

Measurement of 16 polycyclic aromatic hydrocarbons in narghile waterpipe tobacco smoke

Elizabeth Sepetdjian^a, Alan Shihadeh^b, Najat A. Saliba^{a,*}

^a Department of Chemistry, American University of Beirut, Beirut, Lebanon

^b Department of Mechanical Engineering, American University of Beirut, Beirut, Lebanon

Received 24 May 2007; accepted 30 December 2007

Abstract

An analytical method for the determination of 16 polycyclic aromatic hydrocarbons (PAHs) in the mainstream of narghile smoke is presented. The smoke was generated using a digital waterpipe smoking machine connected to the mouthpiece of a narghile that was loaded with 10 g of a popular flavored tobacco and kept alight with quick-light charcoal briquettes that are commonly used for this purpose. A standard smoking regimen consisting of 171 puffs of 530 ml volume and 2.6 s duration spaced 17 s apart was used, and the smoke condensates were collected on glass fiber filters. PAHs were extracted with toluene assisted by sonication. For purification, the extract was passed through a silica cartridge and eluted with hexane. The eluent was preconcentrated, reconstituted in acetonitrile, and analyzed using a GC–MS–SICP method. The method showed good selectivity, repeatability, accuracy and sensitivity. The limit of detection ranged from 15 to 96 ng for benzo[*a*]pyrene and indeno[1,2,3-*cd*]pyrene, respectively. It was found that a single narghile smoking session delivers approximately 50 times the quantities of carcinogenic 4- and 5-membered ring PAHs as a single 1R4F cigarette smoked using the FTC protocol. The pattern of PAH concentrations suggested that formation pathways differ from those of the cigarette, possibly reflecting the differing combustion conditions of the two smoking devices.

© 2008 Elsevier Ltd. All rights reserved.

Keywords: PAH; Narghile smoke; Ma'assel tobacco; Carcinogens; Analytical separation tobacco smoke

1. Introduction

A sharp rise in the popularity of the narghile waterpipe has been noted in recent years in regions where it is culturally rooted, as well as in Europe and North America, where waterpipes are drawing new and young smokers (Memon et al., 2000; Mohamed et al., 2003; Shediach-Rizkallah et al., 2002; Smith-Simone, 2007; WHO, 2005). The new appeal of the narghile waterpipe appears to be correlated with the marketing of an array of heavily flavored and sweetened tobacco mixtures (Rastam et al., 2004), which list “molasses” as an ingredient, and which burn with a strong aroma of caramelizing sugar. These flavored and

sweetened tobacco mixtures are commonly referred to as *ma'assel*. Despite its long history and recent revival, there have been few studies on narghile smoke constituents (Hoffman et al., 1963; Rakower and Fatal, 1962; Sajid et al., 1993; Shihadeh, 2003; Shihadeh and Saleh, 2005), and standard methods and instruments for its study have yet to be developed.

A recent study by Shihadeh and Saleh (2005) attempted to analyze the polycyclic aromatic hydrocarbons (PAHs) in *ma'assel* narghile smoke condensates using an HPLC method previously developed for cigarette smoke analysis (Brunnemann et al., 1994). It was found that the chromatograms were heavily populated with overlapping peaks that made it possible to quantify only three PAH compounds: phenanthrene, fluoranthene, and chrysene, all 3- or 4-ring compounds. None of the more carcinogenic 5-ring compounds could be identified. The authors speculated

* Corresponding author. Tel.: +961 1 350000; fax: +961 1 365217.
E-mail address: ns30@aub.edu.lb (N.A. Saliba).

that the large quantities of flavorings in *ma'assel* introduced interferences not normally seen in cigarette smoke chromatograms, and concluded that a method specific to the flavored tobacco mixtures needed to be developed.

The purpose of the current study was to develop a method capable of quantifying 16 PAH compounds in *ma'assel* narghile smoke, and to use it to report PAH yields from a standard waterpipe machine smoking regimen.

2. Experimental

The PAHs analyzed in this study were naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[*a*]anthracene, benzo[*k*]fluoranthene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, benzo[*g,h,i*]perylene, di-benzo[*a,h*]anthracene, indeno[1,2,3-*cd*]pyrene. Sixteen EPA priority pollutant PAH standards of 2 mg/ml of methylene chloride was obtained from Absolute Standard Inc. (USA). The glass fiber filters

