Environment International 36 (2010) 763–771

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Environmental and biological monitoring of exposures to PAHs and ETS in the general population

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ARTICLE INFO

Article history:
Received 7 October 2009
Accepted 30 May 2010
Available online 29 June 2010

Keywords:
PAH monophenolic metabolites
ETS biomarkers
personal exposure
LC-MS/MS
GC/MS
1,3-Butadiene
3-ethenylpyridine
MATCH project

ABSTRACT

The objective of this study was to analyse environmental tobacco smoke (ETS) and PAH metabolites in urine samples of non-occupationally exposed non-smoker adult subjects and to establish relationships between airborne exposures and urinary concentrations in order to (a) assess the suitability of the studied metabolites as biomarkers of PAH and ETS, (b) study the use of 3-ethenypyridine as ETS tracer and (c) link ETS scenarios with exposures to carcinogenic PAH and VOC. Urine samples from 100 subjects were collected and concentrations of monophenolic metabolites of naphthalene, fluorene, phenanthrene, and pyrene and the nicotine metabolites cotinine and *trans*-3′-hydroxycotinine were measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS) to assess PAH and ETS exposures. Airborne exposures were measured using personal exposure samplers and analysed using GC–MS. These included 1,3-butadiene (BUT), 3-ethenylpyridine (3-EP) (a tobacco-specific tracer derived from nicotine pyrolysis) and PAHs. ETS was reported by the subjects in 30-min time–activity questionnaires and specific comments were collected in an ETS questionnaire each time ETS exposure occurred. The values of 3-EP (>0.25 μg/m^3 for ETS) were used to confirm the ETS exposure status of the subject. Concentrations as geometric mean, GM, and standard deviation (GSD) of personal exposures were 0.16 (5.50) μg/m^3 for 3-EP, 0.22 (4.28) μg/m^3 for BUT and 0.09 (3.03) ng/m^3 for benzo(a)pyrene. Concentrations of urinary metabolites were 0.44 (1.70) ng/mL for 1-hydroxypyrene and 0.88 (5.28) ng/mL for cotinine. Concentrations of urinary metabolites of nicotine were lower than in most previous studies, suggesting very low exposures in the ETS-exposed group. Nonetheless, concentrations were higher in the ETS population for cotinine, *trans*-3′hydroxycotinine, 3-EP, BUT and most high molecular weight PAH, whilst 2-hydroxyphenanthrene, 3 + 4-hydroxyphenanthrene and 1-hydroxyphenanthrene were only higher in the high-ETS subpopulation. There were not many significant correlations between either personal exposures to PAH and their urinary metabolites, or of the latter with ETS markers. However, it was found that the urinary log cotinine concentration showed significant correlation with log concentrations of 3-EP ($R = 0.75$), BUT ($R = 0.47$), and high molecular weight PAHs (MW>200), especially chrysene ($R = 0.55$) at the $p = 0.01$ level. On the other hand, low correlation was observed between the PAH metabolite 2-naphthol and the parent PAH, gas-phase naphthalene. These results suggest that (1) ETS is a significant source of inhalation exposure to the carcinogen 1,3-butadiene and high molecular weight PAHs, many of which are carcinogenic, and (2) that for lower molecular weight PAHs such as naphthalene, exposure by routes other than inhalation predominate, since metabolite levels correlated poorly with personal exposure air sampling.

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) and volatile organic compounds (VOC) are ubiquitous in outdoor and indoor air, and therefore of public health concern. PAH are formed and emitted into the environment as a result of incomplete combustion of organic materials from natural and human activities. VOC sources are mainly industrial processes, fossil fuel combustion in transportation and heating, solvent use, building materials and environmental tobacco smoke (ETS), also known as second-hand smoke (Harrison et al., 2009). Due to their adverse health effects, including carcinogenicity (IARC, 2006), it is important to assess their concentrations in both occupational and environmental settings (Han et al., 2008).

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doi:10.1016/j.envint.2010.05.015

PAH absorbed into the human body are metabolized to their monohydroxylated PAHs and finally to glucuronides and sulfates, which are excreted in urine and bile (Chetiyanukornkul et al., 2006). As regards nicotine, most is converted in the liver to cotinine which is further metabolized to *trans*-3′-hydroxycotinine. Nicotine, cotinine and hydroxycotinine are conjugated to form nicotine-N-glucuronide, cotinine-N-glucuronide and hydroxycotinine-O-glucuronide. Demethylation of nicotine and cotinine are minor metabolic pathways. Nicotine, cotinine, hydroxycotinine, nornicotine and respective glucuronides account for 74–99% of a nicotine dose excreted in an adult smoker's urine (Hukkanen et al., 2005).

Biological monitoring has been increasingly viewed as a desirable alternative to air sampling for characterizing environmental exposures, not only because it accounts for all possible exposure routes but also because it covers unexpected or accidental exposures and reflects inter-individual differences in uptake or genetic susceptibility (Lin et al., 2005). The use of urinary biomarkers as a non-invasive means for assessing exposure to air toxics concentrations has been widely adopted in occupational exposures (Rossbach et al., 2007; Forster et al., 2008).

1-Hydroxypyrene is widely considered as an appropriate biomarker for exposures to PAH on the basis that pyrene is rapidly distributed, metabolized and eliminated from the body; 1-hydroxypyrene in urine represents a constant fraction (2%) of total pyrene intake (Bouchard et al., 1998); and the half life of urinary 1-hydroxypyrene excretion ranged from 4 to 35 h (Jongeneelen et al., 1990), declining to a baseline within 48-h (Buckley and Lioy, 1992). Whilst many studies have traditionally reported 1-hydroxypyrene in urine (Pastorelli et al., 1999; Han et al., 2008), only recently a few have reported concentrations of the other PAH metabolites in occupationally exposed populations (Serdar et al., 2003; Campo et al., 2006; Chetiyanukornkul et al., 2006; Rossbach et al., 2007; Forster et al., 2008; Rossella et al., 2009). However, very few have reported levels of urinary biomarkers concurrent with PAHs in ambient air of a non-occupationally exposed population (Leroyer et al., 2010) whilst no study has yet reported concurrent measurement of PAH urinary biomarkers and personal exposure levels of PAHs in the general population.

