Beiträge zur Tabakforschung International/*Contributions to Tobacco Research* Volume 23 · No. 2 · August 2008
DOI: 10.2478/cttr-2013-0851

Determination of Polycyclic Aromatic Hydrocarbons In Exhaled Cigarette Smoke*

by

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SUMMARY

The retention by humans of 20 polycyclic aromatic hydrocarbons (PAHs) from mainstream cigarette smoke was evaluated. The analysis was done by a new technique using solid phase extraction (SPE) for the cleanup and the concenration of PAHs. The new technique has excellent sensitivity and accuracy, which were necessary for the analysis of the very low levels of PAHs present in the exhaled cigarette smoke. The study was done on a common commercial cigarette with 10.6 mg 'tar' by U.S. Federal Trade Commission (FTC) recommendation. The results were obtained from ten human subjects, each smoking three cigarettes. The exhaled smoke was collected using a vacuum assisted procedure that avoids strain in exhaling. The study showed that the PAHs with a molecular weight lower than about 170 Daltons are retained with high efficiency. The heavier molecules are less retained, but even compounds such as indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene, and benzoperylene are retained with efficiencies around 50%. The dependence of retention efficiency for PAHs (in %) on their octanol-water partition coefficient ($LogP_{ow}$) was found to be nonlinear and showed considerable variability for several compounds that have very close $LogP_{ow}$ values. Better correlation was obtained between the retention efficiency and PAHs vapor pressure (Log VP). [Beitr. Tabakforsch. Int. 23 (2008) 85–97]

ZUSAMMENFASSUNG

Die Retention von 20 polyzyklischen aromatischen Kohlenwasserstoffen (PAHs) durch den Raucher aus dem Hauptstromrauch von Zigaretten wurde untersucht. Die Analyse wurde mit einem neuen Verfahren durchgeführt, bei dem zur Reinigung und Konzentrierung der PAHs Festphasenextraktion (Solid Phase Extraction, SPE) verwendet wurde. Dieses neue Verfahren zeichnet sich durch exzellente Sensibilität und Präzision aus, was für die Analyse der in sehr niedrigen Konzentrationen im exhalierten Zigarettenrauch vorkommenden PAHs notwendig ist. Es wurde eine handelsübliche Zigarette mit 10,6 mg Kondensat gemäß den Abrauchnormen der Federal Trade Commission (FTC) untersucht. Es wurden die Ergebnisse von 10 Rauchern ermittelt, die jeweils drei Zigaretten rauchten. Der exhalierte Rauch wurde in einem Vakuum-unterstützen Verfahren gesammelt, so dass ein Widerstand beim Exhalieren vermieden wurde. Die Untersuchung zeigte, dass die PAHs mit einem Molekulargewicht unter 170 Dalton in hohem Maße retiniert wurden. Die schwereren Moleküle wurden in geringerem Maße retiniert, aber selbst Moleküle wie Indeno[1,2,3-*cd*]pyren, Dibenz[*a,h*]anthracen, and Benzoperylen wurden zu etwa 50% retiniert. Es wurde festgestellt, dass die Korrelation zwischen der Retentionseffizienz der PAHs (in %) und ihr Oktanol-Wasser-Verteilungskoeffizienz ($LogP_{ow}$) nicht linear ist und bei mehreren Verbindungen mit geringen $LogP_{ow}$-Werten eine hohe Variabilität aufweist. Die Korrelation zwischen der Retentionseffizienz und dem PAH Dampfdruck (LogVP) war größer. [Beitr. Tabakforsch. Int. 23 (2008) 85–97]

RESUME

La rétention par le fumeur de 20 hydrocarbures aromatiques polycycliques (PAH) de la fumée principale de cigarette a été évaluée. Cette analyse a été conduite selon une nouvelle procédure, utilisant l'extraction en phase solide (SPE) pour la purification et dosage des PAHs. Cette nouvelle procédure possède une sensibilité et précision excellente, qui sont nécessaires pour l'analyse des hydrocarbures présents à moindre teneur dans la fumée de cigarette exhalée. L'étude

*Received: 22nd January 2007– accepted: 8th May 2007

a été réalisée avec une cigarette commerciale de 10.6 mg de goudron fumée selon la recommandation de la Federal Trade Commission (FTC). Les résultats ont été obtenus avec 10 fumeurs, chacun fumant trois cigarettes. La collecte de la fumée exhalée a été partiellement réalisée sous-vide pour éviter trop de résistances durant l'exhalation. L'étude a montré que les PAHs ayant un poids moléculaire inférieure à 170 Dalton sont retenues avec efficacité. Les molécules plus lourdes sont moins retenues, mais mêmes des composées comme l'indeno[1,2,3-*cd*]pyrène, le dibenz[*a,h*]anthracène et le benzoperylène sont retenus à environ 50%. La corrélation entre l'efficacité de rétention des PAHs (en %) et le coefficient de partage entre l'octanol et l'eau ($LogP_{ow}$) s'est révélée non linéaire et démontre une variabilité considérable pour plusieurs composants ayant des valeurs ($LogP_{ow}$) très proches. La corrélation obtenue entre l'efficacité de rétention et la pression de vapeur des PAHs (LogVP) est meilleure. [Beitr. Tabakforsch. Int. 23 (2008) 85–97]

INTRODUCTION

The retention by cigarette smokers of nicotine and of total particular matter (TPM) has been reported in the literature as early as 1908 (1) and continued to be thoroughly studied (2–16). However, very few published papers evaluated individual compounds in exhaled cigarette smoke. A recent study reported the retention efficiency in humans for 160 compounds that can be directly measured in the chromatographic profile of the particulate phase of cigarette smoke (17). The study showed that although all the compounds found in the delivered smoke were also present in the exhaled smoke, the composition of the exhaled smoke was different than that of the delivered smoke. Depending on their chemical nature, some compounds were almost completely retained, some compounds were partially retained, and some were retained very little. For example, long chain hydrocarbons (saturated or squalene type) and phytosterol type compounds were practically not retained by the smokers. However, in the chromatographic smoke profile study, only a limited number of compounds were analyzed, other compounds being left unexamined although they were known to be present in smoke. Such compounds required special procedures for analysis. In particular, for compounds known to have potential biological activity, the evaluation of their retention by the smokers was important, even if their level in smoke is very low. These compounds cannot be detected directly in a chromatographic profile of smoke, and require analytical techniques specific for their determination. For example, carbonyls can be measured in exhaled smoke using derivatization with 2,4-dinitrophenylhydrazine followed by high performance liquid chromatography (HPLC) analysis, as recently reported (18). Another class of compounds from smoke that requires a specific, more sensitive quantitative analytical procedure is that of polycyclic aromatic hydrocarbons (PAHs), which are found at very low levels in smoke. This study describes for the first time in literature the findings regarding the retention by humans of PAHs from mainstream cigarette smoke. For the analysis, a sensitive and accurate analytical technique was necessary. A considerable number of analytical techniques are reported in the literature for the quantitation of PAHs in particulate phase of cigarette smoke (19–29). These include procedures for sample preparation such as solvent extraction, solid phase extraction (SPE), simultaneous distillation and extraction (SDE), etc. and analytical techniques such as HPLC and gas chromatography (GC) with mass spectrometry (MS) detection. Some of these techniques allow only the analysis of benzo[*a*]pyrene (B*a*P), other were conducted for the analysis of a series of PAHs (26–28), or even for the analysis of PAHs with alkyl side chains (29). For the analysis of PAHs in exhaled smoke it was necessary to develop a more sensitive technique than the previous ones, which met the requirements for this measurement.

EXPERIMENTAL

The experiments for the evaluation of PAH retention by human smokers were done on a common commercial cigarette with 10.6 mg Federal Trade Commission (FTC) 'tar', where FTC 'tar' is defined as the weight of total wet particulate matter (TPM) minus the weight of nicotine and water when the cigarettes are smoked following U.S. FTC recommendations. These conditions require a puff volume of 35 mL, with a duration of 2 s and each puff taken at 60 s interval (30). [*Note*: The FTC cigarette smoking conditions regarding puff volume, puff duration, and puffing interval are identical with those recommended by the International Organization for Standardization (ISO) (31–33). However, FTC requires the adjustment of the smoking machine to a specific 'tar' value for an Industry Monitoring cigarette, while ISO requires a specific air velocity (200 mm s^{-1}) at a specified point for the smoking machine. Also, the conditioning of the cigarettes (30, 32) is slightly different between FTC and ISO]. The number of smokers used in the study was ten, with three cigarettes smoked by each subject, as further described. A relatively large variability noticed between smokers offered a wide range in the quantity of delivered smoke. The quantity of delivered PAHs was determined based on empirical correlation charts established between the level of PAHs in smoke and the level of nicotine in the cigarette butts. The correlation charts were obtained by machine smoking in conditions that generate different amounts of smoke, as described in detail further in this paper. The nicotine from the cigarette butt for each smoker was measured allowing the back calculation of the amount of delivered PAHs.