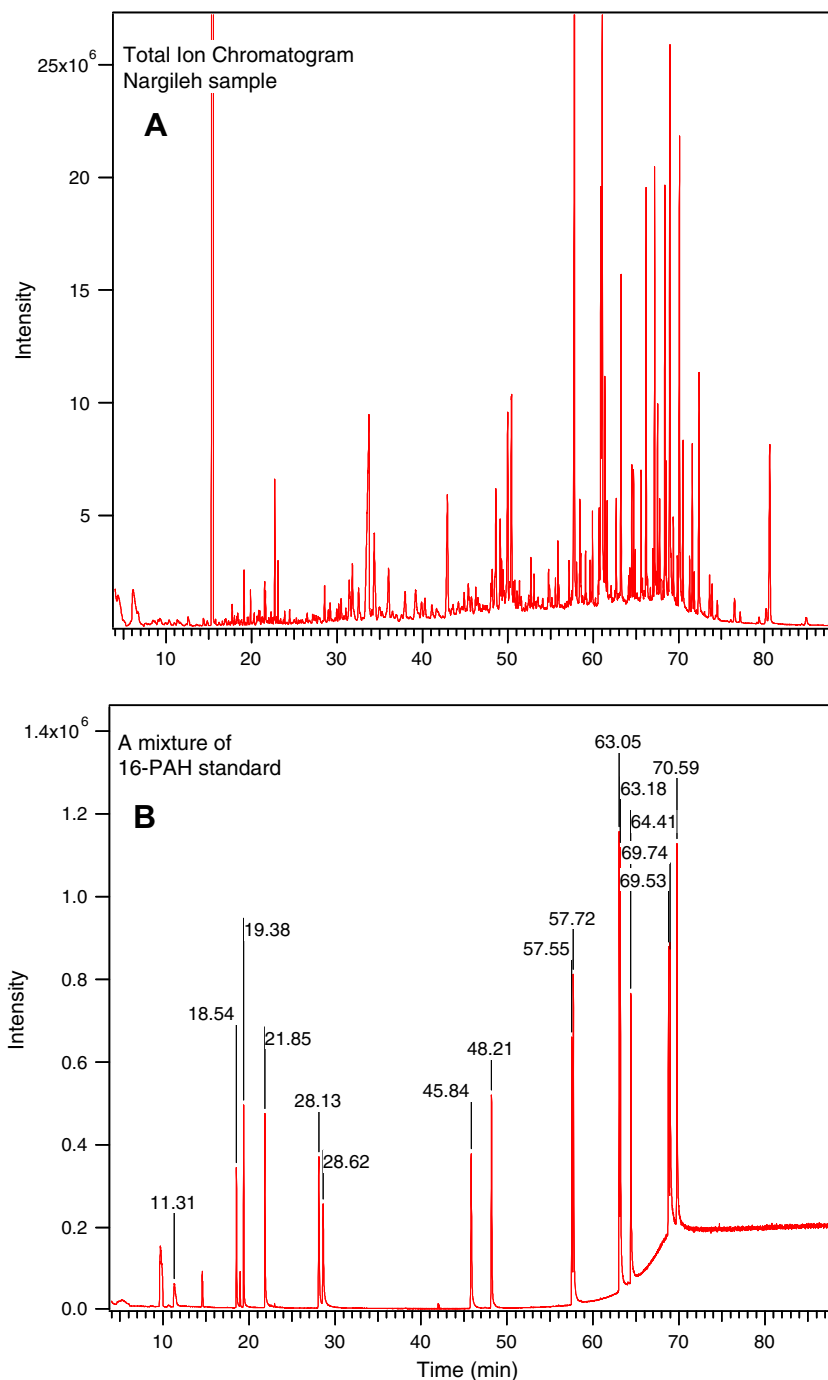


Fig. 1. Comparison between the total ion chromatogram of the narghile sample (A) and the chromatogram of the 16-PAH standard mixture (B).

(47 mm, Gelman type A/E) used for the collection of the total suspended particles (TSP) generated from tobacco smoke, and the silica solid phase extraction (SPE) cartridges (1000 mg/8 ml capacity) used for clean-up were obtained from Alltech (USA). Chemicals and solvents of analytical grade were purchased from Sigma–Aldrich.

2.1. Smoke generation and sampling

The smoke generation and sampling procedures were identical to those presented in *Shihadeh and Saleh (2005)*. In brief, a digital waterpipe smoking machine was programmed to produce a smoking regimen consisting of 171 puffs of 530 ml and 2.6 s duration spaced 17 s apart. These puffing parameters were derived from a field study in which topographies of 52 smokers in a café in Beirut were recorded, and represent an “average” waterpipe smoking session (*Shihadeh et al., 2004*). Ten grams of “two apples” flavor (Nakhla brand, Egypt) tobacco mixture was loaded in the waterpipe head during each smoking session, and covered with a perforated aluminum foil. A single quick-light charcoal disk (Three Kings, Holland) was lit and placed atop the perforated foil at the beginning

of the smoking session, and a second 1/2 disk was added after the 105th puff. The smoke exiting the waterpipe mouthpiece was split into two parallel streams and each stream drawn through a 47 mm glass fiber filter (Gelman type A/E) to collect the particulate phase. To avoid overloading, six pairs of filters were used each smoking session, with filters swapped at 40, 60, 80, 105, 140 puffs. The filters from three replicate machine smoking sessions were analyzed for PAH content.

Total particulate mass collected on each filter was determined gravimetrically by weighing the filter/holder assembly before and after it was used. At the end of each smoking session, after post-weighing, each filter was removed from the holder and stored in the dark in an airtight container at -4°C until extraction. Extraction typically was performed less than 24 h after collection.

2.2. Sample extraction and cleanup

Each filter was removed from its container and placed in a 24 ml vial and sonicated ($f = 40\text{ kHz}$) in 15 ml of toluene, for 2 h at a temperature of 50°C . After sonication, the glass fiber filters were removed and the volume of each vial

