure, as determined in urine. A total of 64 chemicals were measured, including 13 metals, 25 VOCs, 9 nicotine metabolites, 7 PAHs and 10 heterocyclic aromatic amines (HCAA) many of which are toxic and carcinogenic (International Agency for Research on Cancer, 2004). The study included never-smokers, cigarette smokers, waterpipe smokers, and dual users of both cigarettes and waterpipes, and obtained information on direct waterpipe and cigarette use as well as on secondhand exposure to waterpipe and cigarette smoke. This information thus enabled us to dissect the effects of passive vs. active waterpipe smoking as well as the combined effects of waterpipe and cigarette use.

2. Methods

2.1. Study population

We conducted a study of waterpipe tobacco venues and their employees in Istanbul, Turkey, Moscow, Russia, and Cairo, Egypt (Moon et al., 2015). The three countries were selected based on high waterpipe

consumption data from the Global Adult Tobacco Survey (Morton et al., 2014). Due to administrative hurdles to transport the urine samples from the Cairo participants to the United States for analyses, the current study is restricted to Istanbul and Moscow. Within each city, we identified neighborhoods with a high concentration of waterpipe tobacco venues. Although we initially planned a stratified random sample strategy in each city, we switched to a convenience sample due to a low venue response rate. To be eligible to participate, venue owners/managers had to provide oral informed consent to conduct air sampling in the venue and at least one nonsmoking adult employee (≥ 18 years of age) had to provide oral informed consent and be willing to provide urine samples. A total of 26 venues (9 in Istanbul and 17 in Moscow) participated (venue response rates were 32% in Istanbul and 34% in Moscow). Data were collected between January and May 2013 in Istanbul and December 2013 to May 2014 in Moscow. Field staff fluent in the local language conducted all communications with venues and participants. Johns Hopkins Bloomberg School of Public Health Institutional Review Board and the ethics committees at the local co-investigators' institutions approved the study protocol.

2.2. Questionnaire data collection

A total of 176 employees (mean six per venue) participated (96% response rate in Istanbul and 95% in Moscow). The questionnaire was administered by the field staffs using face-to-face interview method. Eleven employees did not provide urine samples during the time of the fieldwork. Urine samples were originally collected from 165 participants (94%) and used for an initial biomarker study. For this study, we used urine from 110 participants from 25 venues (9 from Istanbul and 16 from Moscow). The selection of this subset from the original urine samples was based solely on whether adequate volume (3 mL) of urine remained for conducting all the comprehensive laboratory analyses. At each venue, field staff administered a questionnaire to venue employees to assess information on sociodemographic and occupational factors, smoking status, exposure to SHS at work, home, and other places, and involvement with lighting waterpipes at the venue.

We categorized participant smoking status using data on self-reported tobacco use such as never/former smokers, cigarette only users, waterpipe only users, and dual users in order to distinguish the source of chemicals in the urine samples of the tobacco users. Current smokers reported smoking cigarettes or waterpipe within the past three months either "daily", "less than daily", or "just a few puffs". These definitions were adapted from WHO guidelines by adding the question on smoking "just a few puffs" from another study of bar and nightclub employees (Jones et al., 2013). The reason for adding the "just a few puffs" to the questionnaire was to identify all current smokers and appropriately identify them from workers who were truly exclusively exposed to secondhand smoke. In a work environment where smoking is so common as in a waterpipe venue, employees could be smoking occasionally without a full perception that they are current smokers. As a goal was to distinguish between secondhand smoke exposure and active smoking it was important to make sure that even occasional smokers were categorized as smokers rather than as non-smokers. Never-smokers must have either never tried any kind of tobacco, or have smoked fewer than 100 cigarettes and smoked waterpipe for no more than one 20-minute session in their lifetime. Former smokers reported past tobacco use but did not report smoking cigarettes, waterpipes, or other types of tobacco within the past three months. The frequency of use of cigarette and waterpipe are presented in the supplement Table 1.

