

# Blood Levels of Polycyclic Aromatic Hydrocarbons in Children of Lucknow, India

Vipul K. Singh · Devendra K. Patel · S. Ram · N. Mathur · M. K. J. Siddiqui · Jai Raj Behari

Received: 25 February 2007 / Accepted: 25 June 2007 / Published online: 1 September 2007  
© Springer Science+Business Media, LLC 2007

**Abstract** Polycyclic aromatic hydrocarbons (PAHs) are compounds with two or more fused benzene rings produced by incomplete combustion of organic substances involved in natural and anthropogenic processes. Children are exposed to these compounds through inhalation, dietary ingestion, and, also, soil at the playground. It has been well established that PAHs have carcinogenic, mutagenic, and teratogenic effects. Considering possible health risks due to PAHs exposure among children, the present study was carried out in collaboration with the Pediatrics Department, King George's Medical University (KGMU), Lucknow, to determine its exposure in children by estimating blood PAHs levels. Due to the variable composition of PAHs mixtures emitted from different environmental sources, any single compound or metabolite may not be representative of all exposure conditions. For these reasons, the measurement of blood PAHs levels as a possible biomarker, especially of the EPA (Environmental Protection Agency, USA) priority list, has been proposed. Acenaphthylene, anthracene, phenanthrene, fluoranthene, naphthalene,

pyrene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, and benzo(*a*)pyrene were determined by HPLC-FD/UV. On the basis of the individual compound, the median (50th percentile) of naphthalene (19 ppb) was highest, however, benzo(*a*)pyrene (4.0 ppb) level was found to be lowest among all detected PAHs. The median level of total non-carcinogenic PAHs (113.55 ppb) was higher than the total carcinogenic PAHs (32.35 ppb) in blood samples of children. A significant correlation was found between period of time spent in the surrounding breathing zone of the cooking place and total noncarcinogenic PAHs ( $p < 0.05$ ), while the blood carcinogenic PAHs level in children was found to be associated with lower status of their families ( $p < 0.05$ ). It is speculated that there may be chances of health hazards through exposure to PAHs, those not yet declared hazardous and present at higher concentrations in the Indian environment. Further study with a larger sample size and accompanying environmental data is desired to validate the findings of this pilot study and strengthen the database of PAHs exposure in India.

V. K. Singh · M. K. J. Siddiqui  
Analytical Toxicology Section, Industrial Toxicology Research Centre, P.O. Box 80, M.G. Marg, Lucknow 226001, India

D. K. Patel · J. R. Behari (✉)  
Analytical Chemistry Section, Industrial Toxicology Research Centre, P.O. Box 80, M.G. Marg, Lucknow 226001, India  
e-mail: vipulitrc@rediffmail.com

S. Ram  
Department of Paediatrics, King George Medical University, Lucknow 226003, India

N. Mathur  
Epidemiology Section, Industrial Toxicology Research Centre, P.O. Box 80, M.G. Marg, Lucknow 226001, India

**Keywords** Children · Polycyclic aromatic hydrocarbons · India

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) are compounds with two or more fused benzene rings produced by incomplete combustion of organic substances involved in natural and anthropogenic processes (IARC 1986). It has been well established that PAHs have carcinogenic, mutagenic and teratogenic effects on animals and human (Grimmer 1983; Perera 1997). PAHs and their metabolites are distributed to body tissues through blood; surrogate

markers that estimate levels in target organs, such as protein (Meyer and Bechtold 1996) and hemoglobin (Wei et al. 2000) adducts, might reflect target organ levels due to their abundance in blood than DNA. Children may get exposed to these compounds through inhalation, dietary ingestion and soil in the playground. They spent more time indoors with the mother and it has been reported that indoor exposure to individual PAH compounds is generally much higher than ambient exposure (Pandit et al. 2001). In studies of PAH exposure among children, no difference was found compared with adults (Chuang et al. 1999). Interestingly, they are reported to get higher exposure than adults in the same vicinity (Heudorf and Angerer 2001).

Prenatal exposure to airborne PAHs during pregnancy was found associated with lower birth weight and head circumference in African-American children, which may be correlated with lower IQ and poorer cognitive functioning (Perera et al. 2003; Tsai et al. 2003). Long-term low-level exposure to PAHs and halogenated aromatic hydrocarbons has been associated with a wide variety of effects including irritability, mood instability, short- and long-term memory loss, and lack of concentration in children (Dahlgren et al. 2003). The metabolic activation of PAHs by cytochrome P450 (CYP) 1A1-catalyzed reactions results in the production of xenobiotic metabolites and reactive oxygen species (ROS), which are able to interfere with cell homeostasis (Cavalieri and Rogan 1995; Kim and Lee 1997). ROS have also been shown to be associated with many pathophysiological changes in the body.