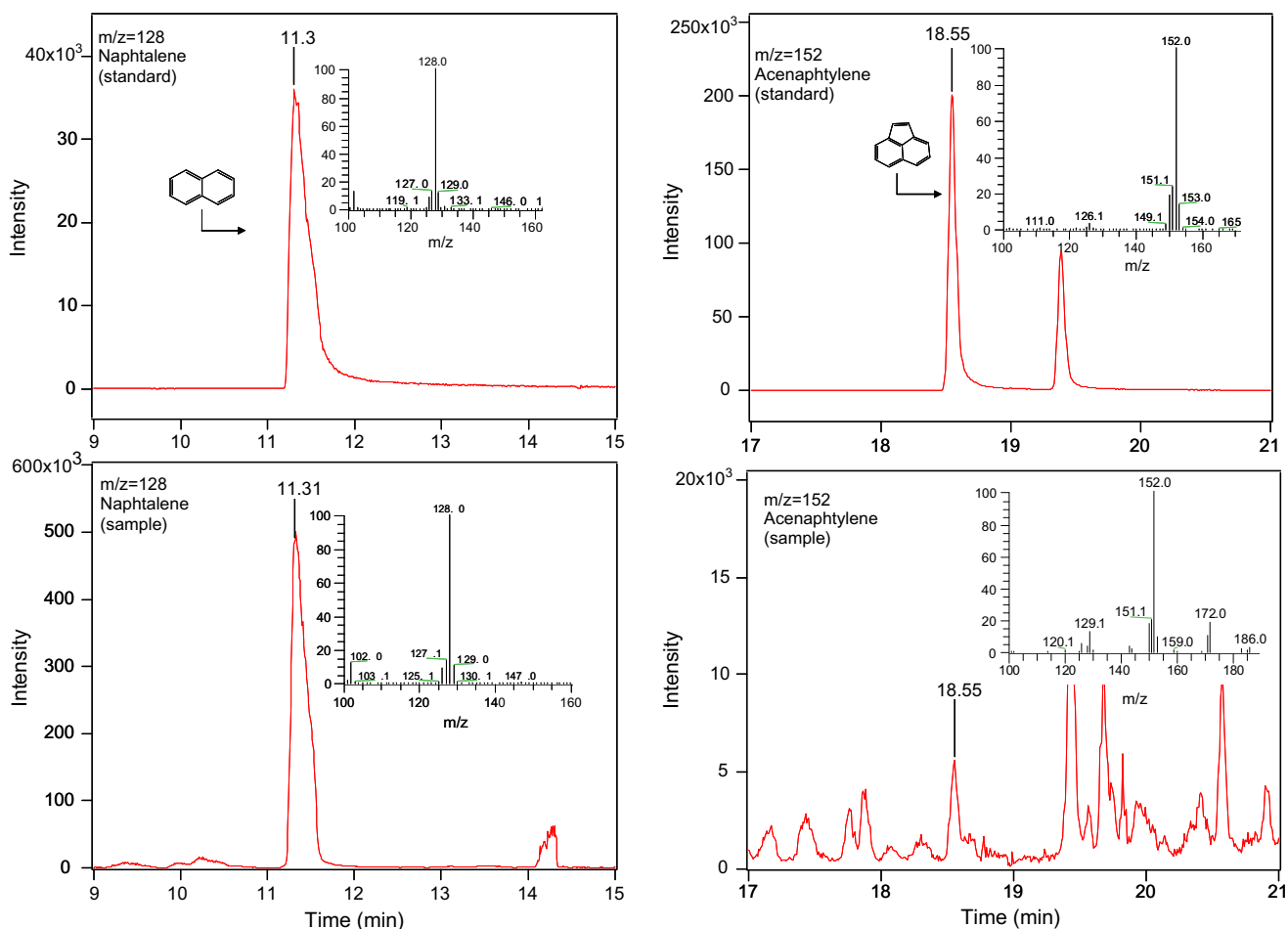


Fig. 2. GC chromatograms for the 16-PAH standard solution and the extracted ion GC chromatograms for the identified PAH in a narghile sample solution. The corresponding MS of the standards and the identified compounds in the samples are inserted.

containing the extracted solution was reduced to 1 ml under a gentle flow of N_2 . The resulting preconcentrated samples were loaded onto separate SPE silica cartridges of 1000 mg/8 ml capacity. It should be noted that dichloromethane and a 50:50 mixture of dichloromethane:toluene were also investigated as extraction media, and it was found that neat toluene provided the highest recoveries for PAH compounds in narghile sampled filters.

The cartridges were preconditioned each with 10 ml of hexane and the loaded samples were eluted using another 10 ml of hexane. Each eluent, collected in a 24 ml vial, was concentrated by reducing the solvent to 1 ml using a gentle flow of N_2 . The concentrated extracts from every four filters of a given smoking session were combined to form a single sample and the sample solution was then evaporated to dryness under a gentle flow of N_2 . It was reconstituted in 0.1 ml of acetonitrile and an aliquot of 1 μ l was injected into the GC. The recovery of the samples was also assessed by an alternative method in which the volume of the solution was reduced to approximately 100 μ l, and adjusted, using a microsyringe, to an exact volume before injection for the calculation of the injected concentration. With this alternative method, similar recoveries were obtained.

2.3. GC–MS instrument and conditions

A Thermo-Finnigan Trace GC-2000 Polaris QMS equipped with a binary pump and a thermostatically controlled AI 3000 autosampler was used in this study. Chromatographic separation was achieved with an Alltech ATTM-5 ms column (30 m \times 0.25 mm, 0.25 μ m film thickness). The mobile phase was helium gas flowing at a constant 1.0 ml/min through the column. A splitless injection volume of 1 μ l was utilized. The injector temperature was set at 290 $^{\circ}$ C and the initial column temperature was set at 80 $^{\circ}$ C for an initial holding time of 4 min. Following this initial holding time, the column was heated at 5 $^{\circ}$ C/min to 170 $^{\circ}$ C, where it was held for 20 min prior to resuming heating at 5 $^{\circ}$ C/min to 300 $^{\circ}$ C. It was held at this temperature for an additional 20 min. The total analysis time was 88 min.

Mass spectrometric detection was performed using an external ion source operated in electron impact ionization mode with an ionizing energy of 70 eV and quadrupole ion trap MS analyzer (Polaris Q-MS) scanning from m/z 100 to 300 at 0.52 s per scan. The ion source temperature was 225 $^{\circ}$ C. Data acquisition was set on a scan mode between 100 and 300 amu. Quantification was done using