2.3. Urine sample collection

The urine samples (~50 ml first morning void or spot urine sample, whichever was possible) were collected in the morning in the privacy of the workers' home. Each worker was provided with a set of instructions to collect the urine, as well as the sterile urine cup and an opaque

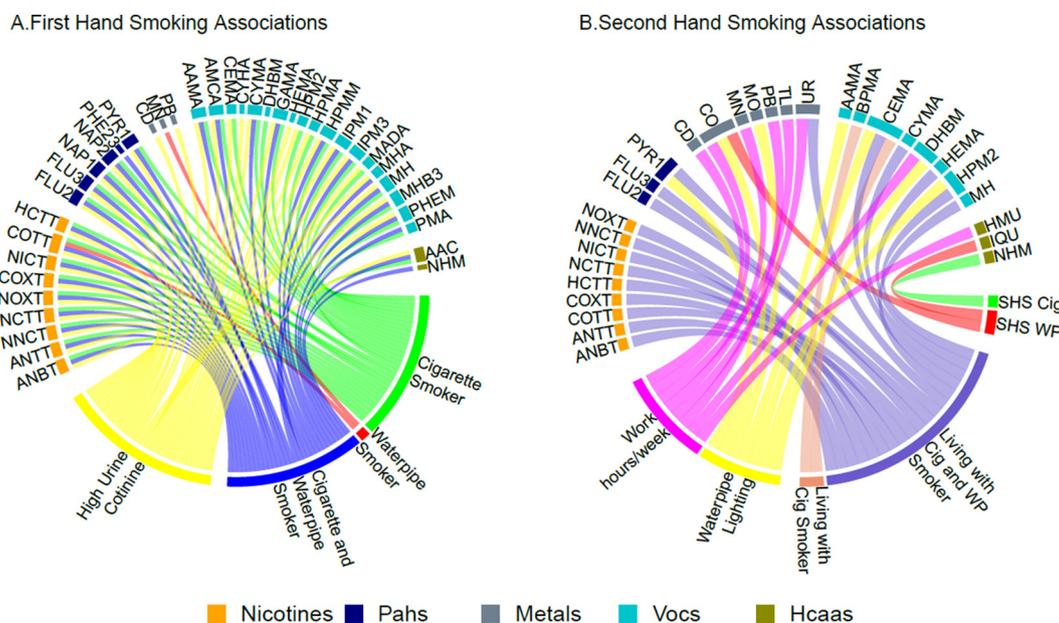


Fig. 1. Geometric Mean Ratios (GMR) of nicotine related metabolites, polycyclic aromatic hydrocarbons (PAHs), metals, volatile organic compounds (VOCs) and heterocyclic aromatic amines (HCAAs) by first and secondhand smoking variables.

All lines represent significant association between the biomarkers and first and secondhand smoke variables. The colors of the lines represent the different first hand and secondhand smoke variables.

*Models adjusted for sex, age, average work hour per week and country. SHS: Secondhand Smoke, Cig: Cigarette, WP: Waterpipe.

container to put the urine cup inside and bring it to work at the time of their regular shift. The collection of morning urine samples minimized variability in urine dilution. The half-life of many chemicals in the urine are longer than several hours (for example, for cotinine the half-life in urine is around 15 h, for metals the half-life is days, months, or even years depending on the metal). Therefore, the collection of the urine sample likely at least 8 h since the last shift is reasonable for the current study. The average time from sampling to incubation in the refrigerator ranged from 2 h to 6 h. Samples were placed in a refrigerator until fieldworkers returned to pick up the samples. Because the original focus of the study was not to measure metals, the urine collection cups were not pre-screened for metals content. Samples were stored at -20°C before being shipped on dry ice to Johns Hopkins Bloomberg School of Public Health.

2.4. Laboratory analysis

Urine samples were subsequently shipped on dry ice to the Centers for Disease Control and Prevention (CDC) Division of Laboratory Sciences for analysis. Urinary tobacco alkaloids (nicotine and its six major metabolites, plus minor tobacco alkaloids anatabine and anabasine) were measured by an isotope dilution high performance liquid chromatography/tandem mass spectrometric (HPLC-MS/MS) method (Wei et al., 2014). The limits of detection (LODs) for these alkaloids ranged from 0.39 to 10.5 ng/mL, depending on the analyte. The 7 PAH metabolites were quantified by online solid phase extraction coupled with high-performance liquid chromatography-isotope dilution tandem mass spectrometry, as previously described (Wang et al., 2017). The LODs for PAHs ranged from 8 to 90 ng/L. Urinary VOC metabolite concentrations were measured using ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS) according to a published procedure (Alwis et al., 2012). LODs for VOCs ranged from 0.500 to 15.0 ng/mL. Total urine element concentrations of beryllium, cadmium, cobalt, manganese, lead, strontium, thallium, and uranium were measured using inductively coupled plasma mass spectrometry (ICP-MS). The limits of detection for these elements in urine range from 0.002 to

