



Mainstream smoke of the waterpipe: Does this environmental matrix reveal as significant source of toxic compounds?

Jens Schubert^{a,*}, Jürgen Hahn^b, Gerhard Dettbarn^c, Albrecht Seidel^c, Andreas Luch^a, Thomas G. Schulz^a

^a German Federal Institute for Risk Assessment (BfR), Department of Product Safety, Thielallee 88-92, 14195 Berlin, Germany

^b Official Chemical & Veterinary Surveillance Institute Sigmaringen, Hedingerstraße 2/1, 72488 Sigmaringen, Germany

^c Biochemical Institute for Environmental Carcinogens (BIU), Prof. Dr. Gernot Grimmer-Foundation, Lurup 4, 22927 Grosshansdorf, Germany

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ABSTRACT

In recent years the number of waterpipe smokers has increased substantially worldwide. Here we report on the concentrations of tobacco-specific nitrosamines (TSNAs) and polycyclic aromatic hydrocarbons (PAHs) in waterpipe smoke and the analysis of selected biomarkers indicative for the body burden in waterpipe users. We further identify high amounts of unburned humectants (glycerol and propylene glycol) in the waterpipe smoke as main part of the so-called “tar” fraction. These results give cause for serious concern. For standardization we applied a machine smoking protocol. Smoke was collected on glass fiber filters and analyzed for nicotine, water, humectants, TSNAs, and PAHs. In addition, we determined carbon monoxide and found high amounts in the smoke being causative for high levels of carboxyhemoglobin (COHb) in the blood of smokers. In comparison to the reference cigarette 3R4F, the nicotine contents were 10-times higher, but TSNA levels were found lower in waterpipe smoke. This finding explained the low levels of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol detected in the urine of waterpipe smokers. Finally, the levels of benzo[*a*]pyrene were three times higher in waterpipe smoke compared to the reference cigarette. Altogether, the data presented in this study point to the health hazards associated with the consumption of waterpipes.

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1. Introduction

The waterpipe is a traditional aid for tobacco consumption in Asia and Northern Africa (Maziak et al., 2004a). Although exact numbers are missing to date, web blogs, “hookah bar” advertisements and the advent of popular waterpipe stores indicate that both the general interest and the number of young people smoking waterpipes have considerably grown in recent years in European countries and North America (BZgA, 2008; Jackson and Aveyard, 2008; Primack et al., 2008).

There are major differences in the consumption of waterpipes and cigarettes: The flavored tobacco, mainly used for waterpipes in Europe and the US, contains huge amounts of humectants such as glycerol and propylene glycol. The humectants prevent the tobacco from burning thereby yielding a smooth and pleasant smoke. Furthermore, the heat for the waterpipe is generated using charcoal, which is placed on top of the tobacco head. Studies from Lebanon showed that smoking habits differ greatly between waterpipe and cigarette smokers (Shihadeh et al., 2004). For instance, average inhalation volumes were about 530 ml for single waterpipe puffs

whereas the puff volumes found for cigarette smoking were in the range of 35–60 ml (Hammond et al., 2007). Furthermore, the smoking time differs greatly. For a waterpipe with 10 g tobacco the smoking time amounted to almost 60 min and 171 puffs whereas the time for a cigarette is between 5 and 10 min (6–11 puffs). Shihadeh and colleagues established a machine-smoking protocol, which is based on the investigations of smoking behavior of waterpipe smokers in Lebanon, and investigated several constituents of waterpipe smoke such as carbon monoxide (CO), polycyclic aromatic hydrocarbons (PAHs), various aldehydes (e.g., formaldehyde, acetaldehyde, acrolein), and certain metals (e.g., lead, chromium, arsenic) (Al Rashidi et al., 2008; Monzer et al., 2008; Sepetdjan et al., 2008; Shihadeh, 2003; Shihadeh and Saleh, 2005).