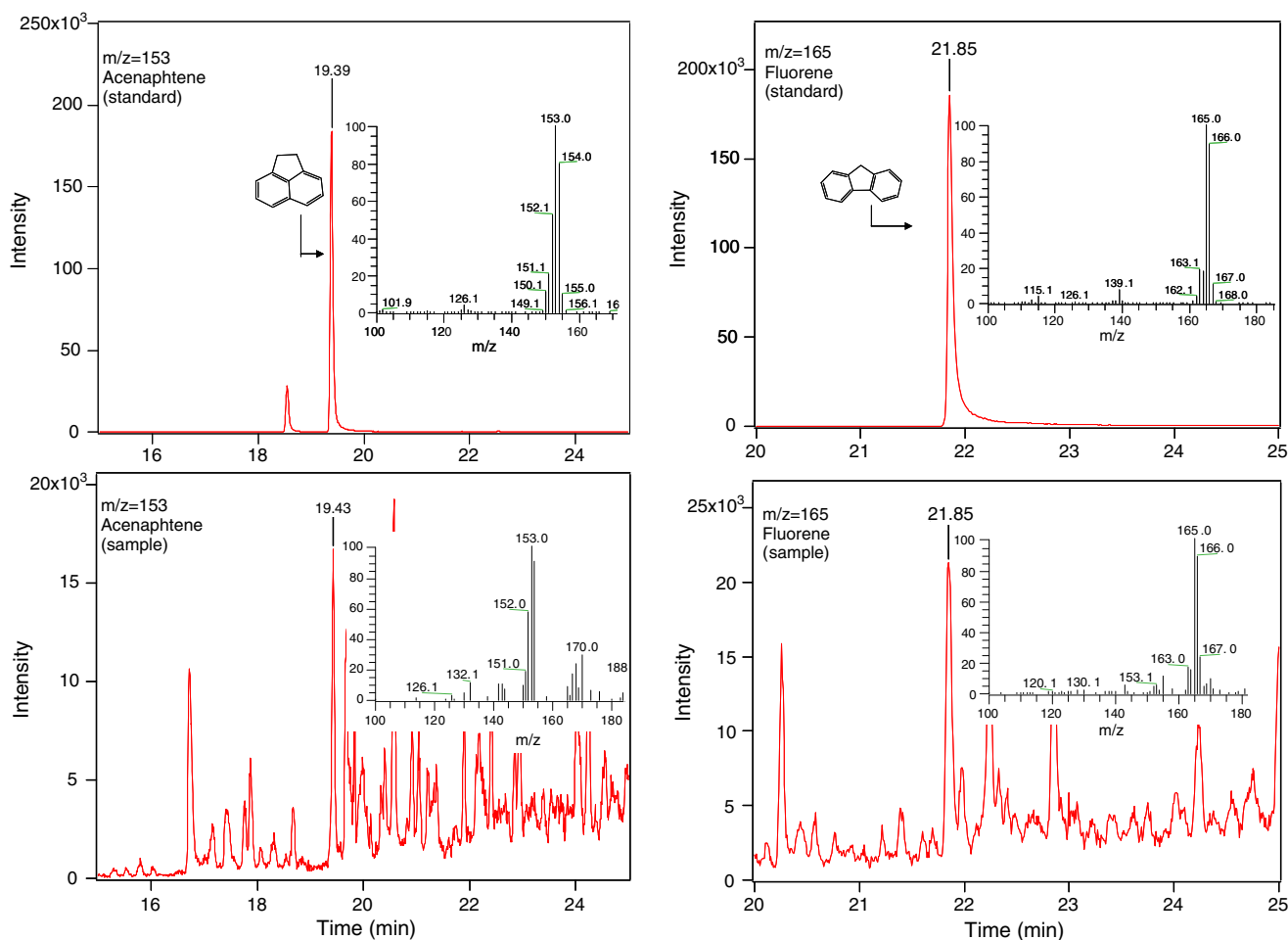


Fig. 2 (continued)

selected ion current profile (SICP), which provides the intensities of each monitored ion mass-to-charge ratio as a function of time. SICP is commonly used to isolate peaks of specific m/z in crowded chromatograms. Analyses were performed using one target ion (the molecular ion), and their abundances were determined by comparison to injection of standards under the same chromatographic conditions. Collected peaks were fitted to Gaussian profiles before integration using the Grams© software.

3. Results

3.1. Specificity

The total ion chromatograms of the narghile analyte and reference standards for the 16 PAHs that were identified by ion mass-to-charge ratio and quantified during the analysis are shown in Fig. 1. In comparison to the reference standard, the total ion chromatogram of the mainstream smoke extract showed more complexity because of additional background and chemical interferences. However, the method yielded selected ion chromatograms which were compared to the PAH standards demonstrated good separation for the 14 analytes (Fig. 2). In order to avoid any

fluctuation in the retention times between the sample and the standards due to a variation in the column over time, a standard calibration curve was prepared for every narghile sample. On any given day, all our standards gave RT's repeatable to less than 0.1 min as shown in Fig. 2. Good sensitivity and chromatographic resolution was exhibited for all analytes, except for the overlapping peaks of chrysene/benzo[*a*]anthracene, and the benzo[*k*] and [b]fluoranthenes. These overlapping components were subsequently quantified by combining their chromatogram areas and reporting the total concentrations.

3.2. Linearity

The linearity of the method was assessed by regression of selected ion peak area to PAH concentration (0.3, 0.5, 1, 2, 3, 4 and 5 ppm) in the injected sample for each of the 16 PAH standards. Correlation coefficients (R^2) ranged between 0.985 and 0.999.

3.3. Recovery and repeatability

Recovery and repeatability were estimated by extracting and analyzing blank filters that had been spiked with