Considering the toxicological properties of PAHs, sufficient interest has generated recently to evaluate the total PAH uptake in the body of children through different exposure pathways, namely, respiratory, dermal, and gastrointestinal routes. In order to achieve this goal, the quantification of PAHs and their metabolites in body fluids is recommended, which would be compatible with environmental PAH exposure. One of the aims of the present work is also to evaluate the possibility of using unmetabolized PAHs in blood as biological markers of exposure to low levels of PAHs. Due to the varying composition of PAHs mixtures from different sources, any single compound may not be truly representative of all exposure conditions (Strickland and Kang 1999). For these reasons, the measurement of unchanged PAHs in blood samples of children has been proposed in this study, with particular attention to noncarcinogenic PAHs on the EPA priority list (acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene) for their abundance in PAH mixtures in the environment and other carcinogenic PAHs, like naphthalene, benzo(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, dibenzo(*a,h*)anthracene, and indeno(*1,2,3-cd*)pyrene,

for their toxic potential (Keimig and Morgan 1986; Bieniek 1997; Gundel and Angerer 2000).

## Materials and Methods

### Subject Selection

A total of 56 healthy children (2–12 years) from the general population in and around Lucknow, the capital of the most populous state of India, Uttar Pradesh, were enrolled in this study. The children were not suffering from any chronic disease and their blood profiles fell within the normal range. Subjects were interviewed by a trained physician and/or nurses in order to obtain demographic data such as age, gender, height, weight, socioeconomic status, and area of residence; any history of exposure to chemicals, type of kitchen fuel use, duration of exposure near the breathing zone in the cooking place, smoking habits of the parents or family members, distance of residence from the highway, and other relevant information for known sources of PAH exposure were also included in the questionnaire. None of the subjects reported any occupational/accidental indication of exposure to PAHs. Therefore, the main source of the detected blood PAH levels was expected to be food chain contamination/inhalation. However, water and dust may also contribute to exposures to these compounds. Parents/guardians of the children were informed about the study and their consent was obtained. Additionally, institutional ethics committee clearance was obtained for collecting human blood samples.

### Sample Collection and Storage

All blood samples used in the study were collected at the Department of Paediatrics, King George's Medical University (KGMU), Lucknow, over the study period of 1 year (September 2005–August 2006). Approximately 2 ml venous blood was withdrawn from all subjects and stored in preheparinized glass vials. All the samples were numbered and transported under ice-cold conditions to the Analytical Toxicology Lab (ITRC), Lucknow, and stored at  $-20^{\circ}\text{C}$  until analyses of PAHs.

### Chemicals and Reagents

All chemicals, solvents, and water used were of analytical or HPLC grade. Acetonitrile, *n*-hexane, di-chloromethane (DCM), and SPE cartridges (LiChrosep, RP-18) for cleanup were procured from Merck, Darmstadt, Germany. Individual standards of all 13 polycyclic aromatic hydrocarbons on

the EPA priority list, namely, naphthalene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(*a*)anthracene, benzo(*k*)fluoranthene, benzo(*b*)fluoranthene, benzo(*a*)pyrene, dibenz(*a,h*)anthracene and indeno(*1,2,3-cd*)pyrene were purchased from Supelco (Bellefonte, PA, USA) for preparation of the mix external reference standard. Purity of all individual PAH standards was in the range of 93.4%–99.7%. All individual standards were dissolved separately in acetonitrile to make a stock solution; the working standard solution was made by mixing the stock solution of each compound at different concentrations in amber-colored volumetric flask (to avoid light exposure) and storing them at 4°C in the refrigerator.

#### Extraction of PAHs

Extraction of PAHs from blood was carried out according to the method reported by Van Schooten et al. (1997). Liquid-liquid extraction of PAHs was performed by taking 2 ml of blood with 3 ml *n*-hexane in an amber-colored, capped conical flask (25 ml), which was kept in a shaker for 15 min. After this step, the upper organic layer was collected in a stoppered glass tube; this extraction process was again repeated twice with *n*-hexane (10 min each time) and the resultant extracts were pooled with the previous one. Then the pooled extract was evaporated to 0.5 ml under a gentle steam of nitrogen and kept in the refrigerator until cleanup to avoid losses of lighter PAHs by volatilization.