2.34 ng/mL, depending on the analyte (Caldwell et al., 2005; Jarrett et al., 2008). The protocol for urine metal analyses was the same with the protocol used in the U.S. National Health and Nutrition Examination Survey (CDC, 2016). Ten heterocyclic aromatic amines (HCAAs) (i.e. AαC, MeAC, Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, IQ, PhIP, harman and norharman) in urine were measured by an isotope-dilution high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (ID HPLC-ESI MS/MS) using a method of Zhang et al. (2016). The limit of detection for urinary HCAA ranged from 0.31 to 0.83 pg/mL, depending on the analyte. Finally, creatinine was measured by a commercial automated, colorimetric enzymatic (creatinase) method implemented on a Roche/Hitachi Cobas 6000 Analyzer. The list of all metabolites with abbreviations and their LOD values are shown in the supplement Table 2. Values below the LOD were replaced with the LOD/square root of two (Maziak et al., 2015).

2.5. Data analysis

Urinary biomarker concentrations were right-skewed and were log-transformed; medians or geometric means were reported. We examined the correlation between chemicals and cotinine using Spearman rank correlation coefficients. We assessed the median, 25th percentile, and 75th percentile of chemical concentrations overall and by age, sex, country, average work hours per week, smoking status, living with a smoker, average numbers of hours of SHS exposure from cigarettes and from waterpipes per week (separately), and involvement with lighting waterpipes at work. The variables SHS from cigarette and waterpipe were right-skewed and dichotomized by their median values. We divided the concentrations of urine biomarkers by concentrations of urinary creatinine to correct for variability in urine dilution.

In a separate model for each of the 64 biomarkers (modeled as log-transformed and corrected by urinary creatinine), we estimated their association with smoking status, SHS exposure variables, and lighting of waterpipes (a total of 6 exposure variables) using multivariable linear regression with robust variance and an independent correlation structure within venues. The beta coefficients and 95% confidence intervals from each model were exponentiated to obtain geometric mean ratios

(GMRs) and corresponding 95% confidence intervals (CI). All models were adjusted for city, age, sex, and average work hours per week. Models for SHS exposure variables and for lighting of waterpipes were further adjusted for smoking status. In a sensitivity analysis, we also reported the adjusted GMRs (95%CI) for SHS exposure variables and lighting of waterpipes only among never/former smokers.

To facilitate the description and assessment of the numerous models run, all statistically significant GMRs at the nominal p -value of 0.05 were graphically displayed in two circle plots (Fig. 1). Each line in the circle plot represents a statistically significant GMR between a biomarker and the connected tobacco related exposure variable. The magnitude of the GMR is not shown in the circle plots.

Statistical analyses were performed with Stata Version 13.1 (StataCorp, College Station, TX) and R Version 3.2.2 (R Foundation for Statistical Computing, www.r-project.org, Vienna, Austria). All statistical tests were two-sided and p -values < 0.05 were considered statistically significant.

3. Results

3.1. Participant characteristics

The final study sample included 110 total employees, 61 in Istanbul and 49 in Moscow (Table 1). Most employees were men in Turkey, whereas there were equal proportions of men and women in Russia. The majority of employees had less than a high school education. The median age was 30 years. The most common primary job description was bartender/waiter (58%) and a total of 44% of employees reported being involved with lighting waterpipes. Few employees were never smokers (18%) or former smokers (7%). Current use of both cigarette and waterpipe was more common in Istanbul (48%), while only cigarette smoking was more common in Moscow (35%). The prevalence of smoking waterpipe only was 16% (26% in Istanbul and 4% in Moscow). Overall, 47% of employees lived with at least one smoker in their household. Employees reported a median of 48 h per week of

exposure to cigarette SHS and 21 h per week of exposure to waterpipe SHS (including both working and non-working hours). Waterpipe users were younger and more likely to be men than sole cigarette users or never/former smokers (Supplemental Table 1). The median (IQR) number of cigarettes smoked per day was 20 (10, 20) and 17 (10, 20) for sole cigarette users and for dual users, respectively. The median (IQR) number of waterpipes per day was 1.5 (1, 5) and 1.0 (0, 15) for sole waterpipe users and for dual users, respectively.

