IJRCE

ISSN 2248-9649

International Journal of

Research in Chemistry and Environment

Available online at: www.ijrce.org

Research Paper

Biological Monitoring of PAHs Exposure Among the Foundry Workers

***Sen Somnath[1], Narayana J.[2], Ravichandran B.[1], Dhananjayan V.[1], Gaikwad A. S.[1]**

[1]Industrial Hygiene & Toxicology Division, Regional Occupational Health Center (Southern), Poojanahalli Road, Kannamangala Post, Devanahalli TK, Bangalore-562110, INDIA

[2]Department of Environmental Science, Kuvempu University, Shimoga, Karnataka, 577451, INDIA

(Received 04th January 2016, Accepted 15th March 2016)

Abstract: *Polycyclic aromatic hydrocarbons (PAHs) are known to be human carcinogens and increased incidences of lung, skin, and bladder cancers with occupational exposure. Foundry workers are exposed to PAHs with other pollutants in the workplace during performing duties. To evaluate the PAHs exposure, biological monitoring was conducted among the foundry works to quantify the urinary levels of 1- hydroxypyrene (1-OHP) and hydroxylated phenenthrenes (OHPHE). Total 154 foundry workers including 26 controls were considered for the study and an urine sample was collected at the end of the shift of end the week to evaluate the urinary level of 1-OHP and OHPHE. It was found that mean urinary 1-OHP levels were significantly (<0.05) higher among foundry workers (1.09±1.31 µmol/mol creatinine) than control (0.38±0.73 µmol/mol creatinine). However, the mean OHPHE levels of the foundry workers (1.77±1.70 µmol/mol creatinine) were also high, but not significant compared with controls (1.65±1.38 µmol/mol creatinine). It was also observed that the mean OHPHE level was higher among the workers in shaking-out & blasting sections and the mean 1-OHP level were significantly higher in the melting and shaking-out section workers. The mean 1-OHP level of the workers with job experience ≤1, >1- ≤5 and >10 years was more significant with an elevated range than control. From the present study it was observed that increased urinary level PAHs metabolites (1-OHP & OHPHE) were increasing with PAHs exposure among the foundry workers and these occupational groups of workers might be at higher risk of a long-term chronic exposure.*

Keywords: Foundry workers, PAHs exposure, urinary biomarker, 1- hydroxypyrene, hydroxylated phenenthrenes.

Introduction

Foundry workers are exposed to PAHs (Organic Pollutants) with a variety of other organic and inorganic chemicals. PAHs release from iron foundries in different processes, including moulding, melting, blasting, finishing sections [1]. It was reported that exposure to PAHs and their derivatives may signify a high risk to human health[2,3] because the PAHs compounds are known or suspected to be mutagenic and human carcinogenic[4,5]. With chronic exposure there were increased risk of lung cancer and it has been reported among foundry workers [6,7].

The biological monitoring of PAHs compounds are the novel approach to examine long-term occupational exposure of PAHs. Inhaled or dermal absorption of carcinogenic PAHs are transformed into monohydroxy and dihydroxy derivatives in liver and other metabolically active tissues. The intake of PAHs in the body may be monitored by different biomarkers such as metabolites in urine, PAH-protein adducts or PAH-DNA adducts. Urinary levels of 1-OHP and OHPHE were widely used as biological indicators of PAHs exposure. A specific metabolite of pyrene, 1-OHP, in urine was suggested as a biomarker of human exposure to PAHs [8-12] and various hydroxylated phenenthrenes have also been reported as biomarker of exposure of phenenthrenes [13,14]. 1-OHP is the principal product of pyrene metabolism [15] and it is an optimal reference substance for biological monitoring of PAHs exposures [16].

Jongeneelen [17] proposed a biological limit of 2.3µmol/mol 1-OHP for cokeoven workers. Ny et al [18] proposed 4.33µmol/mol for Soderberg potroom workers. Lauwerys [19] proposed a tentative urinary 1-OHP limit value was 1.4 µmol/mol. These limits are based on TLV of airborne PAHs concentration and the relationship between airborne

PAHs level and urinary 1-OHP concentration. Very few studies have reported the OHPHE values in among people after intake of PAHs. In the present study it was aimed to measure the urinary levels of 1-OHP and total OHPHE among the foundry workers who performed their duties in different sections.