#### Cleanup of Sample by SPE

During the extraction process a few fat and lipid impurities can be coextracted along with the sample, which can interfere with the subsequent separation and identification during chromatography. For their elimination, cleanup of the extract was performed using SPE cartridges (RP-18; Merck) before HPLC analysis. Prewashed SPE cartridges with milli-Q ultrapure water were conditioned with a 10-ml mixture of *n*-hexane:dichloromethane (1:1). Then the sample was loaded on a cartridge and extraction solution was aspirated through the cartridge, resting on a manifold vacuum station (Supelco), with a pressure regulator, at a flow rate of  $\leq 2$  ml/min. Finally, the sorbent was washed with 10 ml of water (milli-Q), dried, and then eluted with a 15-ml mixture of *n*-hexane:DCM (1:1). The extract obtained was concentrated to 1 ml; 0.5 ml of acetonitrile was added to it and the rest of the elution mixture was evaporated. Thus, the final sample was in the acetonitrile phase for HPLC analysis.

#### Analysis of PAHs

The aliquot of final sample extracts was analyzed on an HPLC system (515 series; Waters, Milford, MA, USA) equipped with a fluorescence detector (Model 474; Waters) using a reversed-phased, C-18 ODS analytical column (75 × 4.6-mm i.d., 3.5- $\mu$ m particle size), with a precolumn of the same phase (both supplied by Waters), for all PAHs other than acenaphthylene (due to the lack of response by FD). The HPLC system consisted of a binary pump, an on-line degasser, a thermostatic column housing, and Millennium-32<sup>R</sup> chromatography manager software. The solvent system that constituted the mobile phase was acetonitrile (A) and water (B). Elution conditions and detection wavelength program were adopted as reported by Barranco et al. (2003). The elution conditions were as follows: 0–10 min, 50% A isocratic; 10–24 min, linear gradient 50% A–100% A; and 24–35 min, 100% A isocratic. An aliquot of same sample was reanalyzed in the isocratic mode (mobile phase, 70% acetonitrile and 30% H<sub>2</sub>O) using a UV detector (Model 2487; Waters) at a wavelength of 254 nm, only for acenaphthylene. The flow rate was maintained at 1.0 ml/min throughout the analysis and the injection volume was 20  $\mu$ l.

#### Quality Control

Recovery experiments were conducted to check the analytical quality control; six blood samples in duplicate were spiked with mixed standards of PAHs at 5 to 20 ppb. The average recoveries calculated using observed and spiked concentrations for PAHs were varied from 73.2% to 94.4%; however, coefficients of variation ranged from 3.6 to 7.1. The limit of detection in spiked samples ranged from 0.008 to 0.05  $\mu$ g/L for acenaphthylene and benz(*b*)fluoranthene, respectively, during HPLC analysis. The linear range was determined for each PAH by applying three different concentrations; a best-fit straight line was plotted against the PAH concentrations and their responses. A minimum linear range of 0.4–40 was found for benz(*b*)fluoranthene, however, a maximum range of 8–150 was detected for acenaphthylene.

A blank sample was always prepared and run with each set of samples during PAH analyses by HPLC.

#### Confirmation by GC-MS

For peaks confirmation of detected PAHs in blood samples, a few samples from each batch were randomly selected and analyzed on a gas chromatography-mass spectrometer using a model auto system XL (Perkin-Elmer, USA)

coupled with a Turbo Mass detector, available at the analytical chemistry section of ITRC, Lucknow. This confirmatory GC-MS analysis was performed only for qualitative determination of PAHs in the samples, so selected samples (already analyzed on HPLC) were prepared by exchanging their solvent phase acetonitrile with dichloromethane (DCM) for injection into the GC-MS instrument. GC conditions and temperature programming were as described by Poon et al. (1999) for determination of serum PAH levels. The mass detector was operated in electron impact mode at 70 eV in full scan and target compounds were identified in the scanning mode. The spectrum of an individual PAH was confirmed by matching it with the authentic spectra of the standard PAH on the library list available in the GC-MS instrument.

### Statistical Evaluation

Linear regression analysis was applied to test any statistically significant relation between covariates such as age, sex, BMI, and exposure period, as independent variables, and blood PAH combinations (noncarcinogenic and carcinogenic PAHs), as dependent variables, among children. Statistical analyses were performed using SYSTAT software (version 9.1) supplied by Binary Semantics Ltd. (India) to the Epidemiology Section, ITRC.