3.2. First hand smoke exposure by employee characteristics

Participants who reported using waterpipes exclusively had significantly higher concentration of urinary manganese (Table 2) compared to never or former smokers. After adjustment for age, sex, work hours per week and country, the GMR (95%CI) for manganese comparing sole waterpipe users to never/former smokers was 2.60 (1.24, 5.45) (Table 2) (Fig. 1A). All PAH metabolite concentrations (Supplemental Table 3), and most VOC metabolite concentrations were higher among dual and sole cigarette users compared to never/former smokers (Supplemental Table 5) (Fig. 1A). The concentrations of two HCAA metabolites (2-amino-9H-pyrido[2,3-b] indole (AαC), and 9Hpyrido [3,4-b] indole (norharman)) were significantly higher among dual users than never/former smokers (Supplemental Table 4).

3.3. Second hand smoke exposure by employee characteristics

Being exposed to more hours of SHS from waterpipes was associated with higher concentrations of cobalt. (Fig. 1B). After adjustment, the GMR (95%CI) comparing participants who reported > 21 versus 21 or less hours of SHS exposure from waterpipes per week was 1.38 (1.10, 1.75) for cobalt.

Higher number of work hours per week in a waterpipe venue was associated with higher urinary cadmium, manganese, thallium, and uranium as well as with higher concentrations of 1,3-butadiene (DHBM) and 1-methyl-9h-pyrido-indole (HMU) (Fig. 1B). After

Table 1
Characteristics of employees of waterpipe tobacco venues in Istanbul and Moscow in 2013–2014.

Characteristics	Overall (N = 110)	Istanbul (N = 61)	Moscow (N = 49)	p-value ^a
Number of employees per venue	6 (5, 9)	11 (5, 17)	6 (5, 9)	< 0.001
Age, years	30 (24, 42)	30 (24, 40)	29 (23, 48)	0.97
Male, %	76%	93%	55%	< 0.001
Education, %				< 0.001
Less than high school	68%	88%	43%	
High school	22%	12%	35%	
College/university	10%	0%	22%	
Primary job, %				0.15
Owner/Manager	14%	17%	10%	
Bartender/Waiter	58%	62%	55%	
Cook/kitchen staff	11%	5%	18%	
Other ^b	17%	16%	17%	
Smoking status, %				< 0.001
Never smoker	18%	1%	39%	
Former smoker	7%	7%	8%	
Current cigarette only smoker	26%	18%	35%	
Current waterpipe only smoker	16%	26%	4%	
Current cigarette and waterpipe smoker	33%	48%	14%	
Average time at work, hours/week	54 (48, 66)	60 (54, 72)	48 (36, 48)	0.001
Lives with a smoker				0.017
No	48%	36%	63%	
Yes, Cigarette Smoker Only	45%	56%	31%	
Yes, Cigarette and Waterpipe Smoker	7%	8%	6%	
Secondhand smoke from cigarettes, hours/week	48 (32, 70)	60 (42, 84)	36 (25, 55)	0.001
Secondhand smoke from waterpipe, hours/week	21 (8, 42)	42 (13, 60)	10 (4, 24)	0.001
Involved with lighting waterpipes for customers,% yes	44%	57%	26%	0.001

Notes: Categorical variables are percentages of total sample (N). Continuous variables are median (25th percentile, 75th percentile).

The time of data collection were between January and May 2013 in Istanbul and December 2013 to May 2014 in Moscow.

^a P-values are Pearson's chi-square test of independence for categorical variables and Ranksum for continuous variables.

^b "Other" includes watchman (N = 6) and other miscellaneous primary positions.

Table 2
Ratio of geometric means of employee Urine METAL concentrations by employee characteristics in waterpipe tobacco venues in Istanbul and Moscow.