In the present study three major questions were addressed: firstly, we determined the levels of tar, nicotine and CO in the waterpipe smoke. To assess the internal body burden of these toxins, biomonitoring of nicotine and carboxyhemoglobin (COHb) in the blood and of cotinine in the urine of smokers was performed. Since the amounts of tar were high in waterpipe smoke, we investigated its composition and found high amounts of humectants. Secondly, the levels of tobacco-specific nitrosamines (TSNAs) were measured in tobacco, tobacco smoke and in the urine of consumers. Since *N*'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) have been

* Corresponding author. Tel.: +49 30 18412 3963; fax: +49 30 18412 4928.
E-mail address: Jens.Schubert@bfr.bund.de (J. Schubert).

classified as human carcinogens (IARC, 2007), we determined the contents of these compounds in waterpipe tobacco and smoke. As biomarker of NNK its metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) was analyzed. Thirdly, various PAHs such as benzo[a]pyrene were measured in the waterpipe smoke. Benzo[a]pyrene has been classified as human carcinogen (IARC, 2010) and serves as well-established carcinogenic lead compound of environmental PAH mixtures since decades.

2. Materials and methods

2.1. Reagents

All chemicals used in this study were of analytical grade. A list of potential suppliers is given in the supplementary part (see Table S-1).

CO calibration gas was obtained from Air Liquide (Berlin Germany) and 92 mm glass fiber filter pads were from Borgwaldt KC (Hamburg, Germany). Waterpipe tobacco was purchased from Nakhla Tobacco (Two Apples flavor, Nakhla Tobacco, Egypt). Perforated aluminum foil (\varnothing 15.5 cm, 25 holes) was obtained from Falu, Ballingen, Germany. Quick lighting charcoal (\varnothing 40 mm) was purchased from Three Kings, The Netherlands. 3R4F reference cigarettes were purchased from the University of Kentucky (Kentucky Tobacco Research & Development Center, Lexington, KY, USA).

2.2. Smoking protocol and smoke collection

Smoking was simulated by connecting a Borgwaldt Shisha Smoker machine to a standard laboratory waterpipe (Borgwaldt KC) using a plastic hose. According to a topographical study each smoking session consisted of 171 puffs of 530 ml each and 2.6 s duration every 20 s and therefore resulted in a total length of 57 min and a total puff volume of 90.63 l (Shihadeh et al., 2004). Ten grams of waterpipe tobacco were transferred into the head of the pipe and covered with perforated aluminum foil. A single quick lighting charcoal disk was lit and, after 60 s, placed atop the perforated foil to start the smoking session. The total particulate matter (TPM) was collected by aspirating the smoke of an entire session through a 92 mm glass fiber filter pad. For determination of humectants, TSNAs and PAHs independent smoking sessions were conducted to address the varying extraction conditions (see Sections 2.4, 2.5 and 2.6), whereas levels of nicotine and water were determined from the same smoking session (see Section 2.3).

After a conditioning period of at least 24 h at 22 °C and 60% relative humidity according to German industrial standard norms (DIN-ISO, 2000a), 3R4F reference cigarettes were vaporized in a rotary RM 20 H smoking machine (Borgwaldt KC) by applying the following puff parameters: 60 s interval, 2 s duration, 35 ml volume (DIN-ISO, 2000b).

2.3. Determination of nicotine, water and carbon monoxide

Sample preparation for nicotine determination was according to DIN-ISO, 2000c with slight modifications. In brief, the filter pads were extracted with 100 ml of the extraction solution. The extract was then filtered through a 0.45 μ m PTFE syringe filter and analyzed for nicotine. Nicotine analysis by GC-FID was performed on an HP 6890 gas chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with an FID detector and an autosampler (HP 6890 liquid injector). The chromatographic parameters are provided in the supplementary section (see Table S-2).

For the quantification of water, aliquots of the extraction solution utilized for nicotine analysis were used. Analysis was performed on a Mettler DL 18 Karl-Fischer-Titrator (Mettler-Toledo GmbH, Giessen, Germany).

For CO determination the whole gas phase was collected and quantified by using a non-dispersive infrared absorption (NDIR) CO analyzer (CO/CO₂-Analyzer C24, Borgwaldt KC).