### Results and Discussion

Children have an exceptional vulnerability to both the acute and the chronic effects of environmental hazards and they are disproportionately susceptible by comparison with adults. Studies revealed that children in the youngest age group (6–11 years) are more exposed to PAHs than children in the other age groups and adults. Despite wide spread and toxicological relevance of 1-hydroxypyrene [metabolite of pyrene as well as benzo(*a*)pyrene] only few alternatives are available to assess PAHs exposure. In addition, PAH-DNA adduct level studies in an exposed population gave inconsistent results according to exposure, i.e., elevated benzo(*a*)pyrene-DNA adduct levels were found in nonsmokers versus smokers (Hecht 2002). So our approach, to evaluate unmetabolized PAHs in blood as biological markers, can be valuable because their occurrence does not depend on the biotransformation rate as happens for metabolite excretion in urine, but it is a consequence of the natural integration over time of the fast partition between medium (air, water, food) and blood (Ghittori et al. 1993). So it is less susceptible than metabolite elimination to intra-individual variability (Strickland and Kang 1999).

**Table 1** Sociodemographic features of the children recruited in the study ( $n = 56$ )

Variable	Mean $\pm$ SD ( $n = 56$ )
Age (yr)	5.86 $\pm$ 2.36
Height (m)	1.05 $\pm$ 0.15
Weight (Kg)	18.26 $\pm$ 5.34
BMI (Kg/m <sup>2</sup> )	16.46 $\pm$ 2.42
Head circumference (cm)	49.03 $\pm$ 3.78
Chest circumference (cm)	52.27 $\pm$ 4.23
Average exposure time near kitchen (min)	31.57 $\pm$ 37.82
Distance from highway/traffic (m)	805.20 $\pm$ 562.32

Table 1 shows the demographic features of children ( $n = 56$ ) recruited in the study. All the children were healthy and their growth (as measured by head and chest circumference) was found to be normal. Since children spend more time with the mother (chief cook in family), there may be a chance of being exposed to PAHs from cooking food and burning fuel. Pandit et al. (2001) suggested that during the cooking period, exposure to PAHs is 2–10 times higher than ambient exposure. So their average time spent near the kitchen area was recorded. Vehicular traffic is also one of the chief sources of PAHs in the environment, so distance of residence from the highway was also recorded.

Individual values of all PAHs and their combinations detected in blood samples of children are presented as 25th percentile, 50th percentile (median), 75th percentile, and interquartile range in Table 2. On the basis of the individual compound, the median value of naphthalene (19 ppb) was the highest, however, benzo(*a*)pyrene median (4.0 ppb) was found to be lowest among all detected blood PAHs. Previous biomonitoring studies (Campo 2006; Waidyanatha 2003) also reported higher naphthalene levels, up to 859 ng/L, excreted through urine in comparison to other PAH levels. It is important to mention here that only a smaller amount of PAHs body burden is excreted unchanged in urine (Becher and Bjorseth 1983) so at the same time this amount could be much higher in the body. The higher solubility and volatile nature of lower molecular weight PAHs like naphthalene (Cheung et al. 2006) compared to other congeners of PAHs (with large molecular weight) may be responsible for their elevated blood level. PAH monitoring studies from different region of India also reported high levels of naphthalene in comparison to other PAHs and low benzo(*a*)pyrene levels (Pandit et al. 2001; Sharma 2006) in the ambient environment. The median total noncarcinogenic PAH level (113.55 ppb) was higher than the total carcinogenic PAH level (32.35 ppb) in children. These PAH compounds of low molecular weight

**Table 2** Status of different PAHs estimated in blood samples of children ( $n = 56$ )

Polycyclic aromatic Hydrocarbons	25%	Median	75%	IQR
Naphthalene	1.05	19	160.6	159.55
Acenaphthylene	1.14	8.0	112.47	111.33
Phenanthrene	1.21	9.0	43.51	42.30
Anthracene	1.45	3.6	6.99	5.54
Fluoranthene	1.41	6.0	16.96	15.55
Pyrene	1.93	9.0	27.66	25.73
Benzo( <i>b</i> )fluoranthene	1.01	4.2	10.26	9.25
Benzo( <i>k</i> )fluoranthene	<1.10	4.0	8.00	8.00
Benzo( <i>a</i> )pyrene	<1.10	1.4	3.10	3.10
Noncarcinogenic PAHs	45.42	113.55	289.10	243.68
Carcinogenic PAHs	15.20	32.35	168.10	152.90
Total PAHs	103.83	250.27	434.85	331.02