The MATCH (Measurement and Modelling of Air Toxic Concentrations for Health Effect Studies) Project aimed to provide a significant strengthening of the VOC and PAH personal exposure and microenvironment measurement database through generating new data via direct measurements. The study sought to lead to advances in understanding the causes and magnitude of exposures to VOC and PAH (Delgado-Saborit et al., 2009a; Harrison et al., 2009) and to establish whether collecting lifestyle information is sufficient to model personal exposures reliably when compared with exposures evaluated independently by personal samplers (Delgado-Saborit et al., 2009b). The hypothesis tested in the work described in this paper is that urinary metabolites of PAH and ETS are excreted as a consequence of inhalation exposures to PAH and ETS-related VOCs (e.g. 3-EP), and that subjects exposed to higher concentrations of PAH and ETS-related VOCs will excrete higher levels of PAH and ETS metabolites. If the hypothesis is valid, then ETS and PAH metabolites might prove useful as biomarkers of inhalation exposure to carcinogenic VOCs and PAHs, such as 1,3-butadiene and benzo(a)pyrene, and therefore provide an alternative to costly personal exposure assessment measurements. The second hypothesis tested in this work is that 3-ethenylpyridine may serve as an ETS tracer in environmental samples, which might be useful in those situations where urinary ETS biomarkers (e.g. cotinine) cannot be collected.

2. Experimental section

2.1. Recruitment of subjects

The MATCH Project, recruited 100 healthy adult volunteer subjects for personal exposure and indoor microenvironment (home and office) measurements. Selection took no specific account of age, gender or ethnic background. Potential subjects were excluded if they were (a) smokers, (b) under 18 years old, (c) unhealthy, (d) unable to carry personal sampler for any reason, (e) exposed to PAH/VOC at work, or (f) travelled more than 2 h/day professionally, (g) their journey to work took more than 2 h travelling time for the return trip, or (h) the distance from home to their workplace was more than 20 mi. The self-reported non-smoking status of the subjects was checked with the results of cotinine in the urine analyses, as discussed in the Results and Discussion sections.

Subjects resided in three different areas of the United Kingdom selected for their expected gradient in personal exposures concentrations, namely London, West Midlands and rural South Wales. Subjects were chosen to participate based upon four key determinants (i.e. possible VOC/PAH sources), namely the location where they lived, if they were exposed to ETS, if their house incorporated an integral garage and by the proximity of the house to a major road. Subjects were considered ETS exposed whenever they reported in the activity diary to be in the company of a smoker (friend/relative) or to be in places with smokers (e.g. pub) and this information was validated with the airborne concentration of the ETS marker 3-ethenylpyridine (Hyvarinen et al., 2000). Volunteers were asked to complete a screening questionnaire containing information about personal lifestyle, the study key factors and the exclusion criteria. The subjects finally selected were briefed in the use of the equipment and form filling one week prior to the sampling week.

Ethics Committee approval was secured for this study from the South Birmingham Research Ethics Committee, Birmingham (REC Ref No. 04/Q2707/152).

2.2. Environmental monitoring and analysis

2.2.1. Personal exposure sampling

Collection of the samples was spread over two years, from May 2005 to May 2007, 44% of the subjects were sampled during warm months (April–September) and 56% in cold months (October–March).

Each subject was sampled for a group of 14 VOCs and 1,3-butadiene (separately) for a total of five consecutive 24-h periods using one personal sampler pump (SKC model PCXR8), connected to two different sorbent tubes (one for 14 VOCs and the other for 1,3-butadiene), and during one concurrent day an additional pump sampled PAH on 47-mm quartz filters. The active-sampling setup was enclosed in a small aluminum briefcase and additional power was supplied via camcorder batteries connected to the pumps. The flow rates used were 40 mL min^{-1} for VOC, 30 mL min^{-1} for 1,3-butadiene and 3 L min^{-1} for PAH (Delgado-Saborit et al., 2009a). All collected samples were kept in refrigerated conditions after sampling and prior to analysis. Duplicates and blanks were taken from 3% of the study population as described in detail in the Supporting Information.

Researchers met the subjects daily early in the morning to collect the sampler corresponding to the previous 24-h, to supply a new 24-h sampler ready for the new day and to check that all the questionnaires (as described below) were correctly completed.

2.2.2. Subject related information

The atmospheric sampling was backed up with information related to the subjects. Several questionnaires, completed daily included 30-min activity diaries, travel description sheets, location description sheet and activity questionnaires. Information about the subject's demographics, home and products stored within the house were collected once per subject. An ETS questionnaire was filled out when the subjects were exposed to ETS (once per event). Further details of the questionnaires can be found in Harrison et al. (2009).

2.2.3. Analysis of environmental samples

Three methods were employed for analysing 1,3-butadiene, the rest of the VOC and all the PAHs as described in detail in Delgado-Saborit et al. (2009a).

In this study, only a selected group of VOC compounds mainly related to ETS is presented jointly with the urinary biomarker data, which included 3-ethenylpyridine (3-EP), 1,3-butadiene (BUT) and naphthalene (Naph) in the gas phase. The measured particulate-phase PAH were acenaphthylene (Ac), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fluo), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chry), benzo(b) fluouranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), indeno(1,2,3-cd) pyrene (Ind), benzo(ghi)perylene (BghiP), dibenzo(ah)anthracene (DahA) and coronene (Cor).

2.3. Biological monitoring

2.3.1. Urine collection and storage

As part of the sampling protocol, the subjects provided a urine sample with the purpose of performing urinary biomarker analyses related to the air toxics under study. The first, mid-stream urine sample done in the morning – corresponding to the previous 24-h personal exposure sample – was collected every day in a 100 mL polypropylene bottle from each volunteer in order to analyse a set of biomarkers present in the urine. Urine samples were collected by the researcher during the daily visit to the subjects' house, were then transferred to the laboratory in refrigerated conditions prior to storage in a −80 °C freezer.

2.3.2. Selection of urine samples for analysis

A total of 500 urine samples were collected. Among those samples, a subset of 100 urine samples were chosen for analysis balancing the following criteria: (a) prioritise the analysis of at least one urine sample from each subject; (b) maximise the number of urine samples related to the day where VOC and PAH were sampled concurrently; (c) select wherever possible urine samples representative of days with reported ETS exposure, which was verified with the levels of 3-ethenylpyridine in personal exposure. Six subjects did not give their consent for analysing their urinary biomarkers and four PAH personal samples were not collected due to faulty pumps. From the 100 selected samples, eight samples from five subjects were not considered in the data analysis, as from the high cotinine values (>50 ng/mL), it is highly likely that these subjects smoked at some point prior to the urine collection (Jarvis et al., 1987) as confirmed later. This subset of 92 samples (excluding the 8 samples from smokers) contained samples from two different days for six subjects. To avoid a within-subject effect upon between-subject relationships, the 2-day mean values have been considered in the analysis for each of those six subjects instead of the individual 1-day data. Therefore, the final number of samples in the analysed subset is 86 urine/VOC data. This subset consists of 62 urine–VOC/PAH data and 24 pairs of urine–VOC data (See Table S2 in Supporting Information). As regards ETS exposure, 55 urine samples were from No ETS subjects and 31 were from ETS-exposed subjects, which includes 19 samples from subjects with low ETS exposures and 12 samples from subjects with high ETS exposures. The criteria to classify subjects as No-, low- and high ETS exposure are described in detail in the Results section.