2.4. Determination of humectants

The filter pads were spiked with the internal standard solution (1,4-butanediol) and 50 ml of methanol were added subsequently. Subsequently, the filter pads were agitated for 1 h on an HS 250 basic shaker (IKA Labortechnik, Staufen, Germany). The extract was then filtered into autosampler vials through a 0.45 μ m PTFE syringe filter and analyzed by GC-FID. The chromatographic parameters are provided in the supplementary section (see Table S-3).

2.5. Determination of TSNAs

The filter pads were spiked with the internal standard solutions (NNN-¹³C₆, NNK-¹³C₆), 75 ml of 100 mM ammonium acetate were added subsequently and the pads were agitated for 1 h. The extract was then filtered into autosampler vials through a 0.45 μ m PTFE syringe filter and analyzed by LC-MS/MS.

Waterpipe and cigarette tobacco (3R4F) were analyzed after transferring 0.2 g of a well-homogenized sample into a 20 ml flask, the addition of the internal standard solutions and subsequent extraction with 15 ml of 100 mM ammonium acetate for

1 h. The extract was filtered into autosampler vials using a syringe filter (0.45 μ m PTFE) and analyzed by LC-MS/MS.

For sample analysis a Shimadzu LC-20AD prominence (Shimadzu, Duisburg, Germany) HPLC system coupled with an API 4000 Q TRAP mass spectrometer (AB Sciex Instruments, Applied Biosystems, Darmstadt, Germany) was used. The HPLC system comprised two pumps (LC-20AD), a column oven (CTO-20ACHT), a degasser (DGM-20A5), a controller (CBM-20A), and a temperature controlled autosampler (SIL-20ACHT). The chromatographic parameters are provided in the supplementary section (see Tables S-4.1, S-4.2 and S-4.3).

2.6. Determination of PAHs

Samples for PAH analysis were prepared according to a previously published method (Zha et al., 2002) with some modifications listed next. The filter pads were spiked with the internal standard solution and extracted with 50 ml of methanol. After shaking the filter pads for 1 h, 30 ml of the extraction solution were concentrated to 5 ml using an IR-Dancer 360 (Zinsser Analytic, Frankfurt, Germany) and filtered through a 0.45 μ m PTFE syringe filter. Then 7 ml of deionised water was added. After SPE cartridges (Varian Bond Elut CH 500 mg/3 ml, Varian, Darmstadt, Germany) were pre-conditioned with 2 ml of methanol followed by 2 ml of 65:35 (v/v) water/methanol, the smoke extract was loaded and washed with 4.8 ml of water followed by 1.6 ml of methanol. Then the cartridges were dried under nitrogen and the PAHs were eluted with 2 ml of cyclohexane. The cyclohexane extracts were concentrated to 0.5 ml and analyzed by GC-MS.

Analysis of 3R4F reference cigarettes was performed slightly different. Here only 25 ml of methanol were used for extraction and 10 ml were concentrated to a volume of 5 ml. Subsequent sample clean-up was similar to the clean-up of waterpipe samples.

GC-MS analyses were performed on an HP 6890 gas chromatograph equipped with an Agilent MSD 5975C mass spectrometer (Agilent Technologies), a Gerstel Multi Purpose Sampler (MPS-2), and a Gerstel Cold Injection System (CIS) (Gerstel, Mühlheim an der Ruhr, Germany). The chromatographic parameters are provided in the supplementary section (see Tables S-5.1 and S-5.2).

2.7. Biomonitoring

The 22 participants were age 18 and older. The groups consisted of 10 non-smokers, 10 waterpipe smokers and two cigarette smokers. All participants were interviewed before starting the experiments. Only waterpipe smokers with no additional cigarette consumption were considered and asked for their tobacco consumption during the preceding 48 h. Non-smokers were asked for possible exposure to environmental tobacco smoke during the preceding 48 h. The study was approved by the Ethics committee of the Charité Medical School, Berlin, Germany.