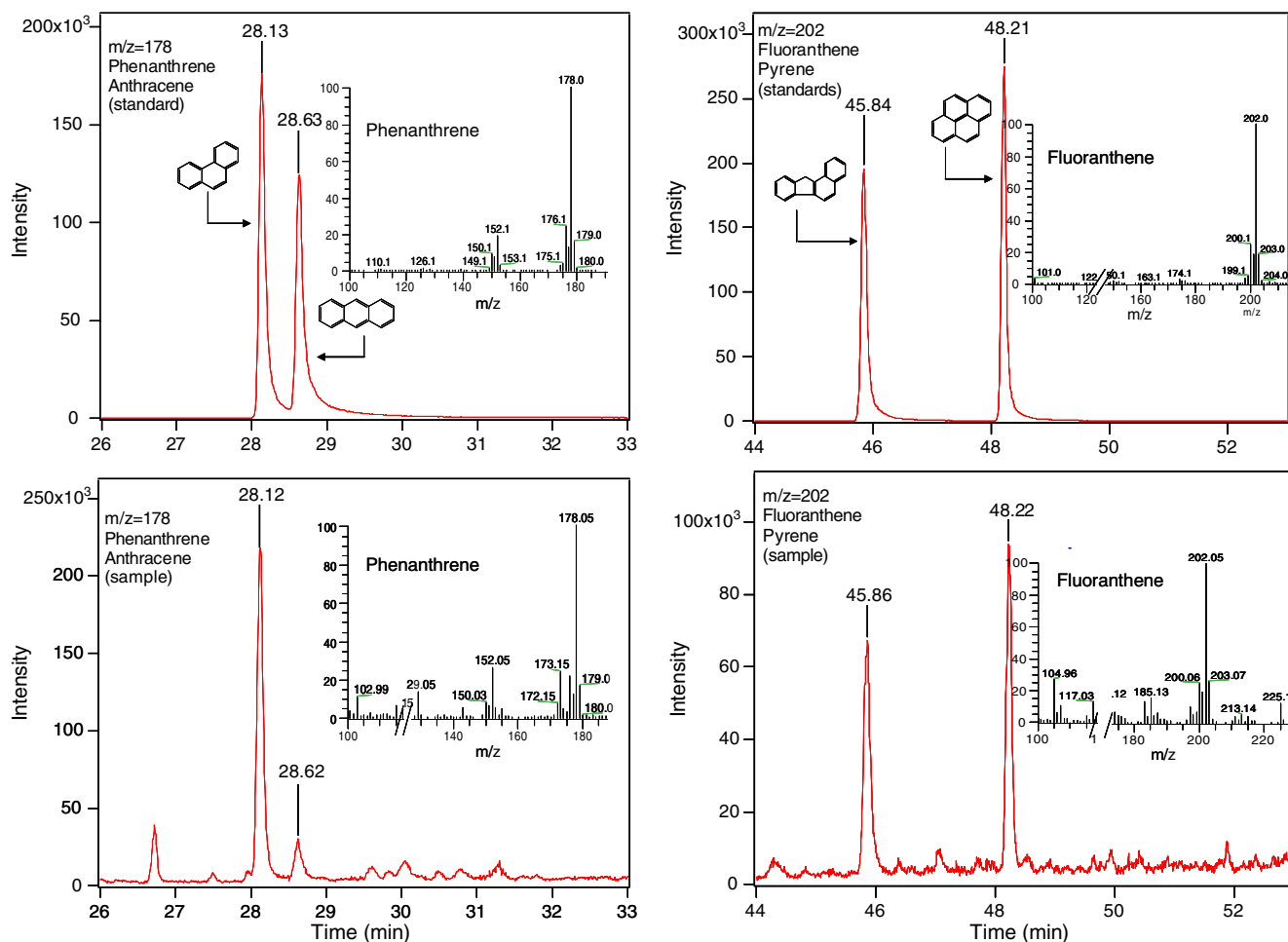


Fig. 2 (continued)

known quantities of the PAH standards. Four replicate solutions, each of 0.5, 1, 2 and 4 ppm concentration were prepared and spiked onto glass fiber filters. These filters were subjected to the complete extraction and analysis procedure in order to assess the average recovery and relative standard deviation at each concentration based on the areas of the selected ion peaks of the GC–MS chromatograms.

As shown in Table 1, the recovery fraction exhibits a sigmoidal relationship with PAH concentration, with a rapid rise in recovery occurring between 2 and 4 ppm. This may be attributed to the fact that solvent droplets adsorbed onto a larger number of particles will retain the analyte during evaporation and, hence, minimize their losses (Ferreira et al., 1995).

The RSD, a measure of repeatability, for each of the PAH compounds is also given in Table 1. It varied between 0% and 10% for lower molecular weight PAHs and between 10% and 20% RSD for high molecular weight PAHs for 0.5, 1 and 2 ppm PAH solutions. At 4 ppm, the RSD dropped to less than 10% for all the 16 PAHs except for di-benzo[*a,h*]anthracene which yielded an 11.2% RSD.

3.4. Limit of detection

The instrumental limit of detection (LOD) for each PAH in the validated spiked method was estimated from the corresponding calibration curve as three times the standard deviations from the three lowest concentrations extrapolated to zero concentration (Harris, 2003). The calculated LOD of PAHs ranged from 15 to 267 ng of naphthalene as shown in Table 2.

3.5. Levels of PAH in narghile smoke

Smoke from three replicate smoking sessions was sampled and analyzed as described above. The TPM mass collected and quantity of tobacco burned during each of these sessions is summarized in Table 3. The inter-trial variations in these variables are inherent to the hand-prepared water-pipe and have been previously documented (Shihadeh, 2003).

The PAH concentrations in the filter extracts for each of the three trials were determined according to the method described above. Concentrations were converted to yields (reported as ng per smoking session) by linear interpolation

