The Research of PAHs Exposure Level Test by Urinary Biomonitoring

Chuan-guo Zhang
Laboratory of Waterway Environmental Protection Technology, Tianjin Water Transport Engineering Science Research Institute, Tianjin 300456, China
Corresponding author: reprobate8@sina.com

Abstract. Some workers had that exposure levels and health effects of polycyclic aromatic hydrocarbons (PAHs) analysed through personal urinary biomonitoring. Personal particulate samples were collected in highway workers’ day shift working time. The spot urine samples were collected on each participant. In these samples, PAHs suggested by US-EPA were measured, and in the urine samples oxidative stress biomarker was analysed. The measured mean occupational exposed concentration of PM2.5 was 230.73 μgm⁻³, and the mean p-PAHs exposed concentration was 319.90 ngm⁻³ for the selected workers. Urinary concentrations of 8-OHdG increased by times following an 8-h work shift in participants. Significant positive associations were found between post-work shift urinary 8-OHdG and p-PAH concentrations. It’s indicated that air exposures for PAHs originating from traffic emissions are important in increasing oxidative burdens in human body.

1. Introduction
The PAHs are a group of widespread persistent organic pollutants (POPs), which originate mostly from incomplete combustion of fossil fuels and organic materials. Although there are a few natural sources (volcanoes and forest fires), human activities contribute the most to PAH emissions (1, 2). In atmosphere of urban areas particularly, the PAH sources are entirely anthropogenic (3–6). Due to PAHs exemplified by benzo -pyrene (BaP) well-known persistence, bioaccumulation and mutagenic effects, the levels and health risks that occupational workers and/or general population exposed to PAHs are of special concern (7–10).

2. Experimental Works
2.1 Study Sites and Subjects
The studied toll station contained 22 tollbooths, which were designed for providing tickets to cars, collecting both cash and prepaid tickets from cars. For each tollbooth, approximately 8,000 to 10,000 vehicles traveled per day. All tollbooths had the same dimension (L×W×H=1.8m×1.2m×2.1m). Each of them had one door (W×H=0.9m×2.0m) and two windows (W×H=0.8m×0.5m). The door was closed but the windows facing the vehicle lane were kept open throughout the work time. There were 24 male workers worked in the highway toll station, and 10 non-smoking healthy male workers (aged from 28 to 35 years, with working history from 2 to 6 years) were recruited in this study. The work time of highway toll station workers is day-night shift. They work 8 h in the day shift (from 9:00 a.m. to 5:00 p.m.), then rest 24 h. After that, the workers undergo night shift working period (from 5:00 p.m. to 9:00 a.m. of the next day), and rest 48 h before next day shift work. During the study period, the
participants were asked not to eat roasted or fried meats and to avoid second hand smoking and alcoholic drinks.

2.2 Collection and Compound Analysis
Sampling was carried out from March to May, 2014. PM2.5 samples were collected on quartz (diameter of 37 mm) by portable pump (Buck Elite-5, USA) equipped with a PM2.5 impactor during highway toll station workers’ day shift working time (from 9:00 a.m. to 5:00 p.m.). During the sampling period, the samplers (operated at a constant flow rate of 4 L min⁻¹) were fixed on the workers’ collars to collect personal samples from their breathing zone. In total, 70 samples were collected (seven samples for each participant) during the observation period. Prior to sampling, all quartz filters were pre-treated by baking in oven for 180 min at 900°C to volatilize any organic contaminants and stored in aluminium foil packages until use. The filters were also maintained under conditions of 45% relative humidity (RH) and 20°C for over 48 h, and were weighed before and after sampling.

| p-PAHs | Mean | 50% | TEF | Detection limit (ng) |
|--------|------|-----|-----|----------------------|
| NAP    | 16.8 | 9.6 | 0.01| 4                    |
| ACE    | 4.0  | 3.4 | 0.01| 4                    |
| FLO    | 4.8  | 4.2 | 0.01| 4                    |
| PHE    | 19.1 | 15.5| 0.01| 3.5                  |
| ANT    | 36.4 | 31.2| 0.01| 4                    |
| FLA    | 12.6 | 106 | 0.01| 4                    |
| PYR    | 21.8 | 18.3| 0.01| 4                    |
| BaA    | 15.8 | 12.7| 0.1 | 4                    |
| CHR    | 17.9 | 14.0| 0.01| 5                    |
| BbF    | 27.8 | 18.8| 0.1 | 5                    |
| BkF    | 61.5 | 53.2| 0.1 | 6                    |
| BaP    | 10.0 | 9.1 | 1   | 6                    |
| IND    | 21.6 | 19.8| 0.1 | 5                    |
| DahA   | 41.9 | 36.5| 1   | 5                    |
| BghiP  | 6.0  | 5.5 | 0.01| 5                    |
| PM2.5  | 330.4| 273.5|     |                      |

2.3 Data and Statistical Analysis
All statistical procedures were conducted by SPSS 16.0 for Windows except for descriptive statistics, which was generated using Microsoft Excel 2013 for Windows. Correlation analysis between airborne PAH exposure and urine 8-OHdG was carried out through Spearman correlation analysis. Paired sample t-test (two-tailed) was performed to detect significant differences of the 8-OHdG concentrations of subjects before and after work shift. Results are considered statistically significant if the p-value is less than 0.05.