Characteristics	N	Adjusted geometric mean ratios (95% confidence intervals)			
		Cd	Co	Cs	Mn
Smoking status					
Never/former (Ref)	28	1.00	1.00	1.00	1.00
Current cigarette only	28	1.07 (0.71, 1.63)	1.07 (0.77, 1.47)	1.01 (0.80, 1.28)	1.69 (0.97, 2.94)
Current waterpipe only	18	0.92 (0.53, 1.61)	1.04 (0.68, 1.61)	0.94 (0.68, 1.29)	2.60 (1.24, 5.45)
Current cigarette and waterpipe	36	1.10 (0.68, 1.76)	1.11 (0.77, 1.60)	1.02 (0.78, 1.34)	1.54 (0.82, 2.89)
Urine Cotinine (ng/g)					
≤ 1277.0 (Ref) (tertile 1)	24	1.00	1.00	1.00	1.00
> 1277.0 (tertiles 2 and 3)	86	1.57 (1.14, 2.17)	0.99 (0.77, 1.29)	0.98 (0.82, 1.19)	0.93 (0.59, 1.45)
Work Hours Per Week (per hour)	109	1.01 (1.00, 1.02)	1.50 (0.96, 2.35)	1.00 (0.99, 1.01)	1.02 (1.01, 1.03)
Living with a smoker					
No (Ref)	53	1.00	1.00	1.00	1.00
Yes, cigarette smoker only	49	1.07 (0.78, 1.47)	1.16 (0.91, 1.46)	0.94 (0.78, 1.13)	1.34 (0.88, 2.04)
Yes, cigarette and waterpipe smoker	8	1.43 (0.80, 2.57)	1.48 (0.95, 2.29)	0.90 (0.65, 1.27)	1.82 (0.84, 3.94)
SHS from Cigarettes Per Week (Hours)					
≤ 48 (Ref)	45	1.00	1.00	1.00	1.00
> 48	65	1.05 (0.43, 2.55)	0.83 (0.43, 1.63)	0.91 (0.67, 1.23)	0.71 (0.39, 1.27)
SHS from WP Per Week (Hours)					
≤ 21 (Ref)	49	1.00	1.00	1.00	1.00
> 21	61	1.14 (0.83, 1.55)	1.38 (1.10, 1.75)	1.14 (0.96, 1.36)	1.11 (0.73, 1.68)
Lighting waterpipes					
No (Ref)	62	1.00	1.00	1.00	1.00
Yes	48	1.13 (0.79, 1.59)	1.43 (1.10, 1.86)	1.21 (1.00, 1.48)	0.82 (0.51, 1.30)
Smoking status					
Never/Former (Ref)	28	1.00	1.00	1.00	1.00
Current cigarette only	28	1.10 (0.77, 1.56)	1.10 (0.77, 1.56)	0.98 (0.74, 1.28)	1.26 (0.91, 1.77)
Current waterpipe only	18	0.96 (0.59, 1.54)	1.31 (0.94, 1.82)	0.91 (0.63, 1.31)	1.11 (0.71, 1.74)
Current cigarette and waterpipe	36	1.23 (0.82, 1.84)	1.23 (0.82, 1.84)	0.80 (0.59, 1.10)	1.32 (0.90, 1.92)
Urine Cotinine (ng/g)					
≤ 1277.0 (Ref) (tertile 1)	24	1.00	1.00	1.00	1.00
> 1277.0 (tertiles 2 and 3)	86	1.19 (0.89, 1.57)	1.30 (1.07, 1.58)	1.15 (0.92, 1.42)	1.02 (0.65, 1.61)
Work Hours Per Week (per hour)	109	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	1.01 (1.00, 1.02)	1.02 (1.01, 1.03)
Living with a smoker					
No (Ref)	53	1.00	1.00	1.00	1.00
Yes, cigarette smoker only	49	0.91 (0.69, 1.19)	0.96 (0.80, 1.16)	0.93 (0.76, 1.15)	0.98 (0.76, 1.26)
Yes, cigarette and waterpipe smoker	8	1.15 (0.69, 1.89)	1.40 (0.99, 1.97)	1.07 (0.73, 1.58)	1.62 (1.02, 2.56)
SHS from Cigarettes Per Week (Hour)					
≤ 48 (Ref)	45	1.00	1.00	1.00	1.00
> 48	65	1.43 (0.83, 2.48)	0.92 (0.60, 1.41)	0.81 (0.55, 1.18)	1.00 (0.54, 1.87)
SHS from WP Per Week (Hours)					
≤ 21 (Ref)	49	1.00	1.00	1.00	1.00
> 21	61	1.02 (0.78, 1.33)	1.02 (0.78, 1.33)	1.08 (0.88, 1.32)	0.80 (0.63, 1.03)
Lighting waterpipes					
No (Ref)	62	1.00	1.00	1.00	1.00
Yes	48	1.45 (1.08, 1.93)	1.08 (0.87, 1.32)	1.17 (0.93, 1.47)	1.18 (0.89, 1.57)

The geometric mean ratios for smoking status and work hours per week were adjusted for age, sex, and country.

The geometric mean ratios for urine cotinine, living with a smoker, SHS from cigarettes per week, and SHS from waterpipe per week and lighting waterpipes were further adjusted for cigarette and waterpipe smoking status.