After taking one blood sample from non-smokers, total urine was subsequently collected for 24 h. Two blood samples were taken from smokers, one sample prior to the smoking of one cigarette or of one waterpipe, and one sample immediately after smoking. Waterpipe smokers consumed 5 g of a flavored waterpipe tobacco (Nakhla Tobacco) in a traditional waterpipe (height: 68 cm) with one piece of quick lighting charcoal (Three Kings). In a well-ventilated room solitary waterpipe smokers inhaled at will for a total time of 30 min and then were requested to abstain from further smoking during the urine collection period. Smoking was finished after 30 min since the smoke lost its specific taste at that time. The total urine volume was recorded and samples were stored at -20 °C until further analysis.

COHb and nicotine levels were determined by the private and accredited analytical laboratory "Labor 28" (Berlin, Germany) using GC for nicotine and headspace GC for COHb. NNAL levels were determined by the Analytisch-Biologisches Forschungslabor, Munich, Germany as described (Kavvadias et al., 2009).

Determination of cotinine was performed as described (Voncken et al., 1989) with some modifications. In brief, 100 μ l urine were transferred into a pear-shaped flask to which 0.3 ml of a 5 N NaOH solution and 0.79 μ g phenanthrene-d₁₀, dissolved in 50 μ l dichloroethane, were added. After shaking (1 min) and centrifugation (2 min at 3000 rpm), the aqueous phase was removed and an aliquot of 2 μ l of the dried dichloroethane layer was used for GC-MS analysis. The chromatographic parameters are provided in the supplementary section (see Table S-6).

3. Results

3.1. Total particulate matter (TPM), nicotine, water, tar and carbon monoxide (CO)

Data on TPM, nicotine, water and CO contents in the smoke of waterpipes and 3R4F reference cigarettes are compiled in Table 1. The average values for TPM, tobacco and charcoal consumption (mean \pm SD) for 15 replicate smoking sessions were 2.71 \pm 0.20 g, 3.87 \pm 0.36 g, and 8.22 \pm 0.10 g, respectively. In comparison to the TPM values for 3R4F reference cigarettes (11.0 mg) reported in the literature (Liu et al., 2009), a waterpipe session yielded 250-

Table 1

Standard parameters (mean values \pm RSD) for the smoke of waterpipes or 3R4F reference cigarettes.

	Waterpipe [mg/session] (RSD, %), (n = 15)	3R4F reference cigarette [mg/cigarette] (RSD, %), (n = 5)
Charcoal consumption	8220 (1.2)	
Tobacco consumption	3870 (9.3)	
TPM	2710 (7.5)	11.0 (-) ^a
Water content	1760 (12)	0.87 (-) ^b
Nicotine content	7.75 (5.1)	0.73 (5) ^a
Tar (NFDPM ^c)	949 (27)	9.4 (6) ^a
CO content (n = 11)	367 (9)	12.0 (5) ^a

TPM, total particulate matter; CO, carbon monoxide; RSD, relative standard deviation.

^a From Liu et al. (2009).

^b Calculated as follows: water content = TPM – tar – nicotine content.

^c NFDPM: nicotine-free dry particulate matter.

fold more TPM. The water content of TPM was 1.76 ± 0.20 g for waterpipe smoke whereas for 3R4F reference cigarette smoke a mean value of 0.87 mg has been reported (Liu et al., 2009). The average nicotine content in waterpipe smoke was measured at 7.75 ± 0.39 mg and the calculated amount of tar was 949 ± 253 mg. For 3R4F reference cigarette smoke, average nicotine content of 0.73 ± 0.04 mg was reported (Liu et al., 2009) and the amount of tar was calculated at 9.4 ± 0.56 mg. The CO levels in waterpipe smoke were determined in 11 replicate smoking sessions at 367 ± 33 mg whereas for 3R4F reference cigarettes a mean value of 12 ± 0.6 mg was reported (Liu et al., 2009).

3.2. TSNAs

To the best of our knowledge this is the first study to report on the contents of TSNAs in waterpipe smoke and waterpipe tobacco. For analysis of TSNAs we established a simple but accurate LC–MS/MS method. The results obtained are compiled in Table 2.

