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High levels of 1-hydroxypyrene and hydroxyphenanthrenes in urine of children and adults from Afghanistan

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Among a health cooperation project, we had the opportunity to study the internal polycyclic aromatic hydrocarbon (PAH) exposure of residents from urban and rural areas in Afghanistan. Urine samples from 13 children (age 2.0–9 years) and 42 adults (age 20–65 years) were collected. A total of 25 participants were from Kabul, and 30 participants lived in a rural area. The determination of 1-hydroxypyrene and hydroxyphenanthrenes was carried out by high performance liquid chromatography and fluorescence detection. Median (range) were as follows (n = 55): 1-hydroxypyrene 1646 ng/l (71–16,288 ng/l) and sum of 1-, 2- and 9-, 3-, 4-OH-phenanthrenes 3602 ng/l (116–19,670 ng/l). These results indicate a high PAH exposure, compared with the general population in more developed countries. The levels of 1-hydroxypyrene in urine of women (all non-smokers) from the rural area were higher than those from Kabul (*N*, median, range): rural 15, 2095, 334–11,357 ng/l; Kabul 11, 748,137–5332 ng/l. All households from the rural area used open fires for cooking and energy. We conclude that populations in low-developed countries may be at special risk to increased PAH exposure due to inadequate control of air pollution from car emissions and due to burning of biomass fuels for cooking and household energy.
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Introduction

Exposure of the general population to polycyclic aromatic hydrocarbons (PAHs) has gained great importance in environmental health. The significance of this class of substances from the environmental medicine point of view is determined by their ubiquitous occurrence in the environment and their carcinogenic nature (ATSDR, 1995; WHO, 1998). The main source of exposure to PAHs of the non-smoking general population is the diet (Van Rooij et al., 1994; EFSA, 2008). Further important sources are air pollution and tobacco smoke. High exposure is observed in workplaces during the conversion and processing of coal and coal tar. After metabolic activation, many PAH have been shown to induce lung and skin tumors in animals by mechanisms that operate also in exposed humans. Benzo(a)-pyrene is classified as carcinogenic to humans (IARC group 1) (Straif et al., 2005). According to this group, several other PAH are classified as probably and possibly carcinogenic (group 2A and 2B); a few PAH seem to be even more potent carcinogens than benzo(a)pyrene.

To assess internal PAH exposure, the determination of 1-hydroxypyrene and hydroxyphenanthrenes in urine turned out to be reliable and sensitive biomarkers in environmental and occupational medicine (Angerer et al., 2007; Rossbach et al., 2007; Hansen et al., 2008).

Populations in low-developed countries may be at special risk to increased PAH exposure due to inadequate control of air pollution from industries and vehicular emissions. Additionally, elevated indoor air PAH, VOCs, CO and $PM_{2.5}$ exposure in developing countries is known to be associated with burning of biomass fuels for cooking and household energy (Albalak et al., 2001; Balakrishnan et al., 2002; Dherani, 2003; Naeher et al., 2007; Pearce et al., 2009). Materials like wood and dung are typically burnt on open fire or simple stoves with incomplete combustion (Rehfuess et al., 2006). Smith et al. (2004) estimated that 1.6 million premature deaths and 3.6% of the global burden of disease were attributed to this indoor air microenvironment pollution from the use of solid fuels. Indoor emissions from household combustion of biomass fuel (primary wood) are categorized as probably carcinogenic to humans (group 2A) by IARC (2010).

Among a health cooperation project, we had the oppurtunity to study the internal PAH exposure of residents from Afghanistan. Here we report for the first time, the levels

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of 1-hydroxypyrene and hydroxyphenanthrenes in urine of children and adults living in Kabul and rural areas. Assessment of tobacco smoke, waterpipe and smokeless tobacco exposure and cotinine levels in urine were included.