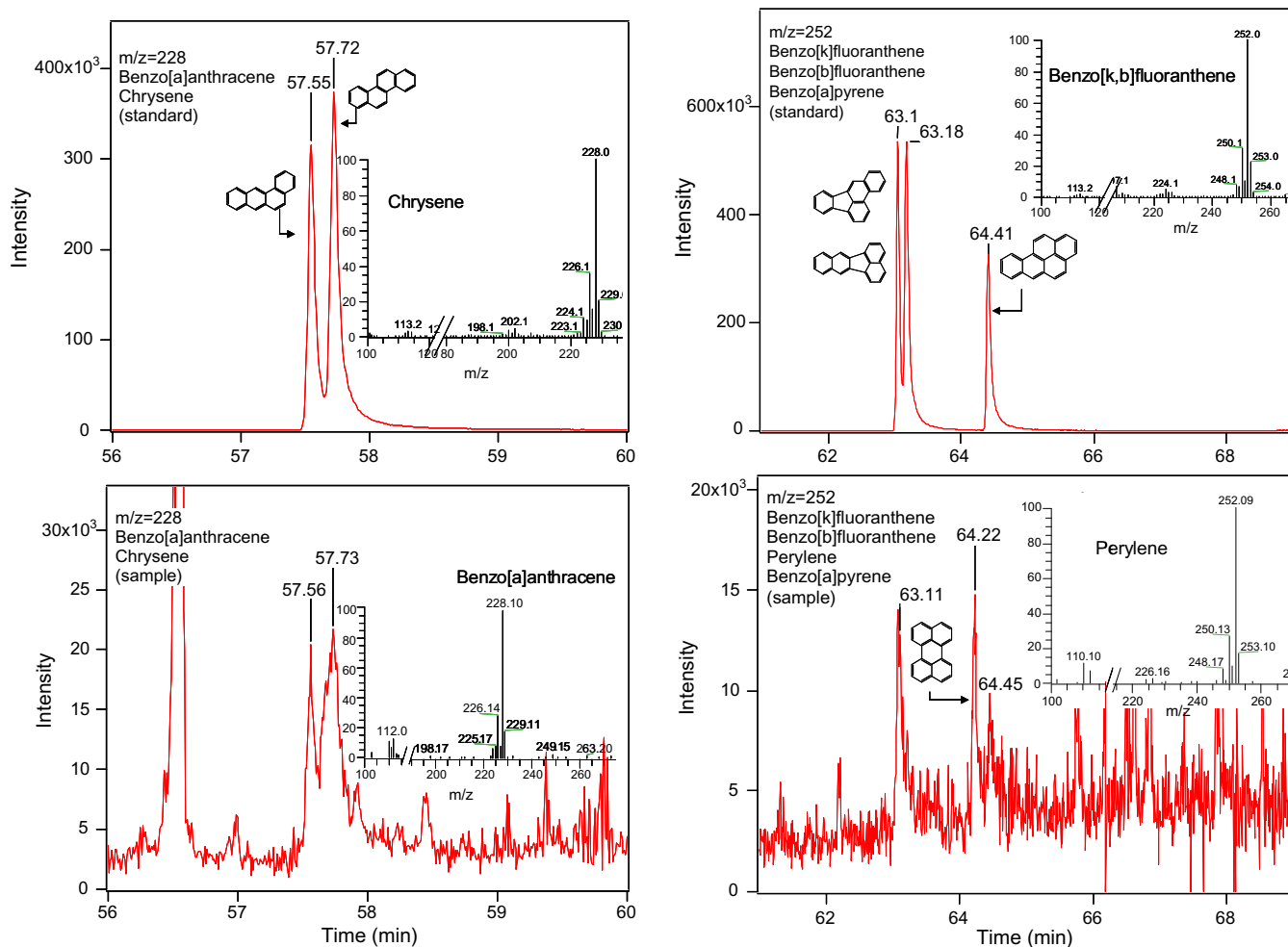


Fig. 2 (continued)

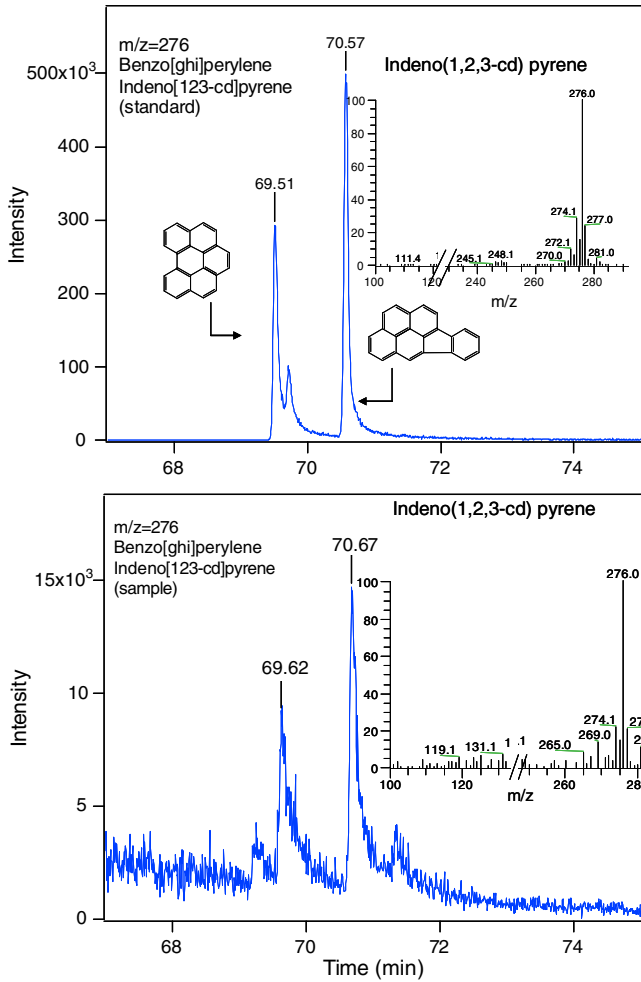


Fig. 2 (continued)

Table 1
Recovery percent for 0.5, 1, 2, and 4 ppm PAH solutions

PAH	Recovery% (RSD,%)			
	0.5 ppm	1 ppm	2 ppm	4 ppm
Naphtalene	61.0(4.5)	54.0(10.0)	110.5(7.4)	88.7(5.4)
Acenaphthalene	43.0(8.2)	40.1(12.1)	62.7(9.4)	68.9(4.5)
Acenaphthene	60.5(5.8)	57.9(20.1)	62.9(9.8)	66.5(4.9)
Fluorene	47.5(5.1)	44.9(9.4)	74.3(8.4)	77.3(7.8)
Phenanthrene	42.4(3.6)	40.2(10.5)	72.7(6.0)	74.9(6.0)
Anthracene				
Fluoranthene	31.6(13.6)	33.5(15.5)	53.3(1.2)	64.6(3.4)
Pyrene	29.1(18.8)	32.3(14.4)	59.3(1.1)	70.3(5.6)
Chrysene +	34.0(10.6)	26.4(16.9)	60.8(14.4)	71.8(2.5)
benzo[a]anthracene				
Benzo[k]fluoranthene	32.6(11.3)	33.1(18.2)	64.8(21.7)	70.4(5.3)
benzo[b]fluoranthene				
Benzo[a]pyrene	30.8(8.4)	38.1(12.6)	70.2(21.3)	78.3(8.4)
Benzo[g,h,i]perylene	20.4(6.8)	20.6(15.9)	58.1(20.5)	62.5(6.0)
Di-benzo[a,h]anthracene	19.4(7.8)	23.9(13.6)	53.7(18.8)	60.3(11.2)
Indeno[1,2,3-cd]pyrene	30.3(12.0)	30.6(19.3)	53.5(16.4)	58.3(7.3)