The applied protocol has been validated by determining (i) the recovery after extraction, (ii) the LOD and LOQ, and (iii) the precision of the LC–MS/MS procedure. The corresponding data are provided in the supplementary part (see Tables S-7.1 and S-7.2). For further validation we determined the contents of certain TSNAs in the smoke of the 3R4F reference cigarette. Since there are no data available in the literature on the TSNAs contents of the 3R4F reference cigarette smoke, the analytes were again measured in the other participating laboratory (J.H., Sigmaringen, Germany). The values obtained are given in Table 2.

All TSNAs revealed with considerably higher concentrations in cigarette tobacco compared to waterpipe tobacco. NNK concentration in waterpipe tobacco was 41.1 ng/g tobacco; in cigarette tobacco it was 19-times higher with 798 ng/g tobacco. Even greater

(167-fold) was the difference for NNN with 18.4 ng/g in waterpipe tobacco compared to 3070 ng/g in cigarette tobacco.

Due to the higher amounts of tobacco used for waterpipe smoking compared to cigarette smoking (10.0 g vs. 0.78 g) the differences in the TSNA contents of the smoke were much smaller. Here the NNK contents of waterpipe smoke (46.4 ng/session) were almost half of the contents detected in cigarette smoke (101 ng/cigarette). For NNN the difference was 4-fold between waterpipe smoke (34.3 ng/session) and cigarette smoke (137 ng/cigarette).

TSNAs in tobacco were transferred into the smoke, ranging from 5.7% in cigarettes for NNN to 28.2% in waterpipes for NAB.

3.3. Biomonitoring

In comparison to non-smokers (1.2%), the COHb levels increased dramatically in the blood of waterpipe smokers up to a mean value of 17% with a maximum of 32%. The blood levels of nicotine in waterpipe smokers were found at $11.4 \mu\text{g/l}$, but were considerably higher in cigarette smokers. Cotinine could be detected in the urine of waterpipe smokers and non-smokers alike. Since two of the waterpipe smokers did already smoke the day before starting the biomonitoring experiment, their cotinine values were very high (623 and 852 $\mu\text{g/24 h}$), thereby causing a large standard deviation of the overall result.

No differences were found between the urinary NNAL contents of non-smokers and waterpipe smokers, but high values in cigarette smokers confirmed the sensitivity of the method applied (see Table 3).

3.4. Humectants

As for waterpipe smoking, usually humectants are determined in the unburned tobacco and not in the smoke. Here we report on a robust GC–FID method for the determination of humectants in waterpipe smoke.

The method was validated in terms of recovery, reproducibility, limit of detection (LOD), and limit of quantification (LOQ), and the results are reported in the supplementary section (see Table S-8.1 and S-8.2).

Determination of humectants in waterpipe smoke revealed high levels of propylene glycol (211 ± 6.0 mg/session) and glycerol (423 ± 19 mg/session), whereas other humectants such as trimethylene glycol, 1,3-butanediol, diethylene glycol and triethylene glycol were not detectable at all. The total amount of humectants detected was 633 ± 13 mg/session.

3.5. PAHs

Here we established a method for the determination of the 16 US-EPA PAHs in waterpipe smoke based on GC–MS instrumentation. The results obtained are compiled in Table 4.

Table 2

Levels of TSNAs in the tobacco and the smoke of the waterpipe or of the 3R4F reference cigarette (mean values \pm RSD).

Analyte	Waterpipe smoke	Waterpipe tobacco	3R4F reference cigarette smoke	3R4F reference cigarette tobacco	3R4F reference cigarette smoke ^a
	[ng/session] (RSD, %), (n = 5)	[ng/g] (RSD, %), (n = 5)	[ng/cigarette] (RSD, %), (n = 5)	[ng/g] (RSD, %), (n = 5)	[ng/cigarette] (RSD, %), (n = 5)
NAT	103 (11)	48.3 (10)	175 (3.3)	2910 (5.5)	n.d.
NNK	46.4 (18)	41.1 (5.6)	101 (3.5)	798 (7.0)	98 (4.0)
NNN	34.3 (18)	18.4 (15)	137 (4.4)	3070 (8.0)	113 (9.2)
NAB	8.45 (14)	3.00 (17)	12.3 (3.6)	166 (5.1)	n.d.