Materials and methods

Study Area and Population

The cross-sectional study was carried out in Kabul city and in the rural area Ningarhar province-Shinwary district, 175 km east of Kabul city, in December 2007. Kabul is the capital city of Afghanistan with more than four million inhabitants. In recent years, the air quality in Kabul has deteriorated due to the rapid increase in the population, vehicular emissions and inadequate infrastructure. Participants were chosen from a health cooperation project. More details on the study population and on the recruitment of the individuals are given in Hemat et al. (2010). Alltogether, 55 participants could be enrolled in the study. Thirteen children (mean age 5.5 ± 2.3 years; range 2.0–9 years; 5 boys and 8 girls) and 42 adults (mean age 31.6 ± 8.6 years; range 20–65 years; 16 men, 26 females) took part. A total of 25 participants (11 women, 9 men and 5 children) were from Kabul and 30 (15 women, 7 men and 8 children) from the Ningarhar province-Shinwary district. The participants were provided with a letter containing information on both the purpose and the methods of this study. For logistic reasons, examination of the healthy participants took place in the hospitals located in Kabul and Ningarhar province, respectively. After a week, the participants came to the hospitals ''Cluster Hospital of District Shinwari'' and ''Malalay Zegantoon'' located in Ningarhar province-Shinwary district and Kabul city, respectively, where the collection of urine samples was performed.

During the time of enrollment at the hospital, the participants were given a questionnaire about age, sex, location, height, weight and smoking habits (cigarettes, moist snuff and waterpipe), as well as their clinical history, which they filled out with the assistance of the physician. Local village-based study staff helped us to gather additional information about the participants' fuel for cooking, household items and electricity generators. The households from rural areas used solid fuels for cooking and heating. Wood and dung were used as fuel, mostly collected from the local environment. In all households from the rural areas, fuels were burnt on open fires; no stoves were available due to the lower socio-economic status. However, in Kabul, the participants used stoves and solid fuels such as wood and dung for cooking and heating. Additionally, diesel generators were used to provide electricity to the household. The manufacture of the cook stove was not directly evaluated, but most of the participants used the cooking stove inside the house without external ventilation. The male participants from both areas showed habits of taking oral smokeless tobacco such as moist snuff and smoking cigarettes. Moist snuff was referred to as ''naswar'' by the people in Afghanistan. Smoking of the waterpipe using the traditional unflavored ''ajami'' pure dark paste tobacco was also reported by the participants. The people in Afghanistan called this type of waterpipe as ''chalem''. Informed consent was obtained from the parents of the children and from the other participants. The study protocol was submitted to and approved by the Ethics Committee of the Medical Council North Rhine, Düsseldorf, Germany (No. 2007364).

Sampling of Urine

Standard materials for urine collection (URITOP S, Braun, Melsungen, Germany) were taken from Germany, and the material was tested for PAHs contamination before the start of the study. No contamination was found. Transporting the samples from Afghanistan to Germany was challenging due to transport restrictions, sample load, as well as the fragile security of Afghanistan. The samples were frozen in a cold-storage room at $-20\,^{\circ}C$, which was provided by the Ningarhar Hospital, Jalalabad, Afghanistan. After 2 weeks, the frozen samples were embedded in ice plastic bags in a cold box and subsequently transported from Kabul to Düsseldorf airport, and transported immediately to the laboratory of the Ruhr-University Bochum and stored at $-20\,^{\circ}C$. The samples were later transported frozen to Erlangen, where the samples were analyzed for PAH metabolites.

Analysis

The determination of 1-, 2-, 3-, 4-hydroxyphenanthrene and 1-hydroxypyrene was carried out using a modified method, proofed and published by the German Research Foundation (DFG) working group ''Analyses of hazardous substances in biological materials'' (DFG, 1999). Urine sample of 6 ml were buffered with 12 ml 0.1 M sodium acetate buffer, pH 5.0, and hydrolyzed with 80 μl β-glucuronidase/arylsulfatase for 16 h in a waterbath at $37\,^{\circ}C$. To separate the particulate matter, the solutions were centrifuged at 3000 r.p.m. for 15 min, and 12 ml of the supernatant fraction was transferred into an autosampler tube. Then, 3 ml of this solution was injected into the high performance liquid chromatography (HPLC) system by an autosampler. The HPLC system consisted of a quaternary gradient pump (L 7100), a single-channel pump (L6000), a column thermostat (L 7350), an autosampler (AS-4000), a fluorescence detector (L 7480), an automatic six-way valve and a PC for integration. All HPLC instruments were from Merck-Hitachi (Darmstadt, Germany). The metabolites were enriched on a special precolumn consisting of copper phthalocyanine-modified silica gel, and were transferred on an RP-C 18 column (LiChrospher PAH 5 μm 250–4 with precolumn) by a column switching program.