2.3.3. Analysis of urinary biomarkers

About 15 mL of urine from each of the selected 100 samples was sent in dry ice to the Division of Clinical Pharmacology at the University of California San Francisco. The urine samples were analysed for the nicotine metabolites cotinine (Cot) and *trans*-3′-hydroxycotinine (T3HCot) and the PAH metabolites 2-naphthol (2-Nap), 1-hydroxy-fluorene (1-HFl), 2-hydroxyfluorene (2-HFl), 3-hydroxyfluorene (3-HFl), 1-hydroxyphenanthrene (1-HPhe), 2-hydroxyphenanthrene (2-HPhe), 3 + 4-hydroxyphenanthrene (3 + 4-HPhe) and 1-hydroxypyrene (1-HPyr).

Monohydroxy metabolites of naphthalene, fluorene, phenanthrene, and pyrene were determined by the method of Jacob et al., (2007). Concentrations of cotinine and *trans*-3′-hydroxycotinine in urine were determined using liquid chromatography–tandem mass spectrometry (LC-MS/MS). The method (Jacob et al., in preparation) is similar to a published method for determining cotinine concentrations in plasma of non-smokers (Bernert et al., 1997) (Supporting Information contains a detailed description).

The methods have been fully validated, using the criteria of Shah et al. (2000), for precision, accuracy, and to determine the lower limits of detection. These are precision (CV) of ± 15% and accuracy within ± 15% of the expected amount, except at the lower limit of quantitation, for which ± 20% is considered acceptable. These criteria are widely used in drug development studies and are acceptable to the US Food and Drug Administration (Shah et al., 2000).

2.4. Statistical analysis

Data were analysed using SPSS 15.0 for Windows (SPSS Inc.1989–2006), Excel 2002 (Microsoft Corporation, 1985–2001) and Access 2007 (Microsoft Corporation, 2006). Data below the detection limit (See Table S3, supporting information for LOD) were replaced with half the value of the detection limit for the purpose of statistical analysis. When urinary data was available for a subject for more than one day, the arithmetic mean of both measurements was used for the data analysis. Low-, Medium- and High-molecular weight (MW) PAH were calculated adding the concentrations of Naph to An, Fluo to Chry and B(b)F to Cor, generating new variables labelled as SumMW120-180, SumMW200-250 and SumMW250-300 respectively. The sum of the first 16 PAH was also calculated adding the concentrations from Naph to B(ghi)P (Sum16PAH). These newly calculated values were considered as independent variables in the data analysis.

Personal exposures and microenvironment concentrations were tested for normality using the skewness statistic. All the VOC concentrations, including 1,3-butadiene, PAH and urinary biomarker (ETS and PAH metabolites) concentrations were found to have right-skewed distributions. For this reason geometric means and geometric standard deviations are reported and all the environmental and biological concentrations were logged ($\log_{10}$). The datasets were normally distributed after log transformation. Measures of the association between PAH and VOC in environmental samples and ETS and PAH metabolites in biological samples were characterised by Pearson correlation coefficients (R) for logged data. Statistical differences between two strata (i.e. ETS vs. No ETS concentrations) were tested in the logged database with a *t*-test for equality of means and Kolmogorov–Smirnov in those cases where the variance was heterogeneous. General Linear Models were used to search for interaction between different subject characteristics (e.g. gender, age) and excretion of creatinine levels. Results were considered significant with p values less than 0.05. Variance was considered homogeneous provided that $p > 0.05$ in Levene's test.

3. Results

3.1. Description of subjects

The main sources of exposure that were examined in this study were inhalation exposures. Therefore information regarding activities and characteristics that might affect the airborne concentrations relating to personal exposure was collected. This information consisted of subjects' demographics (e.g. age, gender, occupation), time spent in different microenvironments, activities that may affect the PAH and/or VOC personal exposures (e.g. fireplace use, candle burning), home characteristics and ETS exposure characterization. This information collected through questionnaires is summarized in Table S4 for the subjects that participated in the study as well as for the sub-sample of subjects whose urinary samples were analysed. As dietary exposures were not in the scope of this study,

information about diet lifestyles (e.g. vegetarianism), cooking styles or duplicate food samples was not collected.

3.2. ETS classification criteria

The ETS status self-reported in the initial screening questionnaire was confirmed with information reported in the questionnaires daily and with the personal exposures to 3-ethenylpyridine, an ETS tracer.

No ETS subjects were those who declared his/her No ETS status in the screening questionnaire and no ETS events were registered in the time–activity diaries. This information was later confirmed with the levels of 3-ethenylpyridine (3-EP) in air samples. Those subjects whose 3-EP was $<0.25\ \mu g/m^3$ (maximum 3-EP concentration measured in the No ETS subpopulation) were classified as No ETS. In fact the 3-EP value in the No ETS population was $0.07 \pm 0.06\ \mu g/m^3$.

ETS subjects were those who reported ETS events in the time–activity diary on the day preceding urine collection. This information was confirmed with the levels of 3-ethenylpyridine (3-EP) in air samples. Those subjects whose 3-EP was $0.25<3\text{-EP}<1.4\ \mu g/m^3$ were classified as low ETS. Those subjects whose 3-EP was $>1.4\ \mu g/m^3$ were classified as high ETS. The value of $1.4\ \mu g/m^3$ was selected from the frequency distribution of 3-ethenylpyridine in the ETS population (Fig. 1). In Fig. 1 the distribution of ETS subjects appears to be bimodal, with the value of $1.4\ \mu g/m^3$ as the cutpoint between both modes.

3.3. Levels of ETS and PAH metabolites in urine and personal exposures to PAH and selected VOCs

Table 1 presents the geometric means (GM) and geometric standard deviation (GSD) of the urinary ETS biomarker and PAH metabolite concentrations, with the corresponding personal exposure concentrations to selected VOCs (i.e. 3-EP, BUT, Naph), corresponding parent PAH (i.e. Fl, Phe, Pyr) and non-parent PAH compound for the analysed samples excluding those from subjects which had extremely high cotinine concentrations (i.e. smokers). Arithmetic values, minima and maxima as well as the creatinine corrected concentrations are available in Table S5, supporting information.