Note. 25%, 25th percentile; median, 50th percentile; 75%, 75th percentile; IQR, interquartile range (75th–25th percentile). Values are in parts per billion (ppb)

**Table 3** Relation between blood PAH combinations (dependent variables) and individual characteristics of children ( $n = 56$ )

Independent variable	PAH combinations vs characteristics: regression coefficient ( $p$ value)	
	Noncarcinogenic	Carcinogenic
Age	18.71 (0.660)	-16.05 (0.393)
Sex	3.88 (0.973)	69.06 (0.173)
Height	97.49 (0.803)	88.98 (0.605)
Weight	-24.93 (0.284)	4.12 (0.686)
BMI	24.72 (0.246)	-2.55 (0.784)
Diet	-25.84 (0.830)	41.79 (0.433)
Ethnicity	-116.39 (0.408)	-8.207 (0.894)
Lower family status	-119.15 (0.341)	137.33 (0.015) <sup>a</sup>
Cooking fuel (kerosine, coal, wood)	-95.14 (0.491)	-45.68 (0.452)
Smoking	159.40 (0.165)	-76.81 (0.130)
Exposure period	4.82 (0.002) <sup>a</sup>	0.56 (0.273)
Traffic distance	-0.05 (0.642)	0.01 (0.849)
Separate cooking place	190.13 (0.128)	-19.14 (0.724)

<sup>a</sup> Statistically significant,  $p < 0.05$

and size are always present at higher levels in the environment (Fang et al. 2004). Higher molecular weight PAHs (carcinogenic in nature) are bound to particulate matter and also become settled along with particles, whereas low molecular weight, noncarcinogenic PAHs, which eventually bind to smaller particles, may remain suspended in the air more than high molecular weight PAHs, thus making low molecular weight PAHs available for respiration than higher molecular weight PAHs. This may be a possible

reason (difference in exposures) for their higher blood levels in children in comparison to other carcinogenic PAHs. These findings suggest that environmental air pollution could be responsible for PAH exposure to children than any other known source. Total noncarcinogenic and total carcinogenic PAH combination load in individual samples was formulated as follows: (i) the sum of all noncarcinogenic PAH levels detected in an individual blood sample was considered their total noncarcinogenic PAH load; (ii) the total carcinogenic PAH load in blood was formulated in the same way.

Table 3 reports the parameters determined during the recovery experiment conducted in spiked blood samples for analytical quality control. Satisfactory recovery of all analyzed PAH compounds was found.

Table 4 reports the correlation between PAH combination and different individual sociodemographic features of the children. Linear regression analysis was performed between PAH combinations (as dependent variable) and different characteristics of children such as age, gender, dietary habit, ethnicity, socioeconomic status of family, and kitchen fuel used (as independent variables). Noncarcinogenic (mostly two- to three-ringed PAHs) was significantly correlated with the duration of exposure near the cooking area. These findings are consistent with the results of Zhu et al. (2003), who reported higher level of noncarcinogenic PAHs in air of domestic kitchens compared to carcinogenic PAHs having a higher molecular weight. There was also a significant correlation between carcinogenic PAH level and lower family status. In our opinion this may be due to regular use of inexpensive biomass fuels such as coal, wood, and kerosine for food preparation, which generates a high level of carcinogenic PAHs. Dave (1987) also reported that low-income families from both rural and urban regions of India usually reside in poorly ventilated houses and utilize these types of fuels. On the energy ladder, they are cheap and readily available alternate energy sources compared to electricity and LPG (liquid petroleum gas). These results support the findings of a previous study conducted in rural areas of the Lucknow region, which reported high levels of carcinogenic PAHs emitted during combustion of biomass fuel (Bhargava et al. 2004).