Materials and Methods

Total 154 including 26 controls male foundry workers were considered for the study in the years. 2012-14. An interview was conducted using a questionnaires collected data on age, work experience, nature of works and life styles (including smoking, alcohol, tobacco chewing). The controls were selected in the same factories who were working outside the manufacturing plants including canteen staffs, securities and store people. At the ends of the shift of weekends, urine samples were collected from all the participants. Before collection of urine, the participants were asked to wash their hands to avoid contamination. All subjects provided informed consent. The samples were stored in -25°C before analysis. The ethical clearance was obtained from the institutional ethics committee.

Sample Processing and analysis of 1-OHP & OHPHE in Urine: The determination of 1-OHP and OHPHE were carried out followed by using previously developed high performance liquid chromatographic (HPLC) method [14, 20]. Aliquots (6 ml) of urine were buffered with 12 ml 0.2(N) sodium acetate buffer (pH 5.0) and hydrolyzed with 30µl of arylsulfatase for 16 h by keeping inside the incubator at 37°C with constant shaking condition at 210 rpm. Once conditioning was achieved after overnight incubation, the metabolites were enriched on a special RP-C 18 cartridge (500 mg/3CC cartridge procured from M/s AnalChem Pvt Ltd, Allahabad). The column was conditioned with 5 ml of methanol and 10 ml of water and then the hydrolyzed urine samples were passed through the C18 cartridges without letting the cartridges to get dried. The extracts were dried with nitrogen purgings until dryness. The residue was re-dissolved in 2ml of methanol with ultrasonic bath. The aliquots were filtered through 0.45 µm syringes filter prior to HPLC analysis. The 20µl of samples was injected into HPLC to quantify 1-OHP & total OHPHE metabolites. All the isomers of OHPHE (2, 3, 4 and 9-OHPHE) were measured. The limit of detection (LOD) of 1-OHP and total OHPHE was 0.05 and 0.10 µg/l respectively and the relative standard deviation (RSD) of 1-OHP was less than 10%, Mobile Phase: Methanol & Water (1:1) with flow rate 1ml/min, Column: C18 reversed phase column (250mm x 4,6 mm, 5 µm), Detector: Fluorescence Detector, Wavelength for OHPHE metabolites: excitation: 244 nm, emission: 370 nm; Wavelength for 1-OHP: excitation: 241 nm, emission: 386 nm), Temperature of the columns 40°C.

Urinary 1-OHP and OHPHE concentration were expressed as µmol/ mol of creatinine. The urinary 1-OHP level of each individual was corrected according to urinary creatinine values, which was measured using an automated method based on the kinetic Jaffe's reaction.

Statistical Analysis

The Statistical calculation was performed using the Statistical package for social sciences (SPSS, Version17.0), Result of all test were considered as significant when $p<0.05$.

Results

The demographic information of the foundry workers and controls were shown in table 1. Mean age of the foundry workers were higher compared with the control. The foundry workers were approximately 5yr older on average (workers: 40.4±11.8 yr and control: 34.5±11.6 yr). Also the percentage of current smoking status, tobacco chewing and alcohol taking were significantly higher among the workers.

Table 1: Demographical and lifestyle characteristics of the foundry workers and control

Variables		Workers N=128 n (%)	Control N=26 n (%)
Age(yr)	≤25	30(23.4)	3(11.5)
	26-35	40(31.3)	4(15.4)
	36-45	34(26.6)	12(46.2)
	≥45	24(18.8)	7(26.9)
Mean age ±SD		40.4±11.8	34.5±11.6
Smoking	Yes	48(37.5)	5(19.2)
	No	80(62.5)	21(80.8)
Tobacco Chewing	Yes	29(22.7)	3(11.5)
	No	99(77.3)	23(88.5)
Alcohol	Yes	40(31.3)	7(26.9)
	No	88(68.8)	19(73.1)

Table 2 shows the mean urinary 1-OHP levels were significantly (<0.05) higher among the foundry workers (1.09±1.31 µmol/mol creatinine) than the control (0.38±0.73 µmol/mol creatinine). However, the OHPHE levels of the workers (1.77±1.70 µmol/mol creatinine) were not significantly higher with control (1.65±1.38 µmol/mol creatinine); even it was elevated than the control group.