The analysis of PAHs in exhaled smoke employed an original GC/MS procedure with solid phase extraction (SPE) sample cleanup. The procedure was first evaluated on 2R4F and 1R5F Kentucky reference cigarettes for assuring the method accuracy and precision, and on a cigarette that heats instead of burning tobacco generating smoke very low in PAHs for adjusting the proper sensitivity range for the analytical method.

Collection of samples from smoking machine

The cigarettes tested for PAH analysis were a commercial cigarette with 10.6 mg 'tar', two Kentucky references 2R4F, and 1R5F (University of Kentucky, Tobacco Research and Development Center), and a cigarette that

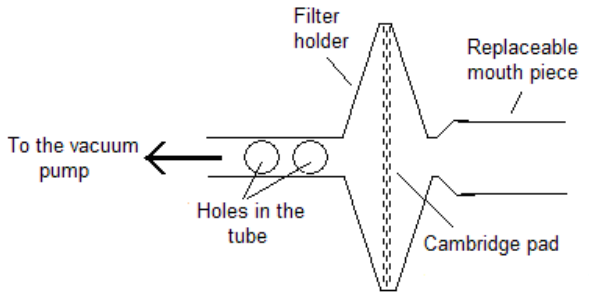

Figure 1. Schematic drawing of the device used for the collection of exhaled cigarette smoke

heats instead of burning tobacco. The 10.6 mg 'tar' cigarette was also used for the evaluation of the retention of smoke by humans. It was a filter commercial product of 83 mm, with American blend, 0.680 g tobacco, 27 mm filter, and 32% ventilation. The cigarette generated 10.4 mg CO.

For machine smoke collection, the smoking was done in conditions similar to those recommended by FTC (30) but using a Borgwaldt rotary machine RM20/CSR (Schnackenburgallee 15, 22525 Hamburg, Germany). The 10.6 mg 'tar' commercial cigarette was also smoked in several intensive conditions including 60 mL puff volume, with a puff duration of 2 s and each puff taken at 60 s interval (indicated as 60/60 conditions), 45 mL puff volume, with a puff duration of 2 s and each puff taken at 30 s interval (indicated as 45/30 conditions), and 60 mL puff volume, with a puff duration of 2 s and each puff taken at 30 s interval (indicated as 60/30 conditions). Except for the cigarette that heats instead of burning tobacco, the particulate phase that contains the PAHs was collected from five cigarettes on one 92 mm Cambridge pad. Ten cigarettes were used for the cigarette that heats instead of burning tobacco. The cigarette butts from the 10.6 mg 'tar' cigarettes were also collected for the analysis of nicotine content.

Exhaled smoke collection

The collection of exhaled smoke from the human subjects has been described previously (17). A vacuum assisted procedure has been used in order to avoid the excessive strain that would be necessary to otherwise overcome the flow resistance of the Cambridge pad. The device is schematically shown in Figure 1. It consists of a 92 mm Cambridge holder and pad having at one opening a replaceable mouth piece (Atlantic Medical Solutions, Charlotte, NC 28217), and at the other opening being connected to a diaphragm vacuum pump which aspirates 2.2 m^3/h (Vacuubrand GMBH, Wertheim, Germany). The tube connecting the pad holder to the pump has two large holes to the exterior, which can be covered with the fingers. When no smoke is exhaled, the holes in the tube to the vacuum pump are kept open such that air from the surrounding is aspirated by the pump without passing the Cambridge filter. During smoke exhaling, the smoker blows the smoke through the replaceable mouth piece. At the same time the holes in the tube are covered by the smoker, such that the exhaled smoke is aspirated through the Cambridge pad. This allows the exhaled smoke to be collected on the pad, without additional strain on the smoker. Through the pad and the connecting tubing, the pump achieves about 250 mL/s flow. The device shown in Figure 1 has been used by ten human subjects selected to smoke their preferred brand.

One concern regarding the collection of exhaled smoke was the modification of the collection efficiency due to a higher flow rate through the pad compared to that from a smoking machine (with a flow rate between 17.5 mL/ s and 30 mL/ s through the pad). To prove the efficiency for the retention with the collection device for exhaled smoke, a second filter holder with a Cambridge pad was connected in series to the vacuum pump. Also a cigarette holder was connected to the mouth piece end of the first filter holder. Using this setup, three commercial cigarettes with 10.6 mg 'tar' were smoked each for three intervals of 1 s with 30 s smoldering interval. On the first Cambridge pad it was weighed an average of 0.162 mg TPM (from two measurements) while the second Cambridge pad did not show any weight modification. It was concluded that no break-through of the particulate smoke occurs during smoke collection.

Each subject smoked three cigarettes within one hour (consecutively), and the exhaled smoke was collected. The smoking was performed in an environment familiar to the smoker (office) with as little as possible change from typical conditions. The cigarettes were the smoker's preferred brand. No measurements were made on inhalation volume or breath-hold duration. Also, no restriction regarding the butt length left on cigarette was made, and no restrictions regarding smoking before and after the experiment were imposed. The cigarettes were previously conditioned under FTC recommendations (30). The cigarettes butts from the smokers were collected for nicotine analysis. In addition to exhaled smoke, the breath without smoking was collected from one smoker (only) as a background check. The measurement was done by collecting on a Cambridge pad the exhaled air from 24 breaths (mimicking the number of puffs from three cigarettes) after one hour of smoking the last cigarette.

Chromatographic analysis of PAHs in smoke

PAHs were analyzed using a GC/MS procedure with selected ion monitoring (SIM) detection. A cleanup step of the samples using solid phase extraction (SPE) was performed before the analysis. The quantitation was performed using a response factor for each analyte, which was calculated using deuterated internal standards and a control solution with nondeuterated PAHs. These solutions were prepared in two steps, with a stock solution prepared first followed by a dilution to prepare the working solutions. The stock solution for the deuterated standards was prepared in cyclohexane. This solution was diluted in isopropanol to make the working solution of internal standards solution (I.S.). The amounts of deuterated PAHs in solution I.S. were between 70 µg/mL (for naphthalene-d_8, and 2-methylnaphthalene-d_{10}) and 5 µg/mL (for the PAHs with higher molecular weight). Similar to the deuterated solutions, two control solutions (stock and working) were prepared for nondeuterated PAHs. The stock solutions were made in cyclohexane and an aliquot was taken and diluted with isopropanol to obtain the working control solution (solution C). The concentrations of stock nondeuterated solution were chosen to be close to the concentrations gene-

Table 1. Gas chromatography/mass spectrometry (GC/MS) operating parameters

Parameter	Description	Parameter	Description
GC column	BPX-5	Pulse pressure	40.0 psi
Column dimensions	Two 30 m columns connected, 0.25 mm id.	Pulse time	0.5 min
Film thickness	0.25 µm	Carrier gas	Helium
Initial oven temperature	100 ˚C	Flow mode	Constant flow
Initial time	7.0 min	Flow rate	1.1 mL/min
Oven ramp rate	25°C/min	Nominal initial pressure	22.66 psi
Oven final first ramp	150 ˚C	Purge flow	6.3 mL/min
Final time first ramp	0 min	Purge time	1 min
Oven ramp rate	4 ˚C/min	GC outlet	MSD
Oven final temperature	320 ˚C	MSD transfer line	280 ˚C
Final time	15 min	Ion source temperature	230 ˚C
Total run time	66.5 min	Quadrupole temp.	150 ˚C
Inlet temperature	280 ˚C	MSD EM offset	250 V
Inlet mode	Pulsed splitless	MSD solvent delay	12.0 min
Injection volume	5.0 µL	MSD acquisition mode	SIM

rated by the PAHs found in a 2R4F cigarette. The deuterated PAHs were obtained from C/D/N Isotopes Inc. (Pointe-Claire, Quebec H9R 1H1), and the nondeuterated PAHs were obtained from Aldich/Sigma (Saint Louis, MO 63178-9916).