If we consider the ambient air quality in India, PAHs in Delhi, Kanpur, Ahmedabad, Mumbai, and Kolkata (major cities) was between 9.4 and 190.96 ng/m<sup>3</sup>, which is 10–40 times higher than the standard limit (Mohanraj et al. 2003). We were unable to find the status of ambient air PAHs in the Lucknow region, however, in Gomti River water in this area, PAHs ranged between 0.04 and 65.85 µg/L (Malik et al. 2004). These values are higher than the standard limits prescribed by the CPCB (Central Pollution Control Board), India (5 ng/m<sup>3</sup> for air), and BIS (Bureau of Indian

**Table 4** Limit of detection, linear range, coefficient of variation (CV), and recovery of spiked samples of the extraction method

PAH	Detection limit ( $\mu\text{g/L}$ )	Linear range ( $\mu\text{g/L}$ )	CV	Recovery (%)
Naphthalene	0.014	4–100	7.1	73.2
Acenaphthylene	0.008	8–150	6.1	94.4
Phenanthrene	0.02	2.0–75	5.1	91
Anthracene	0.015	0.5–50	4.8	92.5
Fluoranthene	0.019	0.9–100	3.6	87
Pyrene	0.017	0.7–75	4.4	89
Benzo( <i>b</i> )fluoranthene	0.05	0.5–40	3.9	84.5
Benzo( <i>k</i> )fluoranthene	0.024	0.4–40	6.3	92.1
Benzo( <i>a</i> )pyrene	0.018	0.6–50	6.4	81.1

*Note.* Limit of detection was calculated by spiked blood samples with individual PAH standards. Linear range consists of three concentrations showing a linear response. CV; coefficient of variation, Recovery as calculated by observed and spiked concentration of samples

Standards), India (0.2  $\mu\text{g/L}$  for water) (Tyagi et al. 2003; BIS 1982). Also, higher PAH levels have been reported in several common Indian oil-fried and pyrolyzed food items (Sivaswami et al. 1990). So there may be exposure to these PAHs constantly from the general environment and food chain contamination. Children are getting exposed more than adults, which results from their relatively higher uptake, in a mass comparison (as much as six times adults), from inhalation and drinking water. Additionally, dermal contact is also increasingly taken into account (Tsai et al. 2001) because they spent more time at the playground, with continuous contact with the soil. Lower activity of some metabolizing enzymes active in detoxification of these xenobiotics has also been reported in early childhood (Armstrong et al. 2004). This might be the reason for their lesser removal from the body via its natural detoxification mechanism. Lodovici et al. (1998) reported that high exposure is associated with an increased level of aromatic adducts, as biological effective dose in PAH-exposed populations, and suggested that resultant alterations of PAH metabolism may play a crucial role in health hazards induced by free radicals (FR), which are generated during its metabolism (Warshawsky et al. 1984; Greife and Warshawsky 1993; Cheu et al. 1997).

Our findings support the previous Indian studies (Malik et al. 2004; Pandit et al. 2001; Sharma 2006) reporting high levels of low molecular weight PAHs in comparison to high molecular weight compounds in the Indian environment. The two- or three-ring compounds that are always present at higher levels in PAH mixtures may have irritative effects and cause respiratory diseases (Fang et al. 2004) and other health hazards. For example, naphthalene, a two-ringed PAH, has recently been classified as a possible carcinogen

to humans (class 2B) (IARC 2002). So there may be chances of health hazards through exposure of PAHs, those not yet declared hazardous and present at higher concentrations in the Indian environment. Considering this we analyzed blood samples of children for the likely occurrence of different carcinogenic and noncarcinogenic PAH compounds as possible biomarkers. Hence, the present study is unique and probably the first of its kind, as it reports EPA-listed priority PAH levels in the blood samples of children in India. Studies with large sample sizes are needed to strengthen the database and investigate PAH-related risks to health in general and children in particular.

**Acknowledgments** The authors express their sincere thanks to the staff of the Pediatrics Department (KGMU), Lucknow, for their help during collection of blood samples. Thanks are due to the Director, ITRC, for his continuous encouragement and help. One of the authors (V.K.S.) expresses his sincere thanks the University Grants Commission, Government of India, for the Senior Research Fellowship.