Each measurement was repeated four times. Relative standard deviation shown in parentheses.

of recovery fractions reported in Table 1. The resulting yields are presented in Table 4, where it can be seen that

Table 2

The limit of detection (LOD) in ng of PAH for each PAH present in the reference standard mixture

PAH compound	LOD (ng)
Naphtalene	267
Acenaphthalene	30
Acenaphthene	57
Fluorene	66
Phenanthrene	39
Pyrene	36
Fluoranthene	39
Chrysene	21
Benzo[k]fluoranthene	57
Benzo[a]pyrene	15
Di-benzo[a,h]anthracene	84
Benzo[g,h,i]perylene	36
Indeno[1,2,3-cd]pyrene	96

Table 3

Particulate matter collected and tobacco burned during three repeated 171-puff narghile machine smoking sessions

Filter	Puff number at filter change	Particulate matter collected (mg)		
		Trial 1	Trial 2	Trial 3
1	40	96.4	140.1	143.5
2	40	118.2	152	164.7
3	60	174.6	148.1	172.6
4	60	191.2	163.7	196.8
5	80	116.2	107.6	149.7
6	80	93.7	134.2	172.1
7	105	128.9	110.6	154.0
8	105	143.4	142.0	178.1
9	140	173.2	190.3	192.5
10	140	183.0	204.2	191.4
11	171	110.2	128.6	97.4
12	171	87.3	151.3	132.1
Total yield (mg)		1596	1773	1945
Tobacco burned (mg)		4951	5239	5093

Table 4

Mean PAH yields in narghile smoke generated from three replicate 171-puff smoking sessions

PAH	IARC classification	Narghile ng/session (RSD, %)	1R4F cigarette ng/cig
Naphthalene	2B	2130 (25)	236
Acenaphthylene	–	180 (39)	50.4
Acenaphthene	3	487 (20)	25.3
Fluorene	3	437 (18)	119
Phenanthrene	3	2650 (17)	110
Anthracene	3	493 (7)	38.1
Fluoranthene	3	2380 (6)	46.2
Pyrene	3	2510 (2)	33.2
Chrysene + Benz[a]anthracene	2B	677 (15)	35
Benzo fluoranthenes	2B	370 (20)	10.1
Benzo[a]pyrene	1	307 (20)	7.9
Benzo[g,h,i]perylene	3	140 (31)	2.5
Di-benzo[a,h]anthracene	2A	147 (22)	0.6
Indeno[1,2,3-cd]pyrene	2B	183 (44)	3.5
Total PAH with biological activities (ng/session)	1	307	7.9
	2A	147	0.6
	2B	3360	284.6
	3	9097	374.3

Kentucky reference cigarette shown for comparison (Gmeiner et al., 1997).

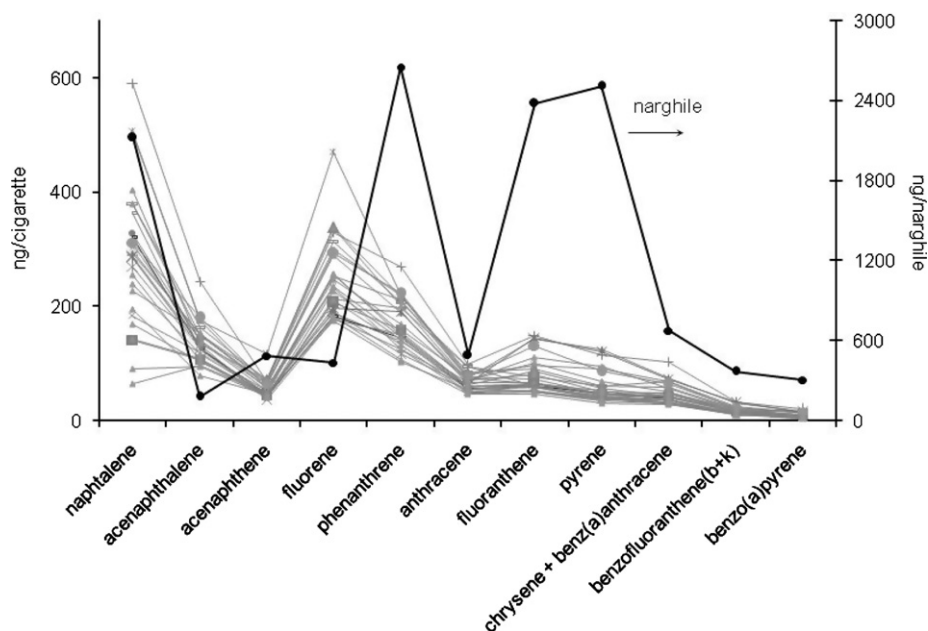


Fig. 3. Comparison of PAH profiles for narghile (this study) and 26 types of cigarettes (Ding et al., 2005).

narghile smoke delivers a range of PAHs. In comparison with previously reported data (Shihadeh and Saleh, 2005), this method shows higher specificity and sensitivity since higher number of PAHs and larger amounts of the identified compounds were detected.