Mo: Molybdenum, Pb: Lead, Tl: Thallium, Ur: Uranium. Ref: Reference group for regression analysis

adjustment for sociodemographic variables and smoking status, each additional hour of work per week in a waterpipe venue was associated with 2% increase in manganese and uranium, 1% increase in cadmium, thallium (Table 2); 1-methyl-9h-pyrido-indole (HMU) (Supplemental Table 4), 1,3-butadiene (DHBM), and acrolein (CEMA) (Supplemental Table 5).

Living with a cigarette and waterpipe smoker was associated with the all nicotine metabolites (Supplemental Table 6), and with several biomarkers of exposure to VOCs (acrolein, acrylonitrile, 34MH (3-methylhippuric acid + 4-methylhippuric acid) (xylene), and propylene oxide) (Fig. 1B) (Supplemental Table 5). Living with a cigarette smoker was associated with nicotine 1'N-oxide, a nicotine metabolite, and metabolites of several VOCs (acrolein, acrylonitrile, and styrene) (Supplemental Table 5).

The associations between SHS exposure variables and the different biomarkers remained similar in magnitude but were less likely to be statistically significant in analyses restricted to the subset of participants who were never/former smokers (Supplemental Tables 7, 8, 9, 10, 11).

3.4. Occupational exposure to first hand waterpipe smoking

Employees involved in lighting waterpipes, compared to those not involved, had higher urine concentrations of cobalt (GMR: 1.43, 95% CI: 1.10, 1.86), cesium (GMR: 1.21, 95% CI: 1.00, 1.48), molybdenum (GMR: 1.45, 95% CI: 1.08, 1.93) (Table 2), 1-hydroxypyrene (GMR: 1.36, 95% CI: 1.03, 1.80) (Supplemental Table 3), acrylamide (AAMA) (GMR: 1.48, 95% CI: 1.07, 2.06), acrolein (CEMA) (GMR: 1.51, 95% CI: 1.07, 2.06), and 1,3-butadiene (DHBM) (GMR: 1.60, 95% CI: 1.17, 2.19) (Supplemental Table 5).

4. Discussion

This is the first study comparing metals, VOCs, PAHs and HCAAs by waterpipe use and secondhand smoke exposure compared to never/former smokers and to cigarette smoking. Sole waterpipe users (no cigarette smoking) had higher manganese concentrations compared to never and former smokers, while manganese concentrations were higher but not statistically significantly different in cigarette smokers, suggesting that waterpipe smoking may be a specific source of

manganese. High urine cobalt concentration was significantly associated with high SHS hours from waterpipe and involved in lighting waterpipe. In addition, employees involved in lighting waterpipes had higher urine cobalt, urine cesium, urine molybdenum, urine 1-hydroxypyrene, AAMA (acrylamide), CEMA (acrolein), and DHBM (1,3-butadiene). Cigarette use was more strongly associated with nicotine-derived biomarkers compared to waterpipe use, possibly because of higher daily smoking intensity among cigarette smokers vs. waterpipe smokers. Overall these findings support that waterpipe smoking and occupational and non-occupational exposure to secondhand waterpipe smoke may be an important source of metal exposure and contribute to increased exposure to certain PAHs and VOCs. Waterpipe venue employees and waterpipe users have higher occupational risk of exposure to these potentially toxic chemicals than other persons.

Waterpipe SHS is derived from combustion of both tobacco and the burning source (usually charcoal). To date, over eighty toxicants have been quantified in waterpipe tobacco smoke (Shihadeh and Saleh, 2005). During recent years, smoke machine experiments (Saadawi et al., 2012, Shihadeh, 2003, Shihadeh and Saleh, 2005, Schubert et al., 2015, Apsley et al., 2011) showed that mainstream waterpipe smoke delivers phenanthrene, fluoranthene, chrysene (Shihadeh and Saleh, 2005), pyrene and naphthalene (Apsley et al., 2011), benzene, toluene and pyridine (Schubert et al., 2015), and numerous chemical elements such as arsenic, cadmium, chromium, manganese, lead (Saadawi et al., 2012) and copper, zinc, boron, chromium, nickel, and lead (Apsley et al., 2011). Smoke machine studies also suggest that waterpipe SHS contains similar or higher concentrations of many carcinogens and toxic chemicals as compared to cigarette SHS, including carcinogenic PAHs, PM_{2.5}, volatile aldehydes, and CO (Daher et al., 2010; Hammal et al., 2015). We previously reported that in the waterpipe venues where our participants worked, there were high concentrations of indoor air markers of SHS, including PM_{2.5}, CO, PAHs, the tobacco specific nitrosamine NNK, and air nicotine (Moon et al., 2015). In a preliminary biomarker study (Moon et al., 2018), we found that among non-tobacco smokers (including both cigarettes and waterpipes) higher work hours were associated with higher urine cotinine and hair nicotine concentrations, showing that employees in these venues are effectively exposed to secondhand waterpipe smoke.