3.4. Effect of ETS on nicotine- and PAH metabolites in urine

The samples have been subdivided as ETS and non-ETS exposed based on the threshold personal exposure to 3-EP of $0.25\ \mu g/m^3$. This cut-off concentration was determined from the comparison of high 3-EP concentrations with the activities noted in the time–activity diaries, indicating exposure to ETS, as described above.

The urinary biomarkers of nicotine exposure (cotinine and *trans*-3′-hydroxycotinine) and the personal exposure concentrations of selected VOC (3-EP and BUT) and some non-parent PAH compounds were higher in the ETS-exposed sub-sample compared with the non-ETS at the $p<0.05$ level. On the other hand, for the PAH metabolite biomarkers and the respective PAH parent compounds, there is no statistically significant distinction between the ETS and non-ETS-exposed groups. However, when comparing the high-ETS subgroup with the other two (i.e. non- and low ETS) there are statistically significant differences at the $p<0.05$ level for 2-hydroxyphenanthrene, 3 + 4-hydroxyphenanthrene and 1-hydroxypyrene. Within-subject cross-correlations between PAH were calculated using the log database. For all compounds except the most volatile (acenaphthene, acenaphthylene and fluorene), cross-correlations were significant at $p<0.01$, giving correlation coefficients typically between 0.35 and 0.95. This is not surprising as PAH exposure is typically to a mixture of compounds.

3.5. Effect of sources other than ETS in urinary PAH metabolites

As outlined above, the subjects participating in this study came from three distinct geographic areas of the United Kingdom (Birmingham, London and South Wales), including urban, suburban and rural dwellers. Additionally, subject selection explicitly took account of whether the subject's home had an integral garage and whether it fronted onto a major highway. When the personal exposures to different levels of PAH and VOC and urinary metabolites of PAH were analysed in relation to these potential influences on exposure, no significant differences were found. We attribute this to the importance of ETS exposure which was not appreciably influenced by the other factors and for some of the compounds, the importance of non-respiratory exposures.

3.6. Correlations between personal exposure levels and biological concentrations in urine

Correlations of the logged concentrations of urinary biomarkers with selected VOC, respective PAH parent compounds and non-parent PAH compounds in personal exposures are shown in Table 2. Normalising with creatinine is a common practice used to correct for differences in urinary flow. Correlations between environmental concentrations and creatinine-normalised logged concentrations of urinary biomarkers (i.e. corrected with creatinine) are shown in Table S6, supporting information.

Correlations nominally significant at the $p<0.05$ level (2-tailed) are highlighted in bold type. The scatter plots corresponding to some of the compounds that correlate ($p<0.05$) with urinary biomarkers are presented in Fig. 2. Suprisingly, in the case of the ETS biomarkers, the correlation of the biomarker concentrations with selected VOC and some non-parent PAH personal exposures is significantly higher in the un-normalised data (Table 2) than in the normalised data (Table S6). Table 2 shows that log cotinine un-normalised has a significant correlation with log 3-EP ($R=0.75$), with log BUT ($R=0.47$) and with log Chry ($R=0.55$), which are all higher than for the respective creatinine-normalised correlations ($R=0.74/0.43/0.44$ respectively) (Table S6). PAH metabolites do not correlate with the parent or non-parent PAH compounds. However, when data from only high-ETS subjects is correlated, 2-naphthol correlates at the 0.05 level with Ph, Pyr, Chry, SumMW120-180, SumMW200-250 and Sum16PAH.

4. Discussion

4.1. Environmental levels of PAH and selected VOCs in personal exposures

The levels of VOC and PAH concentrations in personal exposure observed in this study (Table 1) are substantially lower than those

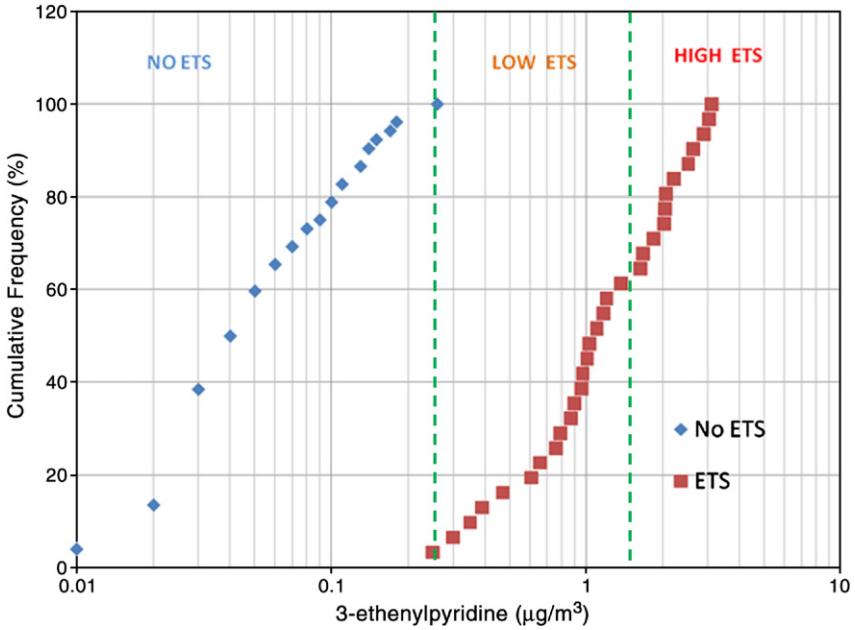

Fig. 1. Cumulative frequency distribution of 3-ethenypyridine ($\mu g/m^3$) in ETS and No ETS subjects.

Table 1
Concentrations of urinary biomarkers (ng mL^{-1}), selected VOC (μg m^{-3}), parent PAH (ng m^{-3}) and non-parent PAH (ng m^{-3}) personal exposure concentrations by key determinant.