Compared to a single 14RF cigarette tested using FTC parameters, a narghile smoking session delivers circa 20 times the total PAH yields, and 50 times the heavy (4- and 5-ring) PAH. Given that most of the 4- and 5-ring compounds quantified here are listed as IARC Class 1 or 2 carcinogens, serious health implications are evident for narghile smokers. While equivalency to the PAH content of 50 cigarettes appears startling, this figure should be considered in light of the fact that approximately 290 times more smoke volume is generated in a narghile use session than for a cigarette smoked using FTC parameters. Thus per unit volume of smoke, the narghile actually provides circa one-sixth the 4- and 5-ring PAH potency. This difference is likely due to the differing combustion conditions and ingredients for the two smoking devices (Shihadeh, 2003). The differing pattern of PAH distributions, shown in Fig. 3, also suggest that PAH sources and formation mechanisms in the cigarette and narghile differ.

4. Conclusions

A method for measuring 16 PAH compounds in the heavily flavored smoke of the narghile waterpipe has been demonstrated. Despite the crowdedness of the narghile smoke matrix, the extraction method showed high selectivity and the GC/MS selected ion chromatograms resulted in good separation for the different PAHs except for the overlapping peaks of chrysene and benzo[*a*]anthracene and benzo[*k*] and [b]fluoranthenes. This method was also accu-

rate with low LOD except for naphthalene; the lowest molecular weight PAH which might have evaporated during the extraction and preconcentration processes. Moreover, method validation indicated acceptable repeatability with an RSD less than 20% and 10% at the low and high spike levels, respectively. A good precision was demonstrated at high concentrations with an average recovery of 70% as compared to around 40% at the lowest concentration. Results of three narghile smoking sessions showed that narghile smokers are exposed to large quantities of carcinogenic PAH, although the concentration of PAH per ml of smoke volume is considerably lower than that of a cigarette. Given that the peak temperatures previously measured in the tobacco are too low to support PAH formation chemistry (Shihadeh, 2003), it is likely that the PAHs measured here originated in the charcoal. Further research is needed to document the relative importance of charcoal in the production of PAHs, and whether PAH yields from the waterpipe vary considerably by charcoal type and user application regimen.

Conflict of interest statement

Nothing to declare

Acknowledgements

This work was funded by the University Research Board at the American University of Beirut, and by the Research for International Tobacco Control Secretariat of the Canadian International Development Research Centre. The authors would like to also thank Mr. Rawad Saleh for his assistance in operating the narghile smoking machine.

References

- Brunnemann, K., Hoffmann, D., Gairola, C., Lee, B., 1994. Low ignition propensity cigarettes: smoke analysis for carcinogens and testing for mutagenic activity of the smoke particulate matter. *Food Chem. Toxicol.* 32, 917–922.
- Ding, Y.S., Trommel, J.S., Yan, X.J., Ashley, D., Watson, C.H., 2005. Determination of 14 polycyclic aromatic hydrocarbons in mainstream smoke from domestic cigarettes. *Environ. Sci. Technol.* 39, 471–478.
- Ferreira, V., Fernandez, P., Melendez, J., Cacho, J., 1995. Analytical characteristics of sample evaporation with the Micro-Kuderna-Danish concentrator. *J. Chromatogr. A* 695, 41–55.
- Gmeiner, G., Stehlik, G., Tausch, H., 1997. Determination of 17 polycyclic aromatic hydrocarbons in tobacco smoke condensate. *J. Chromatogr. A* 767, 163–169.
- Harris, D.C., 2003. *Quantitative Chemical Analysis*, sixth ed. Freeman, New York.
- Hoffman, D., Rathkamp, G., Wynder, E., 1963. Comparison of the yields of several selected components in the smoke from different tobacco products. *J. Nat. Cancer Inst.* 31, 627–635.
- Memon, A., Moody, P., Sugathan, T., 2000. Epidemiology of smoking among Kuwaiti adults: prevalence, characteristics, and attitudes. *Bull. WHO* 78, 1306–1315.
- Mohamed, M., Gadalla, S., Kato, E., Israel, E., Mikhail, N., Lofredo, C., 2003. Water-pipe (Goza) smoking among males in rural Egypt. *Society for Research on Nicotine and Tobacco*, February 23.
- Rakower, J., Fatal, B., 1962. Study of narghile smoking in relation to cancer of the lung. *Brit. J. Cancer* 16, 1–6.
- Rastam, S., Ward, K.D., Eissenberg, T., Maziak, W., 2004. Estimating the beginning of the waterpipe epidemic in Syria. *BMC Public Health* 4, 1–5.
- Sajid, K.M., Akhter, M., Malik, G.Q., 1993. Carbon monoxide fractions in cigarette and hookah (hubble bubble) smoke. *J. Pakist. Med. Assoc.* 43, 179–182.
- Shediak-Rizkallah, M., Affi Soweid, R.A., Farhat, T., Yeretzian, J., Nuwayhid, I., Sibai, A., Kanj, M., El-Kak, F., Kassak, K., Kanaan, N., 2002. Adolescent health-related behaviors in postwar Lebanon: findings among students at the American University of Beirut. *Int. Quart. Commun. Health Edu.* 20, 115–131.
- Shihadeh, A., 2003. Investigation of mainstream smoke aerosol of the Argileh water pipe. *Food Chem. Toxicol.* 41, 143–152.
- Shihadeh, A., Azar, S., Antonius, C., Haddad, A., 2004. Towards a topographical model of narghile water-pipe café smoking. *Biochem. Pharmacol. Behav.* 79 (1), 75–82.
- Shihadeh, A., Saleh, R., 2005. Polycyclic aromatic hydrocarbons, carbon monoxide, “tar”, and nicotine in the mainstream smoke aerosol of the narghile water pipe. *Food Chem. Toxicol.* 43, 655–661.
- Smith-Simone, S., Maziak, M., Ward, K., Eissenberg, T., 2007. Waterpipe use on US college campuses. Poster presented to the 13th Annual Meeting of the Society for Research on Nicotine and Tobacco February, Austin.
- WHO, 2005. *Waterpipe Tobacco Smoking: Health Effects, Research Needs and Recommended Actions by Regulators*. TobReg Advisory Note. World Health Organization. Geneva.