In this study, we found that higher urine cobalt concentration was associated with high SHS hours from waterpipe and being involved in lighting waterpipe. Cobalt likely originates from the charcoal and tobacco used in the waterpipe. Indeed, recent studies have detected cobalt in the waterpipe tobacco (Schubert et al., 2015) and charcoal (Schubert et al., 2015) and also in the main stream smoke of waterpipe (Shihadeh et al., 2015, Apsley et al., 2011). Burning charcoal, as a source of cobalt, can release cobalt to environment air (Elsayed et al., 2016). The employees involved in lighting waterpipe might have been exposed to high level of cobalt from burning coal even though they are not a waterpipe user. The employees involved in lighting waterpipe generally prepare the waterpipe tobacco with bare hands, therefore, they might be exposed to the chemicals through oral (hand-mouth) and dermal routes. Another explanation could be that they usually prepare the burned charcoal in non-ventilated areas of the venues and are exposed to the burned charcoal smoke more than other employees. To the best of our knowledge, this is the first study reporting the association between SHS from waterpipe and cobalt biomarkers, supporting that cobalt is an important metal of concern related to occupational waterpipe SHS. Cobalt and cobalt-containing compounds are classified as Group 2B human carcinogens by the International Agency for Research on Cancer (International Agency for Research on Cancer, 2004). High chronic cobalt exposure by inhalation in humans results in effects on the respiratory system (respiratory irritation, wheezing, asthma, decreased lung function, pneumonia, and fibrosis), cardiovascular system (cardiomyopathy, cardiogenic shock, sinus tachycardia, left ventricular failure, and enlarged hearts), and gastrointestinal system (nausea, vomiting, and diarrhea) (United States Environmental Protection Agency,

2016).

Participants who reported using waterpipes but not cigarettes had higher concentrations of urinary manganese compared to never/former smokers, and working longer hours per week in a waterpipe venue was also associated with higher urine manganese concentrations (Table 2). Manganese has been previously identified in waterpipe tobacco (Saadawi et al., 2012) and waterpipe charcoal (Elsayed et al., 2016). In another experimental study (Schubert et al., 2015), seventeen metals were analyzed in the waterpipe tobacco and charcoal prior to and after waterpipe smoking. The highest amounts in unburned and burned tobacco and charcoal were aluminum, manganese and barium. These findings in waterpipe tobacco and charcoal, paired with the finding of higher manganese concentrations in the urine samples of waterpipe users and with high working hours in a waterpipe venue confirm that waterpipes are an important source of exposure to manganese. This is a particularly important finding given strong evidence on the impact of chronic manganese exposure on neurodevelopmental and neurodegenerative disorders (Lucchini et al., 2018).

Employees involved in lighting waterpipes had higher urine cobalt, urine cesium, urine molybdenum, urine 1-hydroxypyrene, AAMA (acrylamide), CEMA (acrolein), and DHBM (1,3-butadiene) compared to those not involved. Previously, PAH metabolites in the main stream smoke of waterpipe (Shihadeh and Saleh, 2005; Apsley et al., 2011) and higher 1-hydroxypyrene excretion in the urine samples of waterpipe users after a waterpipe smoking session (Jacob 3rd et al., 2013) were reported. Besides, a smoke machine study found that 75–92% of the PAH compounds in the main stream smoke of waterpipe originated in the charcoal and over 95% of the benzo(a)pyrene originated in the charcoal (Monzer et al., 2008). The current study is the first time that PAH biomarkers have been measured in employees involved in lighting waterpipes, finding higher concentrations of 1-hydroxypyrene, a metabolite of pyrene (International Agency for Research on Cancer, 2004). In our preliminary biomarker study, being involved in lighting waterpipes was associated with higher concentrations of urine and saliva cotinine and hair nicotine (Moon et al., 2018). We have substantially expanded the evaluation of real-world exposure to SHS in waterpipe venues and biomarkers of SHS exposure with this study.