For the analysis of PAHs in the smoke, a cleanup procedure was necessary. For this purpose, after smoking, each pad was put in an extraction vial (with the volume of about 100 mL). To each vial there was added 100 µL of a solution of deuterated internal standards (solution indicated as I.S.). After this step the pads were extracted with methanol (Burdick & Jackson, MI, 49442) for 30 min on a wrist action mechanical shaker (Burrell Scientific Co., PA 15219). Two alternatives of extraction were tested for the study. In one alternative, 25 mL of methanol was used for the extraction, and 10 mL of solvent were recovered by filtering the extract in a test tube, through a 0.45 µm PTFE filter (VWR Suwanee, GA 30024). The 10 mL solution was further reduced to 5 mL using a TurboVap LV evaporator (Zymark/Caliper Life Sciences, Hopkinton, MA 01748) at 45 ˚C under a flow of air (for about 20 min). In the other alternative, 15 mL of methanol were used for the extraction and only 5 mL were recovered by filtering the extract through a 0.45 µm PTFE filter (VWR Suwanee, GA 30024). In the first alternative, the ratio (sample volume) vs. (total solvent volume) was 1/2.5 while in the second alternative the ratio was 1/3. A slightly higher amount of PAHs is processed for the sample preparation when the first alternative of pad extraction was used, but the result reported per cigarette was the same. The volume of 10 mL solution that was concentrated to 5 mL can be increased and up to 20 mL solution can be obtained from the filter pad and concentrated to 5 mL, such that the ratio (sample volume) vs. (total solvent volume) can become 1/1.25. A higher amount of PAHs from the pad is processed in this case. However, the concentration of the larger volume of 20 mL to 5 mL was not necessary for this study and was not pursued.

In the test tubes, to the 5 mL methanol solution containing the pad extract and the internal standards are added 7 mL of distilled water. A slight turbidity is typically seen in the solution. The test tubes with 12 mL solution are used for loading the sample into SPE cartridges. The cartridges were Varian Bond Elut CH 500 mg, 3 mL volume (Varian, Walnut Creek, CA 94598). The cleanup process was performed using a Rapid Trace automatic SPE system (Zymark/Caliper Life Sciences, Hopkinton, MA 01748). The steps of the procedure included conditioning the SPE cartridge with 2 mL methanol, then with 2 mL water/methanol 65/35 v/v, followed by loading the cartridge with 11.6 mL sample solution (in two consecutive loadings of 5.8 mL). The loaded cartridge was rinsed with 4.8 mL water, then with 1.6 mL water/methanol 65/35 v/v, and dried for 10 min with a flow of N_2. The sample was eluted with 0.8 mL cyclohexane. The final eluates are transferred into sample vials with 100 µL small volume inserts in order to perform the GC/MS analysis. The analysis of PAHs was performed on a 6890 GC/5973 MS instrument (Agilent, Wilmington, Delaware 19808), in selected ion monitoring mode (SIM), with the parameters for the instrument given in Table 1. Using these parameters the PAHs were eluted at the retention times shown in Table 2. The ions used for the SIM detection and quantitation of each PAH are also indicated in Table 2. The GC/MS parameters shown in Table 1 were selected to obtain good sensitivity. A relatively large injection volume and pulsed splitless injection assure that a large amount of sample is injected in the column. The electron multiplier (EM) offset of 250 V was used to increase the sensitivity of the MS instrument. A clean GC injection port liner and a clean MS source were also important for maintaining good performance of the procedure.

A typical chromatogram for the PAHs in exhaled smoke is given in Figure 2. The details of the chromatogram are shown by expanding a small window of the retention time, and by displaying the trace for the extracted ion 252 in the trace. The four major peaks seen in the expanded window from Figure 2 between 34.0 min and 56.0 min belong to d_{10}-pyrene (at 34.86 min), d_{12}-chrysene (at 42.14 min), d_{12}-benzo[*a*]pyrene (at 49.69 min) and d_{12}-indeno[1,2,3-*cd*]pyrene (at 55.37 min), which are all internal standards. The trace for the extracted ion with *m/z* = 252 show the peaks for benzo[*b*]fluoranthene (48.09 min), benzo[*k*]fluoranthene (48.23 min), benzo[*e*]pyrene (49.56 min), benzo[a]pyrene (49.82 min) and perylene (50.26 min).

Table 2. Retention times and ion monitored for quantitation in selected ion monitoring (SIM) acquisition for the PAHs

No.	Compound	Ret. time	SIM ion
1	d_8-Naphthalene	12.78	136
2	Naphthalene	12.85	128
3	d_{10}-2-Methylnaphthalene	14.89	152
4	2-Methylnaphthalene	15.00	142
5	1-Methylnaphthalene	15.37	142
6	Acenaphthylene	18.59	152
7	d_{10}-Acenaphthene	19.17	164
8	Acenaphthene	19.32	154
9	d_{10}-Fluorene	21.59	176
10	Fluorene	21.74	166
11	d_{10}-Phenanthrene	26.68	188
12	Phenanthrene	26.81	178
13	d_{10}-Anthracene	26.99	188
14	Anthracene	27.10	178
15	Fluoranthene	33.61	202
16	d_{10}-Pyrene	34.86	212
17	Pyrene	34.97	202
18	d_{12}-Chrysene	42.14	240
19	Benz[*a*]anthracene	42.07	228
20	Chrysene	42.29	228
21	Benzo[*b*]fluoranthene	48.09	252
22	Benzo[*k*]fluoranthene	48.23	252
23	Benzo[*e*]pyrene	49.55	252
24	d_{12}-Benzo[a]pyrene	49.69	264
25	Benzo[*a*]pyrene	49.82	252
26	Perylene	50.26	252
27	Indeno[1,2,3-*cd*]pyrene	55.50	276
28	d_{12}-Indeno[1,2,3-*cd*]pyrene	55.36	288
29	Dibenz[*a,h*]anthracene	55.58	278
30	Benzoperylene	57.09	276

The quantitation in this method is done based on specific response factors F for each analyte. For the calculation of response factors, 0.5 µL of the calibrating solution C is mixed with 0.1 µL of solution I.S. (of deuterated standards) and the resulting solution is injected in the GC/MS system. Peak areas of extracted ions for pairs of compounds are used for the calculation of the response factors, considering the appropriate concentration ratios. The factors obtained in this study are given in Table 3. The same values for the factors F were obtained when the mixture of solutions C and I.S. was processed using the SPE procedure and then analyzed by GC/MS. These factors depend on particular sensitivity of the GC/MS instrument, and also imbed any imprecision in the preparation of standards. For this reason, the factors cannot be directly transferred to a different experimental setup, and they must be recalculated when preparing new solutions or when using different instruments.

For the calculation of the amount of a specific PAH the areas of the analyte A_A and the areas of the corresponding internal standard A_{IS} were measured from the chromatogram for the specific measured ions (the molecular ions were used for all compounds). The integration was done using the RTE integrator of the Agilent MS instrument. The calculation of the results was done using the following formula:

$$\text{ng PAH/cigarette} = F \cdot A_A/A_{IS} \cdot (\text{conc. I.S. µg/mL}) \cdot 1000/(\text{number of cigarettes})$$