It was also observed that the higher level of OHPHE among the workers in shaking-out (2.45±2.01 µmol/mol creatinine) & blasting (2.46±1.03 µmol/mol creatinine) sections and 1-OHP level was significantly higher in the melting (1.39±1.58 µmol/mol creatinine) and shaking-out (1.66±1.48 µmol/mol creatinine) section respectively.

According to the experience 1-OHP levels were significant with experience of ≤1, >1- ≤5 and >10 years (Table 3). The OHPHE levels were significantly high among those who are working more than 10yr in the foundry compared with control and it was indicated that the chronic exposure effect of PAHs compounds.

Table 2: Results of biological monitoring of urinary OHPHE and 1-OHP among the foundry workers and control population

Study Group		N	OHPHE µmol/mol creatinine	1-OHP µmol/mol creatinine
Total Workers		128	1.77±1.70	1.09±1.31*
Section wise	**Moulding**	43	1.97±1.65	0.93±1.07*
	Melting	46	1.30±1.65	1.39±1.58*
	Shaking-out	15	2.45±2.01	1.66±1.48*
	Blasting	11	2.46±1.03	0.58±0.58
	Finishing	13	1.98±2.32	0.41±0.58
Control		26	1.65±1.38	0.38±0.73
*Values are represented as mean ± SD ; *P <.,05(1-OHP: Workers Vs Control)*				

Table 3: Average OHPHE and 1-OHP level in the urine of the study group by experience status and control

Experience (years)	N	Age	OHPHE µmol/mol creatinine	1-OHP µmol/mol creatinine
≤1	23	24.95±8.4	1.08±1.10	1.91±1.64*
>1- ≤5	34	31.91±12.7	1.31±1.57	1.12±1.47*
>5- ≤10	45	33.52±7.8	1.51±1.36	0.73±0.90
>10	26	44.64±7.0	2.73±2.10*	0.96±0.96*
Control	26	34.50±11.6	1.65±1.38	0.38±0.73
*Values are represented as mean ± SD, *P values (<0.05) as compared to controls*				

Table 4: The concentration of PAHs metabolites with respect to personal habits among the Study Subjects

Habits			N	OHPHE µmol/mol creatinine	1-OHP µmol/mol creatinine
Control (N=26)	**Smoking**	Yes	5	1.64±1.12	0.78±1.20
		No	21	1.65±1.46	0.28±0.57
	Tobacco Chewing	Yes	3	1.06±0.13	0.04±0.02
		No	23	1.72±1.45	0.42±0.76
	Alcohol	Yes	7	1.98±1.95	0.16±0.23
		No	19	1.72±1.45	0.45±0.83
Workers (N=128)	**Smoking**	Yes	48	1.37±1.53	1.31±1.41
		No	80	2.02±1.74	0.96±1.23
	Tobacco Chewing	Yes	29	1.25±1.20	1.73±1.85
		No	99	1.92±1.79	0.91±1.04
	Alcohol	Yes	40	1.35±1.53	1.55±1.68
		No	88	1.95±1.37	0.88±1.04
Values are represented as mean ± SD,					

The concentration of mean PAHs metabolites with respect to the personal habits was shown in Table 4. Only the higher concentration of mean urinary 1-OHP was observed among the workers with smoking habits (1.31±1.41 µmol/mol creatinine) than non smoker workers (0.28±0.57 µmol/mol creatinine) as well as Controls with smoking habits (0.78±1.20 µmol/mol creatinine); but there was not statistically significant. It was also observed that the mean levels of OHPHE among the non-smoker workers (2.02±1.74 µmol/mol creatinine) were in the elevated range than the non-smoker controls (1.65±1.46 µmol/mol creatinine). The mean 1-OHP levels of the workers with tobacco chewing habits were higher than the control (1.73±1.85µmol/mol creatinine vs., 0.04± 0.02µmol/mol creatinine). The mean level of 1-OHP concentration of the workers with non- tobacco chewing habits was 0.91± 1.04µmol/mol creatinine and it was double compared with control (0.42± 0.76µmol/mol creatinine). It was found that the mean 1-OHP level was higher among the workers with alcohol habits (1.55±1.68 µmol/mol creatinine) than with-out alcohol habits (0.88±1.04µmol/mol creatinine), in the controls (with alcohol 0.16±0.23 µmol/mol creatinine; without alcohol 0.45±0.83 µmol/mol creatinine).