## References

- Armstrong B, Hutchinson E, Unwin J, Fletcher T (2004) Lung cancer risk after exposure to polycyclic aromatic hydrocarbons: a review and meta-analysis. *Environ Health Perspect* 112:970–978
- Barranco A, Alonso-Salces R M, Bakkali A, Berrueta LA, Gallo B, Vicente F, Sarobe M (2003) Solid-phase clean-up in the liquid chromatographic determination of polycyclic aromatic hydrocarbons in edible oils. *J Chromatogr A* 988:33–40
- Becher G, Bjorseth A (1983) Determination of polycyclic aromatic hydrocarbons by analysis of human urine. *Cancer Lett* 17:301–311
- Bhargava A, Khanna RN, Bhargava SK, Kumar S (2004) Exposure risk to carcinogenic PAHs in indoor-air during biomass combustion whilst cooking in rural India. *Atmos Environ* 38:4761–4767
- Bieniek G (1997) Urinary naphthols as an indicator of exposure to naphthalene. *Scand J Work Environ Health* 23:414–420
- BIS (1982) Indian standards, tolerance limits for inland surface waters subject to pollution. IS: 2296, New Delhi
- Campo L, Addario L, Buratti M, Scibetta L, Longhi O, Valla C, Cirila PE, Martinotti I, Foa V, Fustinoni S (2006) Biological monitoring of exposure to polycyclic aromatic hydrocarbons by determination of unmetabolized compounds in urine. *Toxicol Lett* 162:132–138
- Cavalieri EL, Rogan EG (1995) Central role of radical cations in metabolic activation of polycyclic aromatic hydrocarbons. *Xenobiotica* 25:677–688
- Cheu J, Talaska G, Miller M, Rice C, Warshawsky D (1997) Benzo(*a*)pyrene coated ferric oxide and aluminium oxide particles: uptake, metabolism and DNA binding in hamster pulmonary alveolar macrophages and tracheal epithelial cells in vitro. *Carcinogenesis* 18:167–175
- Cheung KC, Leung HM, Kong KY, Wong MH (2006) Residual levels of DDTs and PAHs in freshwater and marine fish from Hong Kong markets and their health risk assessment. *Chemosphere* 66(3):464–473
- Chuang JC, Callahan PJ, Lyu CW, Wilson NK (1999) Polycyclic aromatic hydrocarbon exposures of children in low-income families. *J Expo Anal Environ Epidemiol* 9:85–98