	% Cases<LOD	All subjects (ETS + No ETS)			No ETS subjects			ETS subjects		
		No cases	GM	GSD	No cases	GM	GSD	No cases	GM	GSD
Urinary biomarker concentrations										
Cot	0	86	0.88	5.28	55	0.33	2.7	37	3.72[a]	4.01
T3HCot	0	86	3.13	4.6	55	1.41	3.12	37	10.2[a]	3.47
2-Nap	0	86	2.92	2.52	55	3.13	2.64	37	2.64	2.34
1-HFl	86	11	0.17	2.08	6	0.19	2.63	5	0.15	1.46
2-HFl	0	86	0.28	2.07	55	0.30	2.17	37	0.25	1.90
3-HFl	56	38	0.18	1.77	24	0.16	1.92	14	0.21	1.42
1-HPhe	1	85	0.22	2.21	54	0.22	2.08	37	0.22	2.43
2-HPhe	0	85	0.14	1.66	55	0.14	1.63	37	0.14[b]	1.72
3 + 4HPhe	1	85	0.22	2.16	54	0.22	1.98	37	0.21[b]	2.43
1-HPyr	1	85	0.14	1.66	54	0.14	1.62	37	0.13[b]	1.72
Selected VOC personal exposure concentrations										
3-EP	2	84	0.16	5.5	57	0.05	2.15	27	0.78[a]	3.95
Naph	2	84	0.64	2.63	57	0.57	2.29	27	0.76	3.08
But	1	85	0.22	4.28	57	0.16	4.41	28	0.35[a]	3.63
Parent PAH personal exposure concentrations										
Fl	91	6	0.23	6.21	3	0.22	6.7	3	0.25	8.77
Phe	31	47	0.2	3.18	35	0.21	3.06	12	0.18	3.72
Pyr	21	54	0.22	2.87	38	0.25	2.57	16	0.17	3.67
Non-parent PAH personal exposure concentrations										
Ac	46	37	0.2	4.19	25	0.18	4.27	12	0.23	4.22
Ace	47	36	0.24	3.43	28	0.25	3.17	8	0.2	4.72
Ant	34	45	0.04	3.28	34	0.05	3.28	11	0.04	3.36
Fluo	22	53	0.37	2.8	39	0.37	2.74	14	0.35	3.1
BaA	6	64	0.06	3.27	43	0.05	3.03	21	0.09[a]	3.69
Chry	1	67	0.22	2.72	43	0.18	2.34	24	0.38[a]	3.23
BbF	3	66	0.16	2.94	43	0.14	2.69	23	0.22[a]	3.41
BkF	0	68	0.15	2.5	43	0.13	2.41	25	0.21[a]	2.56
BaP	4	65	0.09	3.03	43	0.08	2.89	22	0.13[b]	3.25
Ind	0	68	0.1	2.84	43	0.09	2.84	25	0.11	2.88
BghiP	21	54	0.03	2.54	38	0.02	2.43	16	0.04[b]	2.6
DahA	0	68	0.14	2.9	43	0.13	2.84	25	0.17[a]	3.08
Cor	12	60	0.09	2.57	42	0.09	2.8	18	0.12	1.93
Σ Low MW [Naph-An]	0	68	0.69	3.07	43	0.60	3.06	25	1.12[a]	2.8
Σ Medium MW [Fluo-Chry]	0	68	0.65	3.76	43	0.61	3.62	25	0.75[b]	4.25
Σ High MW [B(b)F-Cor]	0	68	0.71	2.94	43	0.62	2.9	25	0.98[b]	2.91
Σ 16 [Naph-B(ghi)P]	0	69	2	2.92	43	1.82	2.79	26	2.54[b]	3.24

[a] The concentrations in ETS subset are significantly different than the non-ETS subset at the 0.05 level.
[b] The concentrations in the high-ETS subset (3-EP > 1.4 μg m^{-3}) only are significantly higher than the non- and low-ETS subset at the 0.05 level.

found in similar studies, conducted in different locations in the United States and Europe and at earlier times as discussed in detail in Delgado-Saborit et al. (2009a).

4.2. Biological levels of PAH and ETS biomarkers in urine

Nicotine and PAH urinary metabolites from 100 first morning urine samples of non-occupationally exposed subjects have been analysed (Table 1). Cotinine and *trans*-3′hydroxycotinine levels in non-ETS samples are similar than those reported by Lazcano-Ponce et al. (2007) and Jacob et al. (2007) whilst much lower than those reported by Simoni et al. (2006), Heinrich et al. (2005) and Wall et al. (1988), however cotinine and *trans*-3′hydroxycotinine levels for ETS subjects are substantially lower than previously reported (Wall et al., 1988; Heinrich-Ramm et al., 2002; Heinrich et al., 2005; Jacob et al., 2005; Simoni et al., 2006; Lazcano-Ponce et al., 2007; Matt et al., 2007). Chetiyanukornkul et al. (2006) reported levels in Thailand of PAH biomarkers for taxi drivers and traffic wardens, where both groups were occupationally exposed to VOCs and PAHs, to be in the range of those found in this study, whilst the levels found in Thai rural villagers working in farms were considerably higher. As regards 1-hydroxypyrene levels, our results are in line with results reported in several studies performed in Germany, Italy and France (Merlo et al., 1998; Pastorelli et al., 1999; Heudorf and Angerer, 2001; Leroyer et al., 2010), whilst data reported from Thai rural villagers (in 2006), Dutch adults (in 1994), Polish children (in 1994) and adults in Rome (Italy, in 2001), spanning from the mid 1990s to the mid 2000s, are substantially higher than our 1-HPyr data (Jongeneelen, 1994; Tomei et al., 2001; Chetiyanukornkul et al., 2006). Several extreme cases of urinary biomarkers were identified in our study (Table S5). The information provided in the subjects' activity diaries allowed it to be identified that all the cotinine and tran-3′hydroxycotinine extreme cases were related to higher exposures to ETS, whilst PAH urinary biomarker extremes were related to use of a fireplace (i.e. wood and gas), use of a photocopier and commuting through heavily trafficked roads.

4.3. ETS metabolites as biomarkers of ETS exposure

Previous studies have indicated that the levels of urinary cotinine in non-smokers were usually less than 20 μg/L and the discrimination threshold between active and passive smokers was from 50 to 100 μg/L (Jarvis et al., 1987). In this project the arithmetic mean urinary cotinine level is 3.33 ± 6.39 μg/L and the geometric mean and standard deviation is 0.88 (5.28) μg/L, which shows that our non-smoking subjects are towards the lower end of the range of ETS exposure. However, eight samples from five volunteers were excluded from the analysis as they were well above 50 μg/L of

Table 2
Correlation of urinary biomarkers with selected ETS, VOC compounds, parent PAH and non-parent PAH personal exposures (Pearson R, logged database) (sample size VOC ≤ 86, PAH ≤ 68).