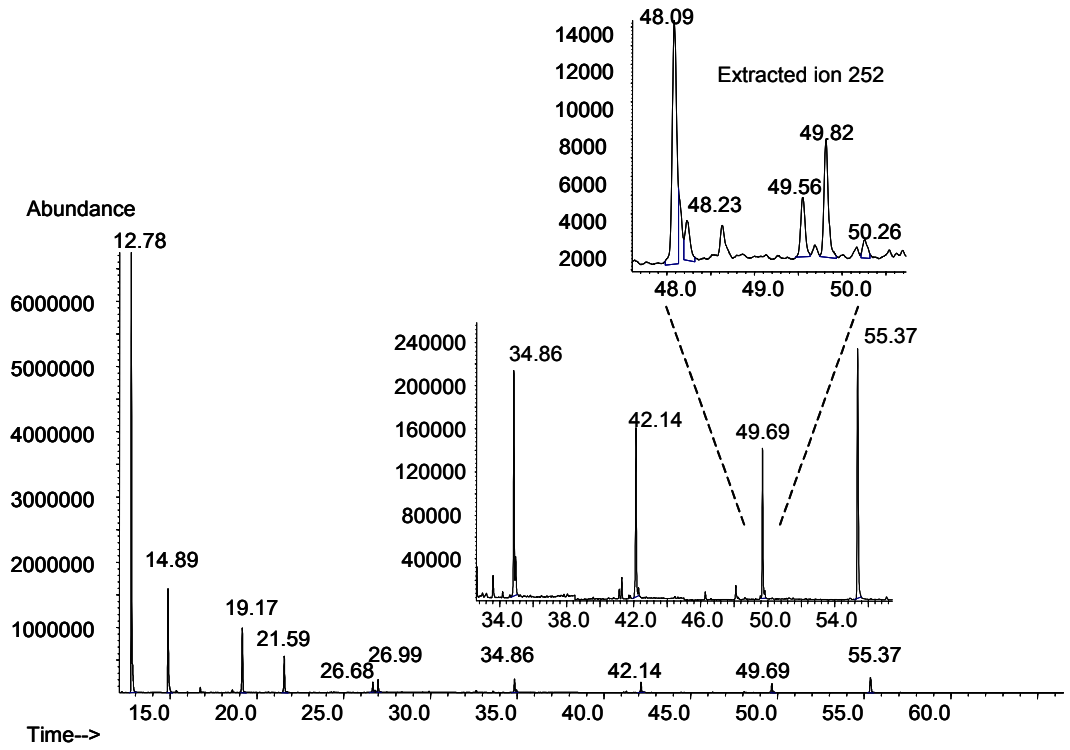

Figure 2. The details of the SIM chromatogram of PAHs in exhaled smoke of a 10.6 mg 'tar' cigarette (retention time in min). Two expanded windows also are shown, one of the retention time between 34.0 min and 56.0 min and the other for the trace of the extracted ion 252.

Table 3. Response factors (*F*) for deuterated/nondeuterated PAHs obtained as the ratio of the areas for the selected ion monitoring (SIM) detected ions of pair of compounds

No.	Compound ratios	Factor *F*
1	d_8-Naphthalene / Naphthalene	0.788880
2	d_{10}-2-Methylnaphthalene / 2-Methylnaphthalene	0.779039
3	d_{10}-2-Methylnaphthalene / 1-Methylnaphthalene	0.818436
4	d_{10}-Acenaphthene / Acenaphthylene	0.804285
5	d_{10}-Acenaphthene / Acenaphthene	0.910053
6	d_{10}-Fluorene / Fluorene	0.855589
7	d_{10}-Phenanthrene / Phenanthrene	0.812661
8	d_{10}-Anthracene / Anthracene	0.931728
9	d_{10}-Pyrene / Fluoranthene	0.847778
10	d_{10}-Pyrene / Pyrene	0.854465
11	d_{12}-Chrysene / Benz[*a*]anthracene	0.913472
12	d_{12}-Chrysene / Chrysene	0.972469
13	d_{12}-Benzo[*a*]pyrene / Benzo[*b*]fluoranthene	1.036063
14	d_{12}-Benzo[*a*]pyrene / Benzo[*k*]fluoranthene	0.894834
15	d_{12}-Benzo[*a*]pyrene / Benzo[*e*]pyrene	1.024822
16	d_{12}-Benzo[*a*]pyrene / Benzo[*a*]pyrene	0.791525
17	d_{12}-Benzo[*a*]pyrene / Berylene	0.783366
18	d_{12}-Indeno[1,2,3-*cd*]pyrene / Indeno[1,2,3-*cd*]pyrene	0.822461
19	d_{12}-Indeno[1,2,3-*cd*]pyrene / Dibenz[*a*,*h*]anthracene	1.197443
20	d_{12}-Indeno[1,2,3-*cd*]pyrene / Benzoperylene	0.969582

Analysis of nicotine in smoke

One additional quantitative analysis performed for this study was that of cigarette butts for nicotine. For the analysis of butt nicotine, the smoked butts were collected and cut at lengths of 1 cm. The 1 cm mouth portions of the cigarettes from the Borgwaldt machine or from each smoker were extracted with 25 mL methanol containing an internal standard (dodecanol). The level of nicotine was measured using a standard procedure (34). The butt nicotine levels were necessary to estimate the amount of smoke PAHs delivered by smokers. A linear dependence has been reported in literature between nicotine in the cigarette butt and the nicotine level on the Cambridge pad, as well as between nicotine in the cigarette butt and the total particulate matter for the cigarette (35, 36). The linear dependence between butt nicotine and the levels of PAHs in smoke also was verified in the present study. However, this dependence should be considered only as an empirical finding for the limited range in which the measurements were done.

RESULTS AND DISCUSSION

Assessment of PAHs analytical procedure

Three characteristics of the analytical method for PAHs analysis were thoroughly investigated before studying the content of PAHs in exhaled cigarette smoke. These characteristics of the analytical method were accuracy, precision, and sensitivity, and they were measured for reference cigarettes and for a cigarette with very low deliveries (a cigarette that heats instead of burning tobacco). These measurements were solely related to the analytical method itself, and not to the evaluation of the smoke retention by humans. The accuracy of the method has been tested for 2R4F cigarettes, for which literature information was available (28, 37). In addition to the literature information for the 2R4F Kentucky reference cigarette, some data for the older 1R4F reference cigarette were also used, since this cigarette is in many respects similar to 2R4F. The literature data regarding PAHs levels for the 2R4F cigarette (37) were obtained using smoking in ISO recommended conditions, while those reported for 1R4F cigarette were obtained using smoking in both ISO [reported in (37)] and FTC [reported in (28)] type conditions. The results obtained in this study for five replicate smoking are given in Table 4. As seen from this table, the agreement with the literature data is good, except for fluorene and phenanthrene that gave higher values in this study. Also, the precision of the method is very good, with all RSD% values less than 10%. The results for 1R5F Kentucky reference cigarette obtained from five replicates in this study are compared in Table 5 with those reported in the literature (28) for cigarettes smoked in FTC conditions. As seen from Table 5, the agreement with literature data is reasonably good, with a few exceptions. Fluorene, phenanthrene, fluoranthene, pyrene, and perylene gave higher values in the present study.

The sensitivity of the PAHs analysis by the method reported in this study was found to be very good. The calculation of the limit of detection from the standard deviation (S.D.) as 3 × S.D. for the lowest measured sample has been applied in this study using a cigarette that heats instead of burning tobacco, which generates smoke very low in PAHs even compared to other cigarettes. The results of the quantitation of PAHs in this cigarette with 10 cigarettes smoked at conditions similar to those recommended by FTC, gave low RSD %, and low limits of detection (for the method) as shown in Table 6. The results were obtained from five replicates of cigarette smoking, each analyzed twice (total of 10 GC runs). Other technique characteristics such as analyte recovery, range of linearity, etc. were not verified in this study.

Generation of the correlation charts between the level of PAHs for machine smoked cigarettes and cigarette butt nicotine

This part of the study was done on the 10.6 mg 'tar' cigarette, which was further used for the evaluation of smoke retention by humans. The analysis of PAHs was performed initially for machine smoked cigarettes using different puffing conditions. At the same time with the analysis of PAHs from the smoke, nicotine was analyzed in the cigarette butts. Correlation charts between the level of PAHs in smoke as a function of the nicotine level in the cigarette butts were obtained. Using these charts the calculation of the level of PAHs in delivered cigarette smoke was possible when the level of nicotine in the cigarette butt was known.