Discussion

In this present study, we measured the urinary levels of OHPHE and 1-OHP of PAHs metabolite among the foundry workers who were exposed to PAHs compound in their workplace. Our results shows that the foundry workers had higher levels of OHPHE (1.77 vs. 1.65 µmol/mol creatinine) and 1-OHP (1.09 vs. 0.38µmol/mol creatinine, $p<0.05$). The OHPHE level was higher among the workers in shaking-out & blasting sections and 1-OHP level was significantly higher in the melting and shaking-out section workers. This data revealed that workers were at more risk in the melting, shaking-out and blasting area due to PAHs exposure than other process sections. The 1-OHP levels among the foundry workers in iron foundry located in rural Denmark reported 0.42 and 0.11 µmol/mol creatinine among smokers and non smoker respectively [21]. The average 1-OHP levels in the finish iron foundry were 2.7, 1.8 and 3.6µmol/mol creatinine among the low, medium and high exposure group of workers respectively [22]. Study by Saranya and Sudha [23] reported 2.15 ±0.87 µmol/mol creatinine of urinary 1 –OHP among foundry workers in Coimbatore, India. The mean 1-OHP levels (0.38 to 1.66 µmol/mol creatinine) were reported in the present study compared with the earlier studies. A study conducted among smoker and non- smoker in general populations of Germany reported 1.83 ±0.84 and 1.50 ±1.05 µmol/mol creatinine respectively [14]. Although there is no study available in foundries for OHPHE to compare the levels with the present study, the levels recorded in the present study were higher than the levels reported elsewhere. In this study, the mean 1-OHP value of the foundry workers was 1.09±1.31 µmol/mol which was below the recommended biological exposure limits (BEL) prescribed by different authors.

Conclusion

The present biological monitoring study indicates that the foundry workers were exposed to carcinogenic PAHs and increased urinary concentrations of OHPHE and 1-OHP among the foundry workers were the supposition. However, due to lack of authorized biological exposure limits (BEL) value, risk involved was not computed. Individual authors have been reported on the relation of airborne PAHs and 1-OHP in urine. The proposed BEL is not valid for all work environments. More over the dermal uptake appears to be a significant pathway of PAHs exposure and extent to dermal absorption to PAHs will differ the occupational setting of BEL value. The epidemiological studies of foundry workers with its urinary metabolites as a dose indicator, will be a more reliable to evaluate the risk, basically in the tropical subcontinent of south Asia.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

The authors are highly grateful to The Director, National Institute of Occupational Health for granting permission to conduct the study. The authors also acknowledge the ROHC(S)'s staffs to conduct the study. We appreciate the management and workers of foundries for their full co-operation to conduct the study. Dr Debjit Chakroborty assistance is highly appreciated for his critical reviews.

References

1. Nelson D., ScheV P.A., Keil C., Characterization of volatile organic compounds contained in coke plant emissions, *In The 84th annual meeting & exhibition of air & waste management association*, **6**, 79–91**, (1991)**

2. International Agency for Research on Cancer, Polynuclear aromatic compounds, part 1, Chemical, environmental, and experimental data, *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Lyon, France*, **32**, 33–45, **(1983)**

3. International Programme on Chemical Safety, Environmental health criteria 202, Selected non-heterocyclic polycyclic aromatic hydrocarbons, *World Health Organization, Geneva* **(1998)**

4. Baek S.O., Goldstone M.E., Kirk P.W.W., Lester J.N., Perry R, Methodological aspects of measuring polycyclic aromatic hydrocarbons in urban atmosphere, *Environ. Technology*, **12**, 107-29, **(1991)**

5. Adonis M., Gil L., Polycyclic aromatic hydrocarbons and mutagenic activity in bacteria and human cells in culture of organic extracts from Santiago, respirable and total suspended particulate matter, *Inhalation Toxicology*, **12**, 1185-204, **(2000)**

6. Ahn Y.S., Park R.M., Stayner L., Kang S.K., Jang J.K., Cancer morbidity in iron and steel workers in Korea, *Am. J. Ind. Medicine*, **49(8)**, 647-57, **(2006)**

7. Rodriguez V., Tardon A., Kogevinas M., Prieto C.S., Cueto A., Garcia M, Menendez IA, Zaplana J, Lung cancer risk in iron and steel foundry workers: a nested case control study in Asturias, Spain, *Am. J. Ind. Medicine*, **38**, 644-50, **(2000)**