	Cotinine	*trans*-3′-Hydroxycotinine	2-Naphthol	2-Hydroxyfluorene	1-Hydroxypyrene
VOC selected compounds					
3-Ethenyl Pyridine	**0.75**[a**]	**0.64**[a**]	−0.09	−0.020	−0.080
Naphthalene (gas phase)	0.050	−0.050	0.030	0.00	−0.060
1,3-Butadiene	**0.47**[a**]	**0.44**[a**]	0.070	0.15	0.15
PAH parent compounds					
Fluorene	−0.23	0.11	−0.73	−0.26	0.48
Phenanthrene	0.11	0.04	−0.13 (**0.90**[b])	0.12	0.10
Pyrene	0.070	0.090	0.17 (**0.88**[b])	**0.31**[a*]	0.18
PAH non-parent compounds					
Benzo(a)anthracene	**0.35**[a*]	**0.32**[a*]	0.20	0.17	−0.12
Chrysene	**0.55**[a**]	**0.52**[a**]	**0.30**[a*] (**0.76**[b])	0.28	0.11
Benzo(b)fluoranthene	**0.42**[a**]	**0.38**[a**]	0.15	0.17	0.10
Benzo(k)fluoranthene	**0.45**[a**]	**0.42**[a**]	0.16	0.26	0.15
Benzo(a)pyrene	**0.38**[a*]	**0.36**[a*]	0.22	0.28	0.14
Indeno(1,2,3-cd)pyrene	0.060	0.090	0.16	0.14	0.050
Dibenz(a,h)anthracene	**0.37**[a*]	**0.32**[a*]	0.080	0.070	0.00
Benzo(ghi)perylene	**0.30**[a*]	**0.31**[a*]	0.20	0.16	0.12
Coronene	0.23	0.21	0.010	0.060	0.080
Sum of low MW PAH [Naph-An]	0.17	0.11	−0.080 (**0.92**[b])	0.080	0.080
Sum of medium MW PAH [Fluo-Chry]	**0.27**[a*]	**0.31**[a*]	**0.33**[a*] (**0.88**[b])	**0.26**[a*]	0.060
Sum of high MW PAH [B(b)F-Cor]	**0.37**[a*]	**0.37**[a*]	0.24	0.21	0.15
Sum of 16PAH [Naph-B(ghi)P]	**0.26**[a*]	**0.31**[a*]	**0.24**[a*] (**0.84**[b])	**0.25**[a*]	0.13

Bold Values represent correlations statistically significant at the 0.05 level (2-tailed).
[a] The correlation between the variables is statistically significant in a 2-tailed test at the 0.05 level (*) or at the 0.01 level (**).
[b] The correlation between the variables is statistically significant in a 2-tailed test at the 0.05 level only in the high-ETS subset ($N = 9$).

cotinine—the cut-off value suggested for smokers. In a subsequent communication, the subjects thus identified admitted that they had been smoking.

4.3.1. Cotinine vs. trans-3′-hydroxicotinine as ETS biomarker

The influence of exposure to ETS has been studied. Cotinine is the primary metabolite coming from nicotine that is very stable in the body (half life is approximately 18 h, CV 30%) and can be measured reliably in blood, saliva and urine for monitoring nicotine exposure in people (Benowitz and Jacob, 1994). However, Tuomi et al. (1999) suggest that although cotinine has been used extensively as a nicotine marker in the urine of both active and passive smokers, *trans*-3′-hydroxycotinine is the predominant nicotine metabolite, corresponding to 40% of the total nicotine excretion and is better used alongside cotinine when monitoring passive ETS exposure (Tuomi et al., 1999). This is consistent with our results, where higher concentrations of *trans*-3′-hydroxycotinine were measured compared with cotinine. However from Table 2 it can be seen that Pearson correlations of 0.75 and 0.64 respectively are obtained when correlating cotinine and *trans*-3′-hydroxycotinine with 3-EP respectively. Thus both nicotine metabolites correlated extremely well, but cotinine correlates most highly with our chemical marker of vapour phase ETS (3-EP), supporting the hypothesis that 3-EP can be used as a chemical tracer of ETS.

4.3.2. Normalising vs. non-normalising metabolite levels with creatinine

The correlations of cotinine and *trans*-3′-hydroxycotinine with 3-EP, BUT and some non-parent PAH were higher when considering the non-creatinine-normalised ETS metabolite data (Table 2 and Table S6 supporting information for creatinine-normalised data). This poses the question of the appropriateness of normalising the ETS urinary biomarkers with creatinine. Hinwood et al. (2002) argue that 24 h composite or spot samples can be influenced by urine output rate or dilution. Viau et al. (2004) advocate creatinine adjustment to correct for urine dilution. On the other hand, Boeniger et al. (1993) and Barr et al. (2005) argue that creatinine excretion varies with meat intake,

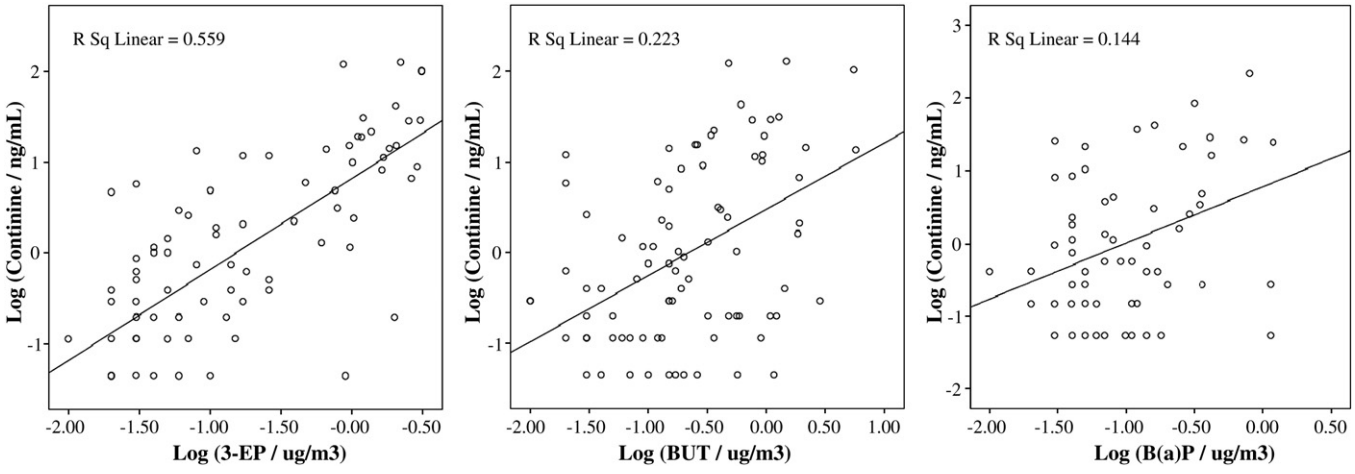

Fig. 2. Scatter log plots of urinary cotinine (in ng/mL) and personal exposure to selected VOC (in μg/m^3) and PAH (in ng/m^3) where significant correlations have been identified.