NAT, (N-nitrosoanatabine); NNK, (4-(methylnitrosoamino)-1-(3-pyridinyl)-1-butanone); NNN, (N'-nitrosoanornicotine); NAB, (N-nitrosoanabasine); n.d., not determined; RSD, relative standard deviation.

^a Values determined in independent experiments by J.H. (Sigmaringen, Germany).

Table 3

Levels of COHb and nicotine in the blood, or of cotinine and NNAL in the 24-h urine of non-smokers, waterpipe smokers and cigarette smokers (mean \pm SD).

Analyte	Non-smokers	Waterpipe smokers	Cigarette smokers
	(SD), (n = 10)	(SD), (n = 10)	(min, max), (n = 2)
COHb (%)	1.2 (0.3)	17.1 (9.0)	3.4 (3.0, 3.8)
Nicotine (μ g/l)	n.d.	11.4 (9.3)	41 (30, 52)
Cotinine (μ g/24 h)	9.5 (9.4)	251 (270)	1238 (753, 1724)
NNAL (ng/24 h)	20.5 (22.4)	13.9 (11.3)	131 (73, 189)

NNAL, (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol); n.d., not determined; SD, standard deviation.

For method validation we determined the PAH concentrations in the smoke of 3R4F reference cigarettes. Comparison to the corresponding levels reported in the literature revealed good agreement except for naphthalene (see Table 4). All values for recovery, reproducibility, LOD and LOQ are provided as supplementary data (see Tables S-9.1 and S-9.2).

It was possible to detect all 16 EPA PAHs in the waterpipe smoke. Naphthalene (3200 ng/session) and phenanthrene (1330 ng/session) revealed with highest values in this matrix. Except for acenaphthene and fluorene all other EPA PAHs showed higher values in waterpipe smoke compared to cigarette smoke. For benzo[a]pyrene the difference between waterpipe smoke and cigarette smoke was found 3-fold.

4. Discussion

To analyze and quantify compounds released from waterpipes during smoking, we applied an adapted and reproducible smoking protocol according to the parameters worked-out in a field study by Shihadeh and coworkers where 52 waterpipe smokers in Beirut were systematically monitored (Shihadeh et al., 2004). We decided to use these smoking parameters since detailed informations for European waterpipe smokers were not available and also for better comparison of our results with published data.

It becomes obvious that the data generated for waterpipe smoking are completely different from those reported for the 3R4F reference cigarette. The TPM generated and inhaled during a single waterpipe session (about 2.71 g) is about 250-fold higher when compared to a single 3R4F reference cigarette (see Table 1). Our val-

ues are in the same range as previously reported by others. Using a plastic hose Saleh & Shihadeh found 2.86 g of TPM produced by one waterpipe (Saleh and Shihadeh, 2008).

A closer look on the composition of TPM generated revealed strong differences between waterpipe and cigarette smoke. While the percentage of water in TPM of cigarette smoke is only 8%, it is about 65% in the TPM of waterpipe smoke (see Table 1). Further, the amounts of nicotine detected in TPM are 10-fold higher for waterpipe smoke than for cigarette smoke, thus resulting in a serious exposure level of waterpipe smokers against this addicting constituent. However, the nicotine levels detected in the blood of waterpipe smokers and the cotinine excretion during a period of 24 h were not as high as for cigarette smokers (see Table 3). It is likely that cigarette smokers consume several cigarettes per day, thus resulting in a higher nicotine uptake. Further, the absorption of nicotine could be higher in the case of cigarette smoke. Nevertheless, waterpipe smokers revealed with considerable nicotine and cotinine levels and therefore it does not come as surprise that regular waterpipe use may lead to nicotine dependence, as has already been reported in the literature (Maziak et al., 2004b).

