Supplementary file (S1)

Health and carcinogenic risk evaluation for cohorts exposed to PAHs in petrochemical workplaces in Rawalpindi city (Pakistan)

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1. Urinalysis

1.1. Analysis of urinary monohydroxy-PAHs metabolites

The quantification of 1-OHPyr was performed within 24 h of sampling collection by enzymatic hydrolysis of the conjugated 1-OHPyr, using a method reported elsewhere (Elovaara et al., 2006). Briefly, 200 µL urine sample was diluted with acetate buffer (pH=5), followed by the addition of β-glucuronidase/arylsulfatase and incubation at 37°C for 5 h. After addition of 700 µL acetonitrile, the solution was centrifuged for 10 min and the supernatant was transferred in Eppendorf tubes. The solution was re-centrifuged, and the supernatant was stored in a vial for further analysis on reverse phase high pressure liquid chromatography (HPLC Figure 2.9-b), as reported in previous studies (Jongeneelen 1989; Kim 1999; Elovaara et al., 2006). A 20 µl volume of sample was injected into an HPLC (HPLC-FD, XS PD-20A VP-Shimadzu Koyoto Japan) equipped with C-18 column (5 µm, 250×4.6; and FD RF-10Ax). Flow rate was 1 mL/min. Wavelengths used were 227-355 nm, 297-455 nm and 242-388 nm for 2-naph, 1-naph and 1-OHPyr, respectively. Mobile phase was a solution of acetonitrile and aqueous 0.5% acetic acid. A 5-8 point calibration (detector response showed good linearity; $R^2 = 0.985-0.99$) curve was drawn by multiple applications of 5-8 external standards in dilution ranging between 0.5 and 100 pg/ml volume data of which was further used for quantification of analyte in samples. Along with each batch, a reagent blank and procedural blanks was run and subtracted from the results. Several method blank samples were run for eliminating chances of potential contamination, while the samples were always run in a batches of (10 samples, along with a set of mixed calibration standards), each time the same standards were run with each batch, the data of which was incorporated to the calibration curve. In order to test accuracy, recoveries and precision of analytical procedure, urine samples were spiked with known amounts of standards, and samples were extracted with the same procedure mentioned previously. Recoveries ranged between 88 and 102 %, whereas LOD for α- naph, β- naph and 1-OHPyr were 7 nmol/l, 4 nmol/l and 0.8 n mol/l, respectively. The day

to day relative standard deviation was less than 14% Urinary creatinine was measured using conventional Jaffe's colorimetric method on a Hitachi-912 (Roche) auto-analyzer.

1.2. Analysis of erythrocyte superoxide dismutase (SOD) activity

The superoxide dismutase (SOD) in erythrocytes was determined using the method described previously (Marklund and Marklund, 1974; Winterbourn et al., 1975); the method is based on the inhibition of autoxidation of pyrogallol by SOD in an alkaline or aqueous medium, consisting in using pyrogallol. for analysis of SOD , freshly obtained whole blood (0.1ml) was hemolyzed by adding 0.9 ml cold deionized water (4°C), and the Hb was removed using Tsuchihashi's method, i.e. rigorous mixing following the addition of chloroform (0.25ml) and ethanol (0.5ml). The resulting solution was then centrifuged at 18000 rpm and the supernatant was used for the analyses of SOD. The reagents used were prepared with chemicals of high purity (analytical grade), including 0.05 M tris-HCl (pH 7.4) tris-HCl buffer solution (containing 1mM EDTA and 50 mM of tris-buffer), 2-mM solution of pyrogallol stock (25.2 mg per ml of tris-HCl buffer, pH 7.4) kept in dark, 2-mM pyrogallol working solution in 0.05 M tris-HCl buffer (0.5 ml stock was diluted to 50 ml) and kept in darkness. Analysis was carried out in control and test samples, for control, a total 3 ml reaction mixture (0.1 ml pyrogallol was added to 2.9 ml of Tris-buffer) was read at 420 nm for 3 min. The change in absorbance was recorded per minutes, so that the pyrogallol was diluted until the rate of change in absorbance was 0.020-0.023 per min. for sample analyses, 2.8 ml of Tris-buffer, 0.1ml of prepared hemolyzate and 0.1 ml of adjusted pyrogallol solution was added, and the change in absorbance was read at 420 nm for 3 minutes. Calculations were based on the Equation I-III), according to which; the one unit of SOD activity equals 50% inhibition of the rate at which pyrogallol is auto-oxidized (i.e. approximately 0.0125 absorbance unit/min) and is defined as 1 unit of activity (Equation I-III).

$$\% \text{ Inhibition} = \frac{\Delta \text{ Abs}_{420} / \text{ min (un-Inhibited)} - (\Delta \text{ Abs}_{420} / \text{ min Inhibited test sample})}{\Delta \text{ Abs}_{420} / \text{ min (un-inhibited)- (Blank)}} \quad (\text{Equation -I})$$

$$\text{SOD (U/ml)} = \frac{\% \text{ Inhibition} \times \text{Dilution factor}}{50\% \times \text{Volume of enzyme sample used}} \quad (\text{Equation -II})$$

$$\text{SOD (U/g Hb)} = \frac{\text{SOD (Units/ml)}}{\text{g (Haemoglobin)/ml}} \quad (\text{Equation -III})$$

Figure S1: Average concentration of sum of total (Σ PAHs), combustion origin (Σ COMB) and sum of 7-carcinogenic PAHs in the dust samples collected from auto-repair workstations (MC) and petroleum-refinery (RF)

Figure S2: Average values of ILCR, from different routes of exposure

Figure S3: rings-based composition of dust-bound PAHs in chemical workplaces (MC and RF combined)

Plate S1: Auto-mechanics, workshop/spare part areas and children apprentices working without proper self protective equipments