The measurement of PAHs in ng/cig for the 10.6 mg 'tar' cigarettes smoked under FTC, 60/60, 45/30, and 60/30 conditions, represented as a function of the corresponding level

Table 4. Comparison of the measured PAH levels in a 2R4F cigarette in ng/cig (five replicates) and the data from literature for a 2R4F and a 1R4F cigarette (28, 37)

No.	Compound	Measured	RSD%	Literature (37) 2R4F	Literature (37) 1R4F	Literature (28) 1R4F
1	Naphthalene	309.99	4.94	271.60	339.6	281.8
2	2-Methylnaphthalene	366.43	1.53			
3	1-Methylnaphthalene	396.49	2.47			
4	Acenaphthylene	69.92	3.92			
5	Acenaphthene	35.94	3.64			
6	Fluorene	169.79	1.76	119.8	116.4	121.2
7	Phenanthrene	146.77	2.26	125.2	94.14	79.2
8	Anthracene	52.52	1.43	45.82	39.16	40.8
9	Fluoranthene	50.96	2.63	56.2	46.04	40.4
10	Pyrene	44.97	5.56	39.2	29.64	27.06
11	Benz[a]anthracene	16.61	3.01	14.48	10.37	8.6
12	Chrysene	16.58	8.12	20.50	15.66	12.2
13	Benzo[*b*]fluoranthene	5.43	8.25	10.24 (sum) [a]	8.02 (sum) [a]	7.4 (sum) [a]
14	Benzo[*k*]fluoranthene	1.37	7.99			
15	Benzo[*e*]pyrene	3.43	6.20	4.67	3.6	3.6
16	Benzo[*a*]pyrene	9.15	4.62	6.96	5.51	4.5
17	Perylene	1.51	9.52	0.88	0.67	0.5
18	Indeno[1,2,3-*cd*]pyrene	1.49	8.71			
19	Dibenz[*a,h*]anthracene	0.56	3.84	0.46	0.38	0.2
20	Benzoperylene	2.04	5.24	1.52	1.16	0.9

[a] The sum refers to benzo[*b*]fluoranthene and benzo[*k*]fluoranthene levels.

Table 5. Comparison of the measured PAH levels in a 1R5F reference cigarette in ng/cig (five replicates) to the data from literature (28)

No.	Compound	Measured	RSD%	Literature
1	Naphthalene	41.28	3.74	39.1
2	2-Methylnaphthalene	44.95	7.56	
3	1-Methylnaphthalene	56.32	6.47	
4	Acenaphthylene	16.41	6.48	
5	Acenaphthene	6.84	4.45	
6	Fluorene	33.81	5.04	21.7
7	Phenanthrene	41.23	2.56	25.6
8	Anthracene	8.13	4.09	7.5
9	Fluoranthene	16.34	4.80	10.9
10	Pyrene	10.25	10.96	6.2
11	Benz[*a*]anthracene	2.79	6.18	2.1
12	Chrysene	4.30	4.66	3.0
13	Benzo[*b*]fluoranthene	1.57	5.90	2.2 (sum) [a]
14	Benzo[*k*]fluoranthene	0.32	9.67	
15	Benzo[*e*]pyrene	0.79	4.97	0.9
16	Benzo[*a*]pyrene	1.31	5.14	1.1
17	Perylene	0.22	7.73	0.1
18	Indeno[1,2,3-*cd*]pyrene	0.22	4.88	
19	Dibenz[*a,h*]anthracene	0.10	8.96	0.06
20	Benzoperylene	0.34	3.96	0.4

[a] See footnote Table 4.

Table 6. The levels of PAHs (ng/cig) in a cigarette that heats instead of burning tobacco smoked under conditions similar to those recommended by FTC, the relative standard deviation % (RSD%), and the corresponding limit of detection (LOD) values (five replicates, each analyzed twice)

No.	Compound	Average	RSD%	LOD ng/cig
1	Naphthalene	53.25	3.58	5.72
2	2-Methylnaphthalene	17.85	3.54	1.90
3	1-Methylnaphthalene	14.06	5.18	2.19
4	Acenaphthylene	1.24	5.99	0.22
5	Acenaphthene	0.42	8.87	0.11
6	Fluorene	1.64	7.67	0.38
7	Phenanthrene	6.58	1.90	0.38
8	Anthracene	1.02	8.09	0.25
9	Fluoranthene	4.66	6.94	0.97
10	Pyrene	4.53	4.12	0.56
11	Benz[a]anthracene	2.14	3.34	0.22
12	Chrysene	2.57	4.30	0.33
13	Benzo[*b*]fluoranthene	1.40	4.37	0.18
14	Benzo[*k*]fluoranthene	0.30	3.20	0.03
15	Benzo[*e*]pyrene	0.72	6.79	0.15
16	Benzo[*a*]pyrene	1.31	4.16	0.16
17	Perylene	0.17	4.97	0.03
18	Indeno[1,2,3-*cd*]pyrene	0.21	6.62	0.04
19	Dibenz[*a,h*]anthracene	0.06	6.27	0.01
20	Benzoperylene	0.47	3.81	0.05

of nicotine in mg/cig in the smoked butts, showed a linear dependence. However, this dependence should be considered only as an empirical finding for the limited range in which the measurements were done. The range of nicotine butt levels for the four machine smoking conditions previously indicated covered the range measured in the cigarette butts from the evaluated human smokers. The equations of the regression lines and the corresponding R^2 values for each analyte are given in Table 7. All the R^2 values for the regression lines were high (> 0.95) proving the good linearity between the nicotine level in the cigarette butt and the PAHs level in the cigarette smoke (different slopes of the regression lines were expected for compounds at different levels in smoke). The validity of the equations from Table 7 can be immediately verified by calculating the PAHs levels using the butt nicotine amount for different machine smoking regimes. The measured levels of PAHs for these regimes, the calculated values, and their differ-

Table 7. The equations of the regression lines and the corresponding R^2 values between the PAHs in ng/cig as a function of nicotine in mg/cig

No.	Compound	Equation	R^2
1	Naphthalene	$y = 4405.8\ x - 391.78$	0.9907
2	2-Methylnaphthalene	$y = 4141.7\ x - 391.07$	0.9740
3	1-Methylnaphthalene	$y = 2984.1\ x - 81.15$	0.9636
4	Acenaphthylene	$y = 398.5\ x + 10.49$	0.9588
5	Acenaphthene	$y = 189.4\ x + 5.31$	0.9570
6	Fluorene	$y = 843.9\ x + 26.19$	0.9860
7	Phenanthrene	$y = 660.0\ x + 13.69$	0.9908
8	Anthracene	$y = 284.8\ x - 16.81$	0.9907
9	Fluoranthene	$y = 312.7\ x - 6.01$	0.9943
10	Pyrene	$y = 223.7\ x + 3.95$	0.9990
11	Benz[*a*]anthracene	$y = 73.0\ x + 3.09$	0.9895
12	Chrysene	$y = 89.6\ x - 1.63$	0.9744
13	Benzo[*b*]fluoranthene	$y = 52.3\ x - 1.80$	0.9998
14	Benzo[*k*]fluoranthene	$y = 8.46\ x + 0.217$	0.9950
15	Benzo[*e*]pyrene	$y = 16.65\ x + 0.942$	0.9850
16	Benzo[*a*]pyrene	$y = 51.31\ x - 0.744$	0.9920
17	Perylene	$y = 10.19\ x - 0.929$	0.9991
18	Indeno[1,2,3-*cd*]pyrene	$y = 6.00\ x + 0.411$	0.9877
19	Dibenz[*a,h*]anthracene	$y = 8.73\ x - 0.80$	0.9616
20	Benzoperylene	$y = 7.51\ x + 1.049$	0.9968

ences (in %) are given in Table 8. As seen from Table 8, the agreement between the experimental and calculated values is very good.

Analysis of PAHs in the exhaled smoke and the calculation of delivered PAHs levels

Before the analysis of PAHs in the exhaled smoke an evaluation of the background level of PAHs in the breath of a smoker when no cigarette was smoked within one hour has been measured. As expected, the PAHs were not detected in the breath without smoking. No need for further sampling of breath without smoking was considered necessary.

The level of PAHs in the exhaled smoke was further analyzed for ten smokers of the 10.6 mg 'tar' cigarette and the results are reported in ng/cig in Table 9 (averages from three analytical measurements). The measurements of the PAHs were affected, as expected, by some analytical errors. These errors are indicated (in parentheses) in Table 9 as RSD%. As seen from Table 9, the analytical errors for the measurements of exhaled smoke were usually below 10%, and only few measurements have RSD% values between 11% and 14%. The table also indicates the amount of nicotine in mg/cig measured in the collected cigarette butts from each smoker. Using the nicotine levels from the cigarette butts for each smoker, and the equations given in Table 7, the delivered levels of each individual PAH can be calculated. The results are given in Table 10.