In addition to nicotine, CO reveals as further serious hazard in the smoke of the waterpipe. In comparison to the 3R4F reference cigarette, the amounts of CO measured in waterpipe smoke were about 30-fold higher (see Table 1). The results obtained in the biomonitoring study confirm high internal exposure of waterpipe smokers against CO. Here, the COHb values measured were 17% (see Table 3), thereby confirming older studies performed in Saudi-Arabia in the 1980s (Al Fayed et al., 1989; Zahran et al., 1985). COHb levels in this range were reported to correlate with mild symptoms of intoxication such as onset of nausea and headache (Kao and Nanagas, 2006). High levels of CO in waterpipe smoke entail the risk that users may get seriously intoxicated, in particular while being exposed in small and insufficiently ventilated rooms. Recently, a study demonstrated that during 4 h of waterpipe smoking the CO concentration monitored in a room of 57 m³ rose up to 65 ppm (Fromme et al., 2009), a value well beyond the maximal threshold limit value of 30 ppm applying for the work place (DFG, 2006).

The levels of NNN, NNK, NAB and NAT were lower in waterpipe smoke when compared to cigarette smoke (see Table 2). This

Table 4

Levels of PAHs in the smoke of the waterpipe or the 3R4F reference cigarette (mean values \pm RSD).

Analyte	Waterpipe	3R4F reference cigarette	3R4F reference cigarette ^a	3R4F reference cigarette ^b	Waterpipe ^c
	[ng/session] (RSD, %), (n = 7)	[ng/cigarette] (RSD, %), (n = 3)	[ng/cigarette] (RSD, %), (n = 7)	[ng/cigarette] (RSD, %)	[ng/session] (RSD, %), (n = 3)
Naphthalene	3200 (5.5)	34.5 (5.8)	n.d.	360.8 (10)	2130 (25)
Acenaphthalene	42.4 (13)	41.4 (3.5)	n.d.	71.6 (11)	180 (39)
Acenaphthene	25.3 (17)	47.4 (7.3)	n.d.	56.8 (7)	487 (20)
Fluorene	25.6 (7.4)	137 (5.3)	n.d.	189.2 (6)	437 (18)
Phenanthrene	1330 (14)	118 (11)	n.d.	138.9 (10)	2650 (17)
Anthracene	133 (18)	40.2 (5.0)	n.d.	62.3 (6)	493 (7)
Fluoranthene	354 (21)	40.0 (2.8)	n.d.	52.7 (11)	2380 (6)
Pyrene	410 (7.5)	40.9 (6.9)	n.d.	44.8 (16)	2510 (2)
Benz[a]anthracene	48.0 (18)	8.44 (8.9)	n.d.	14.1 (10)	677 (15) ^d
Chrysene	62.3 (17)	10.2 (7.0)	n.d.	16.2 (10)	
Benzo[b+k]fluoranthene	22.7 (15)	2.51 (1.0)	n.d.	7.6 ^e	370 (20)
Benzo[a]pyrene	15.7 (26)	5.48 (6.4)	6.9 (5.5)	6.6 (7)	307 (20)
Indeno[1,2,3-cd]pyrene	13.1 (22)	2.03 (10)	n.d.	3.8 (12)	183 (44)
Dibenz[a,h]anthracene	8.71 (16)	0.91 (8.3)	n.d.	n.d.	147 (22)
Benzo[g,h,i]perylene	17.7 (30)	1.95 (1.5)	n.d.	1.9 (11)	140 (31)

n.d., not determined; RSD, relative standard deviation.

^a Values determined in independent experiments by J.H. (Sigmaringen, Germany).

^b From Tarrant et al. (2009).

^c From Sepetdjian et al. (2008).

^d Chrysene & benz[a]anthracene.

^e Combined values for benzo[b]fluoranthene (5.4 ng/cigarette (8)) and benzo[k]fluoranthene (2.2 ng/cigarette (15)).

is due to the much lower contents of TSNAs per gram waterpipe tobacco compared to cigarette tobacco. One reason for the low levels of TSNAs in waterpipe tobacco may be the addition of other ingredients which do not contain TSNAs naturally, such as, for example, humectants, flavors or molasses. The finding that one waterpipe smoking session generates less TSNAs compared to a single cigarette was also confirmed via biomonitoring. In the urine of waterpipe smokers we were unable to detect elevated levels of NNAL, that is, the lead metabolite of NNK (see Table 3). By contrast, the corresponding levels in the urine of the two cigarette smokers were found significantly elevated. The data obtained for NNAL in the urine of non-smokers and cigarette smokers are in good agreement with results published elsewhere (Kavvadias et al., 2009; Shepperd et al., 2009).