After separation, the analytes were quantified by a switchable wavelength fluorescence detector. For the detection of the phenanthrene metabolites, the excitation wavelength was 244 nm and emission wavelength 370 nm. For the detection of 1-hydroxypyrene, the excitation wavelength of 236 nm and the emission wavelength of 386 nm were used. To quantify the co-eluting metabolites, the sum of 2- and 9-hydroxyphenanthrene was quantified by a calibration curve of 2-hydroxyphenanthrene. The limits of quantification (LOQ) were 16 ng/l urine for 1-hydroxyphenanthrene, 4 ng/l for 2-/9-hydroxyphenanthrene, 5 ng/l for 3-hydroxyphenanthrene, 8 ng/l for 4-hydroxyphenanthrene and 12 ng/l for 1-hydroxypyrene.

Cotinine in urine was determined by HPLC after derivatisation with 1,3-diethyl-2-thiobarbituric acid according to Rustemeier et al. (1993). The LOQ was 2 μg cotinine/l urine.

Creatinine in urine was determined photometrically as picrate according to the Jaffé method (Taussky, 1954).

Statistical Analysis

All statistical analysis were performed with the STATISTICA data analysis software system (Version 8 and 9, Statsoft, Tulsa, www.statsoft.com). The Mann–Whitney *U*-test was used to compare the differences in between groups. Correlation analysis was done using Pearson product-moment correlation. *P*-values below 0.05 were used as cut-off for significance.

Results and discussion

Creatinine concentrations in urine ranged from 0.15 to 2.21 g/l (median 0.85 g/l). No sample was excluded from further analysis due to the creatinine level. The levels of 1-hydroxypyrene and phenanthrene metabolites in urine are summarized in Table 1. The data clearly show a high PAH exposure of residents from Afghanistan, especially for children. Compared with data from surveys performed in Germany (Becker et al., 2003) and the USA (Li et al., 2008; CDC, 2009), Afghan children have approximately 10-fold higher PAH metabolite levels in urine. For example, the reference values (reference value is defined within the 95% confidence interval of the 95th population percentile) for PAH metabolites in urine of German children (non-smokers, age 3–14 years, sampling period 2003–2006) are as follows: 1-hydroxypyrene, 500 ng/l; 1-hydroxyhenanthrene, 600 ng/l; 2/9-hydroxyphenanthrene 400 ng/l; 3-hydroxyphenanthrene, 500 ng/l; 4-hydroxyphenanthrene, 200 ng/l; and Σ-hydroxyphenanthrene 1500 ng/l (Schulz et al., 2009). The 95th percentile values (children 6–11 years, sampling period 2003–2004) from the National Health and Nutrition Examination Survey, NHANES (CDC, 2009) are similar: 1-hydroxypyrene, 514 ng/l; 1-hydroxyhenanthrene, 615 ng/l; 2-hydroxyphenanthrene, 225 ng/l; 3-hydroxyphenanthrene, 472 ng/l; 4-hydroxyphenanthrene, 138 ng/l. Also PAH exposure of Afghan adults is several times higher compared with the German or US general population. The 95th percentile values (adults 20 years and older, sampling period

Table 1. Range, median and 95th percentile of 1-hydroxypyrene and phenanthrene metabolite concentrations in urine (ng/l) of all 55 participants (13 children, 42 adults) and of different subgroups. All values were above LOQ.