Calculation of the retention % of PAHs by human smokers

From the results for the PAHs levels in the exhaled smoke given in Table 9 and those calculated for the delivered smoke given in Table 10 the retention of each PAH can be calculated for each smoker, using the expression:

$$\text{Retention \%} = 100 - (\text{Exhaled level})/(\text{Delivered level}) \cdot 100$$

The retentions for each smoker calculated using the above formula from Tables 9 and 10 are shown in Figure 3. The average values for the retentions are given in Table 11 where the RSD% (between smokers) for each compound are also given (the analytical errors are not included). The same table also contains the logarithm of octanol-water partition coefficient ($LogP_{ow}$) [see e.g. (38)] and also the logarithm of vapor pressure Log(VP) (at 20 ˚C in Pa) (39, 40) of each

Table 8. The measured and calculated levels of PAHs in ng/cig for different smoking regimes

	FTC			60/60			45/30			60/30		
Compound	Meas.	Calc.	% Dif.	Meas.	Calc.	% Dif.	Meas.	Calc.	% Dif.	Meas.	Calc.	% Dif.
Naphthalene	446.11	488.50	9.50	761.33	700.86	−7.94	1231.78	1254.52	1.85	1475.06	1470.41	−0.32
2-Methylnaphthalene	414.48	436.44	5.30	689.45	636.07	−7.74	1066.22	1156.55	8.47	1418.44	1359.49	−4.16
1-Methylnaphthalene	545.35	515.07	−5.55	641.66	658.91	2.69	957.71	1033.91	7.96	1243.32	1180.13	−5.08
Acenaphthylene	86.09	88.11	2.35	117.32	106.84	−8.94	149.19	155.66	4.34	185.16	174.70	−5.65
Acenaphthene	42.43	43.15	1.69	54.77	52.28	−4.55	70.49	76.08	7.94	89.20	85.36	−4.30
Fluorene	192.32	194.80	1.29	242.51	235.48	−2.90	327.76	341.53	4.20	392.10	382.88	−2.35
Phenanthrene	150.43	145.56	−3.24	173.14	177.37	2.44	253.21	260.31	2.80	299.10	292.65	−2.16
Anthracene	40.52	40.09	−1.06	53.26	53.82	1.05	89.69	89.61	−0.09	103.64	103.57	−0.08
Fluoranthene	58.65	56.47	−3.73	68.27	71.54	4.78	112.42	110.84	−1.41	125.59	126.16	0.45
Pyrene	49.44	48.65	−1.60	58.40	59.43	1.76	87.68	87.54	−0.16	98.64	98.50	−0.15
Benz[a]anthracene	17.76	17.68	−0.47	21.41	21.19	−1.02	29.30	30.37	3.64	34.76	33.94	−2.35
Chrysene	17.57	16.27	−7.37	19.34	20.59	6.48	30.49	31.85	4.46	37.59	36.24	−3.59
Benzo[*b*]fluoranthene	8.64	8.65	0.11	11.21	11.17	−0.35	17.64	17.74	0.58	20.38	20.31	−0.36
Benzo[*k*]fluoranthene	1.95	1.91	−2.14	2.29	2.32	1.30	3.31	3.38	2.12	3.86	3.79	−1.71
Benzo[*e*]pyrene	4.33	4.27	−1.40	5.08	5.07	−0.10	6.87	7.16	4.21	8.21	7.98	−2.75
Benzo[*a*]pyrene	9.87	9.51	−3.67	11.66	11.98	2.75	17.93	18.43	2.77	21.40	20.94	−2.15
Perylene	1.14	1.11	−2.78	1.55	1.60	2.85	2.89	2.88	−0.41	3.38	3.38	0.01
Indeno[1,2,3-*cd*]pyrene	1.67	1.61	−3.66	1.84	1.90	3.28	2.59	2.65	2.31	3.01	2.95	−2.05
Dibenz[*a,h*]anthracene	0.91	0.94	4.11	1.48	1.37	−7.96	2.22	2.46	10.74	3.06	2.89	−5.41
Benzoperylene	2.53	2.55	0.58	2.91	2.91	−0.07	3.91	3.86	−1.50	4.18	4.22	1.12
Butt nicotine	0.200			0.248			0.374			0.423		

Table 9. The average levels in ng/cig of PAHs in the exhaled smoke, in parentheses the RSD% (three analysis replicates), and the level of nicotine in the cigarette butt (mg/cig) for each of the human subjects

No.	Compound	Smoker 1	Smoker 2	Smoker 3	Smoker 4	Smoker 5	Smoker 6	Smoker 7	Smoker 8	Smoker 9	Smoker 10
1	Naphthalene	22.29	24.58	16.15	27.74	43.98	25.98	14.73	9.98	30.21	18.11
		(2.95)	(3.36)	(1.60)	(2.02)	(0.12)	(3.79)	(0.17)	(1.18)	(1.45)	(0.34)
2	2-Methylnaphthalene	14.62	17.07	10.97	20.51	27.81	20.38	10.76	6.92	22.33	13.25
		(4.00)	(2.59)	(0.12)	(2.56)	(5.19)	(5.07)	(0.72)	(1.39)	(4.35)	(4.48)
3	1-Methylnaphthalene	16.83	17.58	11.65	23.97	32.42	23.22	11.96	7.27	25.34	15.45
		(5.77)	(4.94)	(0.98)	(5.05)	(5.84)	(11.30)	(0.18)	(7.55)	(6.44)	(1.58)
4	Acenaphthylene	13.48	24.38	5.61	20.54	16.88	9.16	4.50	3.02	5.74	4.37
		(6.32)	(3.91)	(5.28)	(1.12)	(3.24)	(9.34)	(3.92)	(4.19)	(0.47)	(1.88)
5	Acenaphthene	1.39	1.76	0.90	3.05	2.06	3.56	0.94	0.53	1.35	0.97
		(6.17)	(8.73)	(4.67)	(8.58)	(7.27)	(12.52)	94.52)	(9.29)	(8.81)	(3.70)
6	Fluorene	5.24	8.42	2.07	11.95	7.20	6.20	2.08	1.73	4.17	2.73
		(7.68)	(4.22)	(9.29)	(1.05)	(1.82)	(5.35)	(2.69)	(1.31)	(8.12)	(3.07)
7	Phenanthrene	13.77	15.93	4.84	18.87	9.76	9.37	4.06	5.86	5.40	2.77
		(2.97)	(2.78)	(2.49)	(5.70)	(3.29)	(10.48)	(1.95)	(2.96)	(1.12)	(0.29)
8	Anthracene	1.95	2.47	0.55	4.88	2.69	1.36	0.77	0.65	1.85	3.57
		(7.98)	(1.44)	(0.46)	(6.65)	(7.04)	(13.18)	(5.14)	(2.66)	(7.60)	(2.06)
9	Fluoranthene	3.60	7.09	0.62	7.70	2.60	1.28	0.55	0.71	1.00	0.61
		(3.50)	(2.75)	(6.31)	(3.78)	(8.22)	(1.82)	(9.28)	(0.03)	(0.38)	(5.34)
10	Pyrene	8.96	12.81	1.26	12.13	3.65	2.06	0.97	1.07	1.70	0.98
		(2.20)	(0.35)	(1.75)	(6.18)	(5.50)	(3.090	(2.15)	(11.06)	(2.06)	(7.07)
11	Benz[*a*]anthracene	2.61	4.29	2.25	5.24	3.81	1.30	1.47	4.73	2.67	0.95
		(3.78)	(4.19)	(9.56)	(7.69)	(11.22)	(11.22)	(2.53)	(1.22)	(6.98)	(2.45)
12	Chrysene	3.37	4.72	3.41	5.74	4.23	3.08	2.30	2.74	3.27	2.78
		(5.02)	(0.43)	(0.14)	(7.59)	(7.28)	(3.64)	(6.75)	(0.89)	(4.78)	(4.44)
13	Benzo[*b*]fluoranthene	4.43	4.66	4.39	4.37	6.45	4.58	3.99	5.26	5.52	3.74
		(6.87)	(4.37)	(5.24)	(8.24)	(9.17)	(8.46)	(3.56)	(2.55)	(0.41)	(4.36)
14	Benzo[*k*]fluoranthene	0.98	0.98	0.64	0.97	1.91	1.00	0.98	1.26	1.15	0.72
		(7.18)	(1.37)	(8.62)	(8.05)	(8.370	(3.60)	(4.01)	(3.23)	(4.43)	(2.49)
15	Benzo[*e*]pyrene	1.19	1.70	1.27	1.34	2.49	1.35	1.16	1.33	0.93	0.86
		(2.70)	(1.64)	(11.16)	(5.30)	(4.33)	(5.36)	(6.74)	(0.34)	(3.55)	(0.08)
16	Benzo[*a*]pyrene	3.41	4.35	3.72	3.47	5.96	3.15	3.50	3.94	4.13	3.09
		(0.67)	(0.87)	(7.33)	(7.75)	(7.21)	(0.78)	(0.87)	(7.28)	(6.82)	(8.43)
17	Perylene	0.46	0.60	0.51	0.57	0.96	0.56	0.53	0.66	0.59	0.32
		(4.10)	(0.03)	(4.58)	(3.90)	(3.55)	(11.60)	(1.42)	(7.11)	(8.40)	(0.63)
18	Indeno[1,2,3-*cd*]pyrene	0.70	0.96	0.98	0.64	1.78	0.91	0.99	1.18	0.97	0.82
		(0.90)	(2.19)	(0.58)	(4.88)	(5.54)	(1.05)	(3.36)	(2.35)	(4.72)	(5.65)
19	Dibenz[*a,h*]anthracene	0.54	0.53	0.35	0.48	1.37	0.43	0.56	0.77	0.56	0.46
		(2.81)	(0.24)	(1.50)	(6.96)	(1.79)	(5.85)	(0.33)	(1.78)	(0.83)	(0.65)
20	Benzoperylene	1.98	2.08	1.51	1.95	3.33	1.50	1.72	2.68	2.52	1.47
		(0.10)	(8.61)	(1.51)	3.85)	(0.33)	(2.94)	(6.95)	(1.22)	(9.61)	(5.96)
	Butt nicotine (mg/cig)	0.228	0.289	0.215	0.247	0.528	0.265	0.288	0.366	0.307	0.203