diurnal factors, age, gender and other factors. This suggests that the creatinine adjustment method may not adequately reduce the measurement variability due to urine dilution, as appears to occur in our dataset (Supporting Information Table S6). Thompson et al. (1990) adjusted cotinine levels based on the observed regression relationship between urinary cotinine and urinary creatinine in a group of smokers instead of the common method of expressing urinary cotinine as a ratio to urinary creatinine (Thompson et al., 1990). Along with the traditional method of normalising biomarker data with creatinine (i.e. ratio of biomarker to creatinine concentration), we tested also the method proposed by Thompson et al. (1990). In our study, however, the correlation between logged cotinine and logged *trans*-3′-hydroxycotinine with logged creatinine is very low ($N=86$; $R_{Cot}=0.06$; $R_{T3HCot}=0.26$; $p>0.10$) suggesting that Thompson's method is not valid in the case of non-smoker subjects. Further investigation of the effect of season, age and gender on the levels of creatinine, cotinine and *trans*-3′-hydroxycotinine by means of a General Linear Model suggests a mild interaction of gender (males) plus age and the interaction of gender (males) plus season (winter) as factors affecting creatinine levels ($p<0.10$), whilst no effect was flagged for cotinine or *trans*-3′-hydroxycotinine concentrations. These results may indicate different excretion mechanisms for creatinine, cotinine and *trans*-3′-hydroxycotinine, consistent with the arguments provided by Boeniger et al. (1993) and Barr et al. (2005) which suggest that the creatinine adjustment method may not adequately reduce the measurement variability due to urine dilution for ETS biomarkers.

4.3.3. Correlation between exposures to PAH and ETS metabolites

Table 2 shows that not only is there a strong association ($p<0.01$) between ETS metabolites and personal exposures to ETS VOC markers (e.g. 3-EP, $R=0.75$ and BUT, $R=0.47$ with cotinine), but also with most of the PAH found predominantly in the particulate phase (≥ 4-rings), with Pearson coefficients ranging from 0.3 (BghiP/Cot) to 0.55 (Chry/Cot). The PAH compound that shows the strongest correlation with the ETS urinary biomarkers cotinine (Pearson $R=0.55$, $p<0.01$) and *trans*-3′-hydroxycotinine (Pearson $R=0.52$, $p<0.01$) is chrysene. These results suggest a direct relation between ETS exposures and exposure to PAH compounds bound to particles. This is consistent with an earlier work where chrysene was emphasized to be a main constituent of sidestream smoke, the primary contributor to ETS (Georgiadis et al., 2001). This suggests that the ETS urinary biomarkers studied are good biological indicators of personal exposure to high molecular weight polycyclic aromatic hydrocarbons, including carcinogenic PAHs such as benzo(a)pyrene, in our study population. This finding may not apply to other populations in which non-ETS exposures to PAH are dominant. Further data obtained in smoking environments during MATCH is being evaluated to identify if Chry in conjuction with 3-EP data can be used as a PAH marker for ETS in indoor environments.

4.4. PAH metabolites as biomarkers of inhaled PAH and ETS exposure

As regards the PAH urinary metabolites, Jacob et al. (2007) reported that the hydroxyfluorenes, hydroxyphenanthrenes, 1-hydroxypyrene and 2-naphthol were all significantly higher in smokers compared to non-smokers (Jacob et al., 2007; Wilhelm et al., 2007). Merlo et al. (1998) found that non-smoker subjects exposed to ETS had higher levels of 1-HPyr than those non-ETS exposed. However in our case, higher concentrations were not observed in our ETS-exposed group (Table 1), either because the ETS exposure was not high enough (dependent on number of cigarettes smoked, time spent in the ETS, distance from smokers, ventilation) to make such a distinction clear or else other sources of PAH contributed to higher exposures in the non-exposed group. In the high-ETS subgroup only, levels of 2-hydroxyphenanthrene, 3 + 4-hydroxyphenanthrene and 1-hydroxypyrene were statistically significantly higher than the levels in the other groups. This suggests that it is only in high ETS environments that ETS is a relevant source of total exposure to PAH, compared with other PAH intake pathways such as dietary sources (Ramesh et al., 2004).

4.4.1. Correlation between PAH and ETS exposures and PAH metabolites

It was also noted that there was no correlation between the PAH metabolites and the ETS metabolites or the ETS VOC markers; nor was there a correlation between the PAH metabolite biomarkers and the respective PAH parent compounds (Table 2). This lack of correlation may arise because the PAH parent compounds (i.e. Naph, Fl, Phe and Pyr) come from a multitude of sources, including both inhalation and the diet. It might also be a consequence of the very low levels of PAH and VOC concentrations to which the subjects were exposed, even those classified as ETS-exposed, compared with exposures in smokers, the latter not having been considered in this project. This is consistent with the fact that only in the high-ETS subset some correlations appeared between 2-naphthol and some PAHs, but not with naphthalene itself, which suggests that it is only in high ETS exposures that 2-naphthol might be an ETS biomarker and that Naph might have additional intake sources. A further cause may be inter-subject variability in host polymorphism in genes for PAH metabolism (Brand and Watson, 2003). Hence, it appears that the metabolites of the PAH compounds cannot be used with confidence to assess personal exposures to PAHs, nor to distinguish between a low and a non-ETS-exposed group. This finding is consistent with results reported by Leroyer et al. (2010), which suggest that 1-hydroxypyrene is not an unequivocal biomarker of exposure to atmospheric PAHs in atmospheric scenarios relevant to the general population. On the other hand, cotinine and *trans*-3′-hydroxycotinine show a significant correlation ($p<0.05$) with the sum of 16 PAH, which include the low molecular weight PAH group (Naph–An) containing the PAH parent compounds.