- Dahlgren J, Warshaw R, Thornton J, Anderson-Mahoney CP, Takhar H (2003) Health effects on nearby residents of a wood treatment plant. *Environ Res* 92:92–98
- Dave JM (1987) Emissions from conventional kerosene stove used in Indian kitchen. In: Proceedings of 4th International Conference on Indoor Air Quality and Climate, 1987, Berlin, 17–21 August, Vol 1, pp 326–329
- Fang GC, Chang KF, Lu C, Bai H (2004) Estimation of PAHs dry deposition and BaP toxic equivalency factors (TEFs) study at Urban, Industry Park and rural sampling sites in central Taiwan, Taichung. *Chemosphere* 55:787–796
- Ghittori S, Fiorentino ML, Maestri L, Cordioli G, Imbriani M (1993) Urinary excretion of unmetabolized benzene as an indicator of benzene exposure. *J Toxicol Environ Health* 38:233–243
- Greife AL, Warshawsky D (1993) Influence of the dose levels of cocarcinogen ferric oxide on the metabolism of benzo(a)pyrene by pulmonary alveolar macrophages in suspension culture. *J Toxicol Environ Health* 38:399–417
- Grimmer GG (1983) In: Grimmer GG (ed) Environmental carcinogens: polycyclic aromatic hydrocarbons. chemistry, occurrence, biochemistry, carcinogenicity. CRC Press, Boca Raton, FL
- Gundel J, Angerer J (2000) High-performance liquid chromatographic method with fluorescence detection for the determination of 3-hydroxybenzo(a)pyrene and 3-hydroxybenzo(a)anthracene in the urine of polycyclic aromatic hydrocarbons-exposed workers. *J Chromatogr B* 738:47–55
- Hecht SS (2002) Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. *Carcinogenesis* 23:907–922
- Heudorf U, Angerer J (2001) Internal exposure to PAHs of children and adults living in homes with parquet flooring containing high levels of PAHs in the parquet glue. *Int Arch Occup Environ Health* 74:91–101
- IARC (1986) Environmental experimental data. In: IARC (ed) IARC monographs on the polycyclic aromatic hydrocarbons. Part 1, Vol. 38. International Agency for Research on Cancer, Lyon, France
- IARC (2002) IARC monographs on the evaluation of carcinogenic risks to humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Vol 82. International Agency for Research on Cancer, Lyon, France
- Keimig S, Morgan D (1986) Urinary 1-naphthol as a biological indicator of naphthalene exposure. *Appl Ind Hyg* 1:61–65
- Kim KB, Lee BM (1997) Oxidative stress to DNA, protein, and antioxidant enzymes (superoxide dismutase and catalase) in rats treated with benzo(a)pyrene. *Cancer Lett* 113:205–212
- Lodovici M, Akpan V, Giovannini L, Migliani F, Dolara P (1998) Benzo (a) pyrene diol-epoxide DNA adducts and levels of polycyclic aromatic hydrocarbons in autoptic samples from human lungs. *Chem Biol Interact* 116:199–212
- Malik A, Singh KP, Mohan D, Patel DK (2004) Distribution of polycyclic aromatic hydrocarbons in gomti river system, India. *Bull Environ Contam Toxicol* 72:1211–1218
- Meyer MJ, Bechtold WE (1996) Protein adduct biomarkers: state of the art. *Environ Health Perspect* 104(Suppl 5):879–882
- Mohanraj R, Azeez PA (2003) Polycyclic aromatic hydrocarbons in air and their toxic potency. *Resonance* Sept:20–27
- Pandit GG, Srivastava PK, Mohan Rao AM (2001) Monitoring of indoor volatile organic compounds and polycyclic aromatic hydrocarbons arising from kerosene cooking fuel. *Sci Total Environ* 279(1–3):159–165
- Perera FP (1997) Environment and cancer: Who are susceptible? *Science* 278:1068–1073
- Perera FP, Rauh V, Tsai W-Y, Kinney P, Camann D, Barr D, Bernert T, Garfinkel R, Tu Y-H, Diaz D, Dietrich J, Whyatt R (2003) Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. *Environ Health Perspect* 111:201–205
- Poon K, Lam PKS, Lam MHW (1999) Determination of polycyclic aromatic hydrocarbons in human blood serum by proteolytic digestion-direct immersion SPME. *Anal Chim Acta* 396:303–308
- Sharma H, Jain VK, Khan ZH (2007) Characterization and source identification of polycyclic aromatic hydrocarbons (PAHs) in the urban environment of Delhi. *Chemosphere* 66(2):302–310
- Silvester DJ (1980) Determination of 3,4-benzopyrene and benzoanthracene (PAH) in phenolic smoke concentrates. *J Food Technol* 15:413–420
- Sivaswamy S, Balachandran B, Sivaramakrishnan YM (1990) Polycyclic aromatic hydrocarbons in south Indian diet. *Curr Sci* 59:480–481
- Strickland PT, Kang D (1999) Urinary 1-hydroxypyrene and other PAH metabolites as biomarkers of exposure to environmental PAH in air particulate matter. *Toxicol Lett* 108:191–199
- Tsai PJ, Shieh HY, Lee WJ, Lai SO (2001) Health risk assessment for workers exposed to polycyclic aromatic hydrocarbons (PAHs) in carbon black manufacturing industry. *Sci Total Environ* 278:137–150
- Tsai SS, Yu HS, Liu CC, Yang C-Y (2003) Increased incidence of preterm delivery in mothers residing in an industrialized area in Taiwan. *J Toxicol Environ Health Part A* 66:987–994
- Tyagi SK, Sengupta B (2003) Polycyclic aromatic hydrocarbons (PAHs) in air and their effects on human health. *Parivesh News Lettter* (November). CPCB, India
- Van Schooten FJ, Moonen EJC, Van der wal L, Levels P, Kleinjans JCS (1997) Determination of polycyclic aromatic hydrocarbons (PAH) and their metabolites in blood, feces and urine of rats orally exposed to PAH contaminated soils. *Arch Environ Contam Toxicol* 33:317–322
- Waidyanatha S, Zheng Y, Rappaport SM (2003) Determination of polycyclic aromatic hydrocarbons in urine of coke coven workers by headspace solid phase microextraction and gas chromatography–mass spectrometry. *Chem Biol Interact* 145:165–174
- Warshawsky D, Bingham E, Niemer RW (1984) The effects of cocarcinogen, ferric oxide, on the metabolism of benzo(a)pyrene in the isolated perfused lung. *J Toxicol Environ Health* 14:191–209
- Wei Q, Cheng L, Amos CI, Wang LE, Guo Z, Hong WK, Spitz MR (2000) Repair of tobacco carcinogen-induced DNA adducts and lung cancer risk: a molecular epidemiologic study. *J Natl Cancer Inst* 92(21):1764–1772
- Zhu L, Wang J (2003) Sources and pattern of polycyclic aromatic hydrocarbons pollution in kitchen air, China. *Chemosphere* 50:611–618