PAH. Some of the $LogP_{ow}$ values were found in literature (41) and others were calculated using a procedure based on atom and conjugated double bond contributions (42). The values for Log(VP) were also either from literature (39) or calculated (40). It was found that the retention of PAHs depends on their chemical nature. The lower molecular weight compounds were retained with high efficiency. The heavier molecules were less retained, but even compounds such as indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene, and benzoperylene that have relatively high molecular weights were retained with efficiencies around 50%. The difference in the retention of PAHs between smokers varied and it was low for the highly retained compounds and as large as about 25% for indeno[1,2,3-*cd*]pyrene, and benzoperylene.

The retention of PAHs was further compared to the $LogP_{ow}$ values. This parameter has been correlated with various observations such as the rate of penetration of non-electrolites through biological membranes in general (43) or through skin (44). The graph showing the dependence of retention efficiency for PAHs (in %) to their $LogP_{ow}$ is shown in Figure 4.

As seen from Figure 4, the dependence of retention efficiency for PAHs (in %) on their $LogP_{ow}$ is not linear and shows considerable variability for several compounds that have very close $LogP_{ow}$ values. This observation may indicate that the retention for PAHs by the human smokers is not a simple partition process. Better correlation was obtained between the retention efficiency for PAHs (in %) on their vapor pressure [as Log(VP)], as shown in Figure 5 (R^2 value of 0.72). This indicated that the vapor pressure may play a role in PAHs retention, although the good correlation is not necessarily a proof of causality.

Table 10. The calculated levels in ng/cig of PAHs (based on butt nicotine) in the delivered smoke for each of the human subjects

No.	Compound	Smoker 1	Smoker 2	Smoker 3	Smoker 4	Smoker 5	Smoker 6	Smoker 7	Smoker 8	Smoker 9	Smoker 10
1	Naphthalene	612.74	881.50	555.47	696.45	1934.48	775.76	877.09	1220.74	960.80	502.60
2	2-Methylnaphthalene	553.24	805.88	499.40	631.93	1795.75	706.48	801.74	1124.79	880.43	449.70
3	1-Methylnaphthalene	599.22	781.25	560.43	655.92	1494.45	709.64	778.27	1011.03	834.97	524.62
4	Acenaphthylene	99.07	122.77	94.02	106.45	215.62	113.44	122.38	152.68	129.76	89.36
5	Acenaphthene	48.49	60.05	46.03	52.09	105.31	55.50	59.86	74.63	63.46	43.76
6	Fluorene	218.60	270.08	207.63	234.63	471.77	249.82	269.23	335.06	285.27	197.50
7	Phenanthrene	164.17	204.43	155.59	176.71	362.17	188.59	203.77	255.25	216.31	147.67
8	Anthracene	48.12	65.50	44.42	53.54	133.56	58.66	65.21	87.43	70.62	41.00
9	Fluoranthene	65.29	84.36	61.22	71.23	159.10	76.86	84.05	108.44	89.99	57.47
10	Pyrene	54.95	68.60	52.05	59.20	122.06	63.23	68.38	85.82	72.63	49.36
11	Benz[*a*]anthracene	19.73	24.19	18.79	21.12	41.63	22.44	24.11	29.81	25.50	17.91
12	Chrysene	18.80	24.26	17.63	20.50	45.68	22.11	24.17	31.16	25.88	16.56
13	Benzo[*b*]fluoranthene	10.12	13.31	9.44	11.12	25.81	12.06	13.26	17.34	14.26	8.82
14	Benzo[*k*]fluoranthene	2.15	2.66	2.04	2.31	4.68	2.46	2.65	3.31	2.81	1.93
15	Benzo[*e*]pyrene	4.74	5.75	4.52	5.05	9.73	5.35	5.74	7.04	6.05	4.32
16	Benzo[*a*]pyrene	10.95	14.08	10.29	11.93	26.35	12.85	14.03	18.04	15.01	9.67
17	Perylene	1.39	2.02	1.26	1.59	4.45	1.77	2.01	2.80	2.20	1.14
18	Indeno[1,2,3-*cd*]pyrene	1.78	2.15	1.70	1.89	3.58	2.00	2.14	2.61	2.25	1.63
19	Dibenz[*a,h*]anthracene	1.19	1.72	1.08	1.36	3.81	1.51	1.71	2.40	1.88	0.97
20	Benzoperylene	2.76	3.22	2.66	2.90	5.01	3.04	3.21	3.80	3.35	2.57

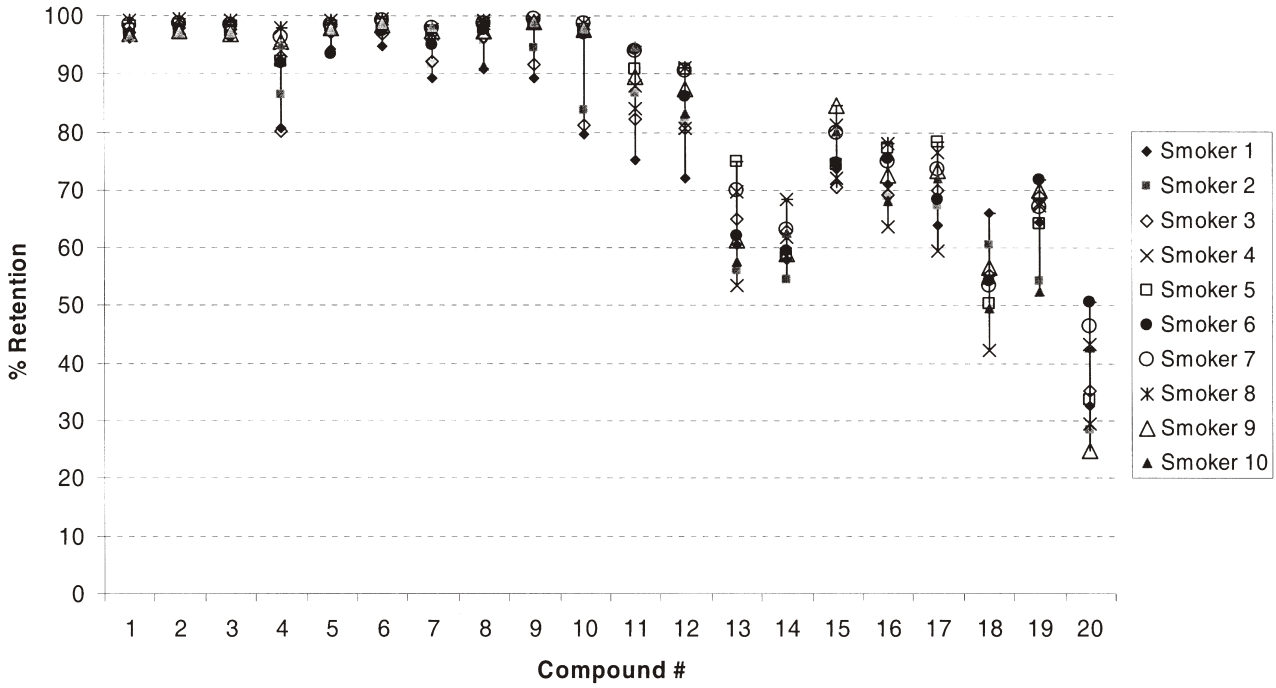

Figure 3. The retention % of PAHs for individual smokers. The chemical name of each compound (given as compound No.) can be seen in Table 9 or 10.