As with TPM, the calculated nicotine-free dry particulate matter (NFDPM, also called “tar”) is still much higher (about two orders of magnitude) in the waterpipe smoke compared to the 3R4F reference cigarette (see Table 1). Up to now there is only little data available on the composition of this matrix (Shihadeh and Saleh, 2005). Applying a GC-FID method enabled us to identify and to quantify a considerable part of the former unknown tar, as humectants. The percentage of humectants measured in waterpipe smoke tar was about 67%, indicating that the majority of humectants added to waterpipe tobacco is just getting vaporized, not burned. However, both propylene glycol as well as glycerol are no innocuous compounds when inhaled. Consumers are exposed to a smoke that contains 4.70 mg glycerol and 2.34 mg propylene glycol per liter volume. In inhalation studies in rats glycerol concentrations of 1.0, 1.93 and 3.91 mg per liter (exposure for 6 h/day and 5 days/week for 2 weeks) led to adverse effects such as minimal to mild squamous metaplasia of the epithelium lining the lower (laryngeal) surface of the epiglottis (Renne et al., 1992). Subchronic exposure of rats (6 h/day and 5 days/week, 13 weeks) in the same study led to the identification of a so-called no-observed-adverse-effect level (NOAEL) of 0.167 mg glycerol per liter air. Thus, the exposure of consumers while smoking a waterpipe reveals 28-fold higher when compared to this threshold level identified in rats. Similar experiments have been performed with propylene glycol in rats. After subchronic and nose-only exposure of animals to 0.16, 1.0 or 2.2 mg propylene glycol/l air (6 h/day and 5 days/week, 13 weeks) adverse effects such as nasal hemorrhage and ocular discharge were observed at all concentrations tested. Starting at 1.0 mg propylene glycol per liter there was a significant increase in the numbers and mucin contents of goblet cells in the nasal cavity as well as decreasing lymphocyte counts in female rats (Suber et al., 1989). Furthermore, a study in volunteers exposed to propylene glycol mist (0.31 mg/l air) revealed increased ocular and throat symptoms and slightly reduced forced expiratory volumes (Wieslander et al., 2001). Similar as with glycerol, waterpipe smokers are exposed to much higher concentrations of propylene glycol when compared to doses that start to trigger adverse effects in experimental studies. Therefore, both humectants present in waterpipe smoke cause concern due to their high concentrations.

The determination of a range of different PAHs revealed that some of these analytes emerge in high concentrations in the smoke of the waterpipe (see Table 4). For benzo[a]pyrene, that is, the carcinogenic lead compound among environmentally important PAHs, the difference between waterpipe smoke and cigarette smoke is 3-fold (see Table 4). PAH levels in the waterpipe smoke have recently been released (Sepetdjian et al., 2008). For instance, the concentration of benzo[a]pyrene determined in our study was 20-fold lower than the value reported by Sepetdjian and coworkers (see Table 4). Since we applied our method twice in two independent measurements of filters generated with cigarette smoke, and both measurements were in accordance with data published by Tarrant and colleagues (Tarrant et al., 2009), we are confident that the val-

ues presented for benzo[a]pyrene and other PAHs in the waterpipe smoke are reliable. Since charcoal has revealed as important source for the generation of PAHs in waterpipe smoke (Monzer et al., 2008), it becomes advisable to use charcoal brands that only produce low amounts of PAHs or to replace the charcoal completely by an electrical heating device.

In summary, the present work reliably demonstrates that the main part of TPM released into the waterpipe smoke consists of chemicals like water, glycerol or propylene glycol. High amounts of the humectants glycerol and propylene glycol emerging in the waterpipe smoke, however, cause some safety concern. Furthermore, there are also other harmful compounds detectable such as nicotine, TSNAs, PAHs or CO, some of them at significantly higher levels when compared to the smoke of cigarettes.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.toxlet.2011.06.017.

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