8. Huang W., Grainger J., Patterson D.G. Jr, Turner W.E., Caudill S.P., Needham L.L., Pirkle J.L., Sampson E.J., Comparison of 1-hydroxypyrene exposure in the US population with that in occupational exposure studies, Int, *Arch. Occup., Environ. Health*, **77**, 491–98, **(2004)**

9. Jongeneelen F.J., Anzon R.B.M., Leijdekkers C.M., Bos R., Henderson P.T., 1– Hydroxypyrene in human urine after exposure to coal tar and a coal tar derived product, *Int. Arc. Occup. Environ. Health*, **57**, 47-55, **(1985)**

10. Jongeneelen F.J., Bos R.P., Anzion R.B.M., Theuws J.L.G., Henderson P.T., Biological monitoring of polycyclic aromatic hydrocarbons, *Scand J. Work Environ Health*, **12**, 137-43, **(1986)**

11. Jongeneelen F.J., Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons, *Ann. Occu., Hygiene***, 45(1)**, 3-13, **(2001)**

12. Strickland P., Kang D., Sithisarankul P., Polycyclic aromatic hydrocarbon metabolites in urine as biomarkers of exposure and effect, *Environ Health Perspectives***, 104(5)**, 927–32, **(1996)**

13. Grimmer G., Dettbarn G., Jacob J., Biomonitoring of polycyclic aromatic hydrocarbons in highly exposed coke plant workers by measurement of urinary phenanthrene and pyrene metabolites (phenols and dihydrodiols), *In. Arch Occup. Environ. Health*, **65(3)**, 189-99, **(1993)**

14. Gtindel J., Mannschreck C., Btittner K., Ewers U., Angerer J., Urinary Levels of 1-Hydroxypyrene, 1-, 2-, 3-, and 4-Hydroxyphenanthrene in Females Living in an Industrial Area of Germany, Arch, *Enviro., Contam. Toxicology*, **31**, 585-90, **(1996)**

15. Brzeznicki S., Jakubowski M., Czerski B., Elimination of 1-hydroxypyrene after human volunteer exposure to polycyclic aromatic hydrocarbons, *Int. Arch Occup. Environ Health*, **70**, 257–60, **(1997)**

16. Jacob J., Seidel A., Biomonitoring of polycyclic aromatic hydrocarbons in human urine, *J. Chromatography* B, **778**, 31–47, **(2002)**

17. Jongeneelen F.J., Biological exposure limit for occupational exposure to coal tar pitch volatiles at cokeovens, Inr, *Arch Occup Environ Health*, **63(8)**, 511-16, (**1992)**

18. Ny E.T., Heederik D., Kromhout H., Jongeneelen F., The relation between polycyclic aromatic hydrocarbons in air and in urine of the workers in a Soderberg potroom, *Am. Ind. Hyg. Assoc. Journal*, **54(6)**, 277-84, **(1993)**

19. Lauwerys R.R., Occupational toxicology, In: Klaassen C.D., Amdur M.O., Doull J., editors, Casarett & Doull's Toxicology: *the Basic Science of Poisons, 5th Ed, McGraw-Hill, New York*, 987–1009, **(1996)**

20. Jongeneelen F.J., Anzion R.B., Henderson P.T., Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine, *J. Chromatography*, **413**, 227-232, **(1987)**

21. Sherson D., Sigsgaard T., Overgaard E., Loft S., Poulsen H.E., Jongeneelen F.J., Interaction of smoking, uptake of polycyclic aromatic hydrocarbons, and cytochrome P450IA2 activity among foundry workers, *Br J. Ind. Medicin,e*, **49**, 197-202, **(1992)**

22. Santella R.M., Hemminki K., Tang D.L., Paik M., Ottman R., Young T.L., Savela K., Vodickova L., Dickey C., Whyatt R., Perera F.P., Polycyclic aromatic hydrocarbon-DNA adducts in white blood cells and urinary 1-hydroxypyrene in foundry workers, *Cancer Epidemiol Biomarkers Prevention*, **2**, 59-62, **(1993)**

23. Saranya R.S., Sudha S., Assessment of genotoxicity in exfoliated buccal epithelial cells of foundry workers occupationally exposed to polycyclic aromatic hydrocarbons, *Asian J. Pharm. Clin. Research*, **6(2)**, 339-42, **(2013).**