It is unfortunate that the PAH metabolites measured in urine derive from the low molecular weight compounds (naphthalene, fluorene, phenanthrene and pyrene), for which our data do not reflect the entire airborne concentration, as the sampling technique collects only the particle phase, which contains a minor proportion of the low molecular weight PAHs. This may partly explain the low correlation of PAH urinary metabolites and parent compounds (Table 2). However, a recent study by Leroyer et al. (2010) also supports our findings regarding the lack of correlation between pyrene (gas + particulate phase) with the 1-hydroxypyrene metabolite in urine. The personal exposure data for naphthalene (in the gas phase) should be reliable as this was measured with the VOC methodology. The relatively weak correlation between naphthalene exposures and urinary 2-naphthol (in Table 2) is probably due to other non-respiratory sources of naphthalene exposure, of which there are many (Price and Jayjock, 2008). It is also notable that while Fustinoni et al. (2010) found positive correlations between respiratory exposure and urinary concentrations of BTEX compounds, naphthalene did not show a significant correlation.

4.4.2. Importance of other routes of exposure to PAH in urinary PAH metabolites

Dietary exposure to PAH might be a greater source of PAH intake into the body than airborne PAHs as the estimated average adult dietary intake of BaP and BaA was 1.6 and 0.8 ng/kg bodyweight/day respectively in 2000 in the UK population (Food_Standards_Agency, 2002). In comparison, at the UK air quality standard for B(a)P of 0.25 ng/m^3, assuming a 70 kg person inhaling 20 m^3 of air daily, B(a)P intake from the atmosphere is much lower at 0.07 ng/kg bodyweight/day. However, the relative absorption efficiencies via the gastrointestinal and respiratory tracts are not known. Nevertheless, collecting information about dietary exposures was not included

in the study design and therefore the interaction between diet, air exposures and other subject characteristics (e.g. age, gender and/or ethnicity) and urinary biomarker excretion would require further research.

4.5. Limitations and strengths

The authors have identified some limitations and strengths in this study. The main limitation was that only particulate-phase PAHs were collected in personal exposures. However, most of the parent compounds of the urinary biomarkers measured are mainly found in the gas phase. Therefore, correlations between PAH urinary biomarkers and parent compounds in the gas phase could not be assessed within this study. On the other hand, the strength of this study is that for the first time, personal exposures to PAHs and VOC compounds and a wide variety of PAH and ETS urinary biomarkers have been measured simultaneously in the non-occupationally exposed population. Only Pastorelli et al. (1999) and Leroyer et al. (2010) report measurements of environmental and biological exposures to PAHs made simultaneously. However, they only reported values for 1-hydroxypyrene and 3-hydroxybenzo(a)pyrene, compared with the wide range of urinary biomarkers that we have reported, including those of ETS exposure (i.e. cotinine and *trans*-3′ hydoxycotinine). Therefore, the fact that we have measured simultaneously PAH and VOCs in personal exposures and PAH and ETS urinary metabolites has allowed us to assess the correlation of environmental concentrations with urinary metabolites for those subjects in the general population, and in particular to those exposed to second-hand smoke.

5. Conclusion

Our study has studied for the first time the relationship between urinary PAH and ETS metabolites and PAH and VOC personal exposures in the general population. The monophenolic metabolites of low molecular weight PAH are not well correlated with the nicotine metabolites or 3-ethenylpyridine, indicating that ETS is probably not the main source of exposure to the parent low molecular weight particle-phase PAH in the non-smoking population. The fact that some PAH metabolites are higher only in the high ETS, but not the low-ETS subpopulation supports this finding. This is most likely because ETS is not the main contributor to airborne concentrations for the non-smoking population or because dietary sources dominate exposure to these compounds. The fact that airborne gas-phase naphthalene concentrations are not correlated with urinary 2-naphthol suggests the latter is the case for this compound. For fluorene and phenanthrene, our measurement data contain high uncertainties as we were only able to measure the minority particle-associated phase of these compounds. These results suggests that PAH metabolites cannot be used as unequivocal biomarkers of airborne ETS and PAH exposures.

Our study also presents novel data showing significant relationships between urinary excretion of the nicotine metabolites, cotinine and *trans*-3′-hydroxycotinine and the gas-phase ETS marker, 3-ethenylpyridine. This serves to confirm the value of 3-ethenylpyridine as a chemical marker of ETS exposure which can be used in studies where it is not practicable to take urine samples.

Furthermore we demonstrate significant associations between the urinary nicotine metabolite concentrations and personal airborne exposures to 1,3-butadiene and benzo(a)pyrene, which IARC classifies as a known human carcinogen. In the context of the measured exposures, it appears that ETS is an important source of 1,3-butadiene and benzo(a)pyrene exposure. In the case of the other higher molecular weight PAH (benzo(a)anthracene, chrysene, benzo(b) fluoranthene, benzo(k)fluoranthene and dibenzo(a,h)anthracene, we found significant correlations between the atmospheric exposures to these compounds and the concentrations of urinary nicotine metabolites. Correlations of the sum of the medium molecular weight PAH (fluoranthene to chrysene) and of the sum of high molecular weight PAH (benzo(b)fluoranthene to coronene) with cotinine and *trans*-3′-hydroxycotinine were statistically significant. These data emphasize the importance of ETS as a medium for exposure of non-smokers to polycyclic aromatic hydrocarbons and 1,3-butadiene. These compounds are likely to play a major role in the elevated cancer risk associated with ETS exposures.

Funding

Research described in this article was conducted under contract to the Health Effects Institute (HEI), an organization jointly funded by the United States Environmental Protection Agency (EPA) (Assistance Award No. R-82811201) and certain motor vehicle and engine manufacturers. The contents of this article do not necessarily reflect the views of HEI, or its sponsors, nor do they necessarily reflect the views and policies of the EPA or motor vehicle and engine manufacturers. Analytical chemistry carried out at the University of California, San Francisco was funded by the Flight Attendant Medical Research Institute, the US National Institutes of Health (DA012393, CA78603), and the California Tobacco Related Disease Research Program (10RT-0215).

Acknowledgements

The authors wish to thank all the 100 subjects who participated in MATCH, and to acknowledge the support provided by agencies in London (Environment Agency and DEFRA), Birmingham and Swansea City Councils and the local media to recruit volunteers. Special thanks go to Dr Ben Armstrong who advised over the statistical analysis and the Biosciences Workshop for building and maintaining the atmospheric samplers. The authors wish to thank the MSc and MPhil students from the University of Birmingham who participated in the sampling campaigns as well as acknowledge the support and help provided by Dr. Colvile, Dr. Clemitshaw and the MSc students from Imperial College during the sampling campaigns in London.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envint.2010.05.015.

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