This study is the first reported in literature to measure PAHs retention from mainstream cigarette smoke. Some related information to PAHs retention is the measurements of the metabolites of PAHs in the urine of smokers (45–47). The retention of pyrene, for example, as shown in this study is about 93% with 8.5% RSD. This value is considerably higher than the level of 23 –29% conversion of pyrene from delivered cigarette smoke in the urine metabolite 1-hydroxypyrene as reported in the literature (45). This result indicates that pyrene from cigarette smoke is only partially excreted in urine as 1-hydroxypyrene. Other paths, such as bile excretion, smoker breath, etc. may contribute to the excretion of the pyrene retained from smoke.

CONCLUSIONS

This is the first reported study that evaluates the retention efficiency of 20 polycyclic aromatic hydrocarbons by

Table 11. Average values and the corresponding RSD% (between smokers) for PAH retention %, and the $LogP_{ow}$ values for octanol-water partition

No.	Compound	Average retention%	RSD%	$LogP_{ow}$	Log(VP) [a]
1	Naphthalene	97.18	1.01	3.3	1.02
2	2-Methylnaphthalene	97.79	0.84	3.87	0.94
3	1-Methylnaphthalene	97.55	0.90	3.86	0.95
4	Acenaphthylene	91.04	7.06	3.48 [b]	−0.05
5	Acenaphthene	97.14	1.90	3.47 [b]	−0.52
6	Fluorene	98.03	1.39	4.18	−1.05
7	Phenanthrene	95.37	3.34	4.46	−1.80
8	Anthracene	96.42	3.12	4.45	−2.85
9	Fluoranthene	96.75	3.80	5.16	−2.89
10	Pyrene	92.89	8.55	4.88	−3.21
11	Benz[*a*]anthracene	87.96	7.01	5.79	−4.57
12	Chrysene	84.44	7.11	5.73	−6.30
13	Benzo[*b*]fluoranthene	63.08	10.76	5.72 [b]	−7.89
14	Benzo[*k*]fluoranthene	60.94	6.21	5.72 [b]	−6.13
15	Benzo[*e*]pyrene	76.55	5.96	5.72 [b]	−6.14
16	Benzo[*a*]pyrene	71.93	6.39	5.97	−6.30
17	Perylene	70.32	8.16	5.82	−6.29 [b]
18	Indeno[1,2,3-*cd*]pyrene	54.32	11.77	6.22 [b]	−8.21 [b]
19	Dibenz[*a,h*]anthracene	64.85	10.04	6.5	−9.43
20	Benzoperylene	36.78	23.37	6.63	−7.89

[a] VP indicates vapor pressure at 20 ˚C expressed in Pa.
[b] Calculated values.

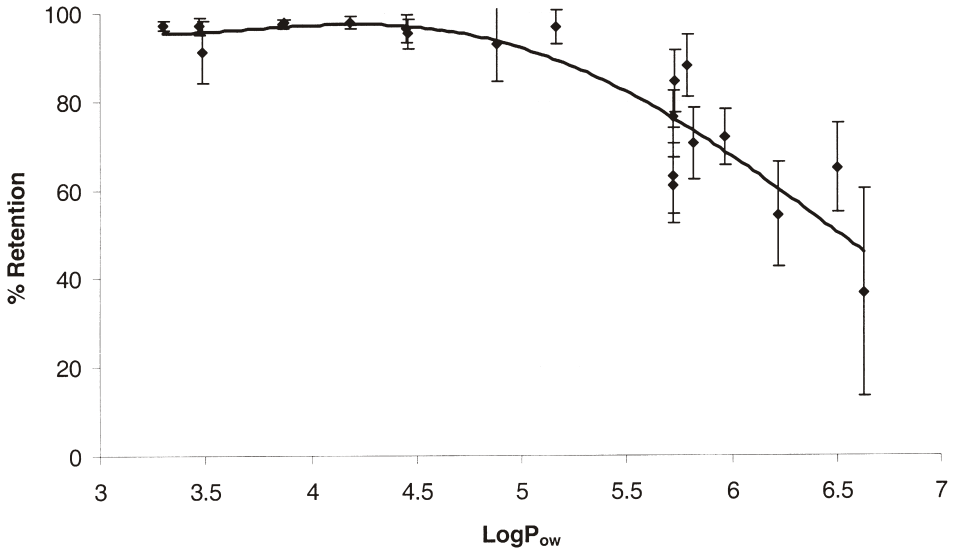

Figure 4. The dependence of retention efficiency for PAHs (in %) on their $LogP_{ow}$

humans from mainstream cigarette smoke. The evaluated cigarette was a common commercial 10.6 mg 'tar' cigarette, and the test was performed on ten subjects each smoking three cigarettes. The lower molecular weight compounds are retained with high efficiency (above 90%). The heavier molecules with the MW > 250 are less retained, but even compounds such as indeno[1,2,3-*cd*]pyrene (MW = 276), dibenz[*a,h*]anthracene (MW = 278), and benzoperylene (MW = 276) are retained with efficiencies around 50%. The result was in some way unexpected, considering the high boiling point of the PAHs with the molecular weight above 250 Daltons. As shown in a study from literature regarding the chromatographic profile of the exhaled cigarette smoke (17), compounds with MW higher than about 250 Daltons are typically retained less than 33% by the smokers. The extremely low level in smoke of most PAHs may contribute to their relatively high retention, although these compounds are hydrophobic and have low partial vapor pressures. A comparison of retention % values of PAHs with their $LogP_{ow}$ values did not show linearity. Better correlation was obtained between the retention efficiency and PAHs vapor pressure (Log VP). The retention of pyrene compared to the level of

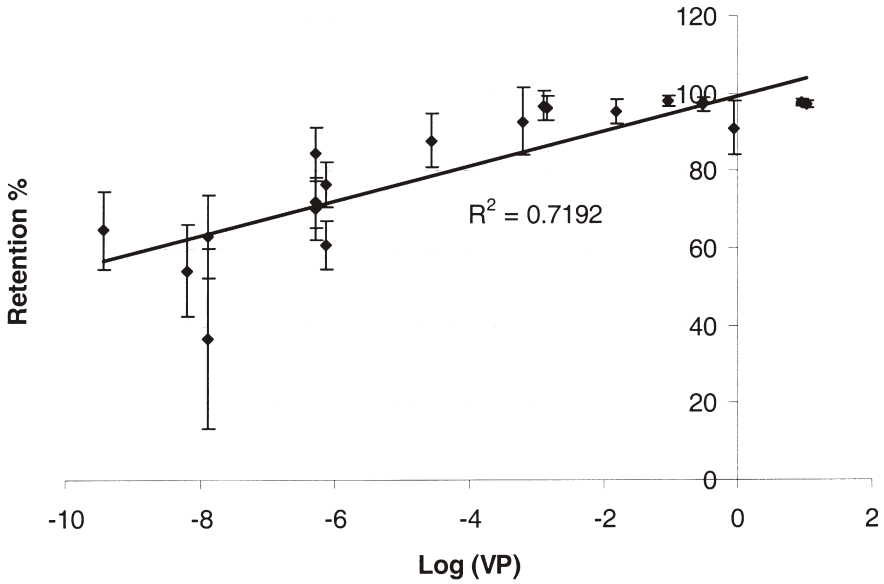

Figure 5. The dependence of retention efficiency for PAHs (in %) on their Log(VP), (VP expressed in Pa)

the metabolite 1-hydroxypyrene reported in literature as analyzed in the urine of smokers, shows a considerable difference (93% retention compared to 26% accounted in urine). This indicates that other paths, such as bile excretion, smoker breath, etc. may contribute to the excretion of the pyrene retained from smoke.

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