	Min	P50	P95	Max
Total (n = 55)				
1-OH-pyrene	71.4	1646	11,357	16,288
1-OH-phenanthrene	46.6	1310	4697	6865
2-/9 OH-phenanthrenes	46.3	783	2251	2903
3-OH-phenanthrene	19.3	1147	3322	8925
4-OH-phenanthrene	4.0	119	444	976
Sum of 1-, 2 + 9-, 3-, 4-OH-phenanthrenes	116	3602	11,421	19,670
Kabul (n = 25)				
1-OH-pyrene	136	1303	6255	11,803
1-OH-phenanthrene	161	1410	2382	2656
2-/9 OH-phenanthrenes	156	939	1599	2122
3-OH-phenanthrene	160	1147	3024	3032
4-OH-phenanthrene	22.3	144	284	519
Sum of 1-, 2 + 9-, 3-, 4-OH-phenanthrenes	581	3820	6875	7728
Rural area (n = 30)				
1-OH-pyrene	71.4	2056	11,357	16,288
1-OH-phenanthrene	46.6	1249	5619	6865
2-/9 OH-phenanthrenes	46.3	681	2667	2903
3-OH-phenanthrene	19.3	1063	4165	8925
4-OH-phenanthrene	4.0	112	444	976
Sum of 1-, 2 + 9-, 3-, 4-OH-phenanthrenes	116	2949	12,052	19,670
Children (n = 13)				
1-OH-pyrene	71.4	3167	16,288	16,288
1-OH-phenanthrene	46.6	1343	6865	6865
2-/9 OH-phenanthrenes	46.3	731	2903	2903
3-OH-phenanthrene	19.3	1133	8925	8925
4-OH-phenanthrene	4.0	114	976	976
Sum of 1-, 2 + 9-, 3-, 4-OH-phenanthrenes	116.2	3739	19,670	19,670
Men (n = 16)				
1-OH-pyrene	136	1560	6255	6255
1-OH-phenanthrene	161	1338	3054	3054
2-/9 OH-phenanthrenes	210	1120	1619	1619
3-OH-phenanthrene	188	1242	2886	2886
4-OH-phenanthrene	4.0	131	519	519
Sum of 1-, 2 + 9-, 3-, 4-OH-phenanthrenes	581	4087	7903	7903
Women (n = 26)				
1-OH-pyrene	137	1504	8513	11,357
1-OH-phenanthrene	166	1276	4697	5619
2-/9 OH-phenanthrenes	228	748	2251	2667
3-OH-phenanthrene	160	809	3322	4165
4-OH-phenanthrene	35.4	112	307	444
Sum of 1-, 2 + 9-, 3-, 4-OH-phenanthrenes	667	2962	11,421	12,052

Abbreviations: LOQ, limits of quantification; Max, maximum; Min, minimum.

Table 2. Comparison of PAH metabolite levels (ng/l) in women (all non-smokers) from Kabul ($n = 11$) and rural area ($n = 15$). Maximum and 95th percentile were identical.

	Min	P50	P95/Max
1-OH-pyrene			
Kabul	137	748	5332
Rural area	334	2095	11,357
1-OH-phenanthrene			
Kabul	166	1007	2083
Rural	404	1303	5619
2-/9 OH-phenanthrenes			
Kabul	261	883	1462
Rural	228	687	2667
3-OH-phenanthrene			
Kabul	160	593	1954
Rural	530	1427	3955
4-OH-phenanthrene			
Kabul	38.6	137	284
Rural	35.4	101	444
Sum of 1-, 2+9-, 3-, 4-OH-phenanthrenes			
Kabul	667	2899	4826
Rural	895	3024	12,052

Abbreviations: Max, maximum; Min, minimum; PAH, polycyclic aromatic hydrocarbon.

2003–2004) from the NHANES (CDC, 2009) are: 1-hydroxypyrene, 553 ng/l; 1-hydroxyhenanthrene, 654 ng/l; 2-hydroxyphenanthrene, 310 ng/l; 3-hydroxyphenanthrene, 696 ng/l;
4-hydroxyphenanthrene, 163 ng/l. The 1-hydroxypyrene reference value for German adults (non-smokers, 18–69 year, sampling period 1997–1999) was set to 500 ng/l (Wilhelm et al., 2008).

Table 2 summarizes the PAH metabolite levels for women. Women from the rural area had higher PAH exposure, compared with those from Kabul. As can be seen, the difference was more pronounced for 1-hydroxypyrene (median values 748 and 2096 ng/l; $P = 0.024$) compared with the hydroxyphenanthrenes in urine (median sum of hydroxyphenanthrenes 2899 and 3024 ng/l; $P > 0.05$). The corresponding creatinine-based values were as follows: 1-hydroxypyrene 1291 and 3820 ng/g creatinine ($P < 0.001$), and sum of hydroxyphenanthrenes 3226 and 4748 ng/g creatinine ($P = 0.027$), respectively.

Tobacco exposure was assessed by questionnaire and measurement of cotinine levels in urine.