The major strength of this study was the application of a comprehensive analytical assessment of harmful and potentially harmful tobacco constituents to biospecimens that have been collected from workers in waterpipe venues and that allowed us to compare non-users with cigarette smokers, as well as active and secondhand waterpipe users. Other strengths included the multi-city design, high employee participation rate, and the detailed exposure characterization based on self-reported data and biomarkers of both cigarette and waterpipe smoking status and on exposure to SHS in the home.

This study has several limitations. The sample size was relatively small, in particular for never/former smokers. Our sample of waterpipe tobacco venues in each city was selected by convenience; therefore, these venues may not be representative of all waterpipe venues in each city. Further, fear of regulation by less compliant venues may have played a role in the low venue participation rate (ranging from 32%–34%) in each city. Identifying a threshold to differentiate active versus passive exposure to tobacco is difficult in waterpipe venues in this setting because of substantial occupational SHS exposure. We used whether the employee lived with a smoker as a proxy for secondhand smoke exposure in the home; however, we could not account for SHS exposures in other places and residual confounding is possible. Also, waterpipe secondhand smoke exposure at home always occurred with cigarettes, so we could not isolate exposure to waterpipes at home. This study was conducted among employees of waterpipe venues in a real-world setting, and we may not have been able to account for other important sources of variability that could explain some of the heterogeneity of our findings across biomarkers. The biomarkers measured in this study encompass a variety of different exposure windows depending on the half-lives and concentrations could likely reflect

aggregate exposures at work, in public places, or in the home. However, employees reported spending a substantial number of hours at work and therefore, we believe that occupational exposure is likely to be an important source of exposure to these toxicants biomarkers or their precursors. We used never/former smokers as a reference group in order to compare with active waterpipe use. Given every participant in this study had at least some exposure to waterpipe smoke, the effect of active waterpipe use might have been underestimated in the study. The standard plastic urine cups were not airtight; further, an internal standard was not performed on the spot before freezing to be able to calculate later on VOC & PAH loss percentage or extraction efficiency. Therefore, the VOC & PAH results might be underestimated in the current study. The lack of information on food consumption of the study participants is a major limitation as different types of foods can be major sources and act as a potential confounder of many of the chemicals analyzed in this study. We combined the never/former smokers into one group despite the differences in percentages reported by both countries percentage of these group are different between countries (1% Russia, 39% Turkey). Urine concentrations of chemicals between the two cities, however, were not statistically different significant after adjustment for sociodemographic characteristics, supporting that combining the two countries for this group is a reasonable strategy. Urine samples underwent freeze-thaw cycles two times, which might affect the quality of sample. However, for most of the chemicals, e.g. metals, the measures are resistant to the number of freeze-thaw cycles (CDC, 2014a). Finally, the effect of passive and occupational exposure to waterpipe was determined mostly by comparing differences in self-reported hours of exposure but a group of participants unexposed to waterpipe SHS was missing.

5. Conclusion

A higher number of work hours per week in a waterpipe venue was associated with higher urinary cadmium, manganese, thallium, and uranium as well as with higher concentrations of DHBM (1,3-butadiene) and HMU (1-methyl-9H-pyrido-indole). Being exposed to SHS from waterpipe in occupational and non-occupational settings and occupational involvement in lighting waterpipe were related to exposure to various metals, PAHs, HCAAs and VOCs. Based on our findings, waterpipe users and nonsmoking employees of waterpipe tobacco venues, and potentially also patrons, may be at an increased risk of health problems as a result of waterpipe use and secondhand exposure. Smoke-free legislation has been successful in reducing exposure to SHS from cigarettes (CDC, 2014b). Expanding clean indoor air regulations to waterpipe venues may reduce potentially harmful exposures to workers.

We consider this study as a screening project to identify potentially relevant chemicals related to waterpipe smoking and SHS exposure. Further studies seeking relevant chemicals in more detail related to waterpipe smoking and SHS exposure are needed.

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Declaration of interests

The authors declare no potential conflicts of interest.

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Disclaimers

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention, the Department of Defense, Department of the Army, U.S. Medical Department, or the U.S. Use of trade names and commercial sources is for identification only and does not constitute endorsement by the U.S. Department of Health and Human Services, or the Centers for Disease Control and Prevention. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory did not constitute engagement in human subjects research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2019.03.074>.

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