According to the questionnaire, all women were non-smokers. Maximum cotinine level in urine of all women was 52 μg/l. Urinary cotinine levels of non-smokers are usually below 100 μg/l (Haufroid and Lison, 1998). We assume that the higher exposure of women from the rural area is probably due to the indoor air pollution via incomplete combustion from the use of open fires for cooking and heating. Similar effects have been found by Mumford et al. (1995) before, when investigating exposure of Chinese women. The participants from Kabul city were directly exposed to exhaust fumes of electricity generators that used diesel as fuel, which may be an explanation of why PAH values are much higher in Afghanistan than in developed countries.

According to questionnaire, 11 adult men were smokers. Nine men had cotinine levels >100 μg/l. The smoke exposure was mainly via waterpipe smoking and, to a lesser extent, through cigarette smoking. We did not find an effect of smoking on PAH metabolites. Median levels of 1-hydroxypyrene, sum of hydroxyphenanthrenes and cotinine in urine for smokers ($n = 11$) and non-smokers ($n = 5$) were as follows: smokers (1655 ng/l, 3602 ng/l, 1482 μg/l), non-smokers (1454 ng/l, 5101 ng/l, 2.5 μg/l). However, considering the small groups, these results should be interpreted with care.

The people of Afghanistan smoke waterpipe using the traditional unflavored tobacco, which is also very popular in Iran (Knishkowy and Amitai, 2005). It is commonly believed that waterpipe smoking has little adverse effects as the water filters most of the toxic components in the smoke. However, waterpipe smokers are exposed to the same kind of risk as cigarette smokers. Even though the smoke from waterpipe is filtered through water before inhalation, 1 h of waterpipe smoking is equivalent to a cigarette smoker inhaling 100 or more cigarettes (Shihadeh et al., 2004; Knishkowy and Amitai, 2005; Eissenberg and Shihadeh, 2009; Cobb et al., 2010; Daher et al., 2010). Diseases such as cancer and ischemic heart disease can manifest themselves quickly in frequent waterpipe smokers. Mothers who smoke waterpipe during pregnancy are at an increased risk of delivering babies with reduced weight due to the high concentration of chemical compounds in waterpipe smoke that could have toxic effects (Kallen, 2001; Kirchengast and Hartmann, 2003; Radwan et al., 2003; Mirahmadizadeh and Nakhaee, 2008).

Unlike cigarettes, waterpipe utilizes charcoal as heating source so that waterpipe smoke does not only contain products from sweetened tobacco mixture, but also charcoal combustion. A single waterpipe smoking session is said to yield 20 times the amount of PAHs found in cigarettes (Sepetdjian et al., 2008, 2010).

In the last decades, waterpipe smoking has increased tremendously in different parts of the world (Cobb et al., 2010). Waterpipe smoking is known in various Asian and African countries under different names: shisha in Egypt, Morocco and Kuwait, narghile in Turkey and Syria, hookah in India, qalyan in Iran and chalem in Afghanistan (WHO Tobacco Free Initiative, 2005). Various types of tobacco are smoked in those areas. The main ones are the traditional unflavored ''ajami'' pure dark paste tobacco, ''Mu' essel''

(30% of tobacco and 70% of honey) and an intermediate form, the Indian Jurak.

Additionally, 11 participants were exposed by oral smokeless tobacco. We found a close association between cotinine excretion and oral tobacco exposure ($r = 0.683$, $P = 0.021$).

Oral smokeless tobacco such as moist snuff is very popular in Afghanistan. Because of the high moisture and salt content, it creates excess saliva and thus requires spitting. Smokeless tobacco is considered by the IARC (2007) to cause cancers of the oral cavity and pancreas. It is estimated that smokeless tobacco contains a list of 28 carcinogenic substances, including PAH (Stepanov et al., 2008). However, in our study, oral tobacco exposure was not associated with increased internal PAH exposure. Stepanov et al. (2008) found great variation of PAH levels in different oral smokeless tobacco products. The study population did not use name-brand products. Therefore, we have no information on PAH levels in the tobacco used here.

We conclude that populations in low developed countries may be at special risk to increased-PAH exposure due to inadequate control of air pollution from car emissions and due to burning of biomass fuels for cooking and household energy. This is of great health significance. For example, indoor use biomass fuel is associated with tuberculosis (Pokhrel et al., 2010) risk of low birth weight and stillbirth (Pope et al., 2010), adverse reproductive outcomes, mortality and respiratory morbidity (Tielsch et al., 2009).

Conflict of interest

The authors declare no conflict of interest.

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