3. Results
3.1 Concentrations of PM and PAHs
The average mass concentrations of particulate matter (PM, reported in μg m⁻³) and particulate PAHs (p-PAHs, reported in ngm⁻³) of PM2.5 exposure samples collected at highway toll station are presented in Table 1. The PM and PAH concentrations obtained through the whole observation campaign were log-normally distributed (p < 0.05, W-test), indicating that the selected highway toll station workers can be regarded as a similar exposure group.
The measured mean exposure concentration of PM2.5 for the highway toll station workers was 230.73 μg m⁻³, and the mean p-PAHs exposure concentration was 319.90 ng m⁻³. Three vehicles emission-related PAHs including BbF, IND and PHE were the most abundant, and their mean concentrations were 61.52 ng m⁻³, 40.90 ng m⁻³ and 36.45 ng m⁻³, accounting for 18.62%, 12.38% and 11.03% of the total PAH concentration, respectively. The high molecular weight PAHs contributed more to total concentration than medium- (4 rings) and low- (2 and 3 rings) molecular weight PAHs (MMW, and LMW, respectively), and their proportions were 47.32%, 24.30% and 28.38%, respectively.

3.2 Concentrations of urinary 8-OHdG

8-OHdG concentrations in urine samples were analysed, and the average concentrations were 3.63 and 7.37 μmol mol⁻¹ for the selected subject’s pre- and post-work shift, respectively. The 8-OHdG in urine at post-work shift is significantly higher than those at pre-work shift (p<0.05). Pre-work shift 8-OHdG concentrations had relatively smaller standard deviations than post-shift concentrations (Table 2).

| Species | Pre-work shift mean | Pre-work shift Std | Post-work shift mean | Post-work shift Std | p-value |
|---------|-------------------|-------------------|---------------------|-------------------|---------|
| 8-OHdG  | 3.63              | 1.95              | 7.37                | 5.06              | <0.001  |

3.3 Discussion

Assessment of human exposure risk to environmental pollutants can be accomplished either through environmental monitoring in which concentrations of pollutants in environmental samples are determined, or through biomonitoring in which internal levels of biomarker in human body are measured as indicators for assessing health effects. Though a considerable number of studies have been conducted on investigating PAHs concentrations in urban environment, little work has been done for population exposure, especially for workers of which the occupational exposure level and ensuing health risks are supposed to be significant. In present study, personal particulate sampling and biomonitoring were used in combination to produce comprehensive exposure assessments for highway toll station workers in Tianjin, China.

Table 3 Spearman correlation coefficients between p-PAH concentrations and excreted-8-OHdG concentrations.

| Species | Post-work shift | 8-OHdG Change in concentration over work shift |
|---------|----------------|---------------------------------------------|
| NAP     | 0.20           | 0.22                                        |
| ACE     | 0.45           | 0.47                                        |
| FLO     | 0.20           | 0.22                                        |
| PHE     | 0.36           | 0.37                                        |
| ANT     | 0.45           | 0.46                                        |
| FLA     | 0.35           | 0.36                                        |
| PYR     | 0.33           | 0.35                                        |
| BaA     | 0.46           | 0.45                                        |
| CHR     | 0.51           | 0.50                                        |
| BbF     | 0.46           | 0.48                                        |
| BkF     | 0.46           | 0.49                                        |
| BaP     | 0.49           | 0.51                                        |
| IND     | 0.41           | 0.44                                        |
| DahA    | 0.51           | 0.41                                        |
Epidemiological studies have demonstrated stronger exposure-response relationships for mortality and morbidity outcomes in association with fine particles than other fractions. The average concentration of PM2.5 measured for highway toll station workers was 230.73 μg m⁻³ during observation period. China Environmental Protection Agency (CEPA) has recently established the PM2.5 National Ambient Air Quality Class 2 Standard at 35 μg m⁻³ for the annual standard and at 75μg m⁻³ for the 24 h standard. Though the PM2.5 exposure concentration of highway toll station workers was much lower than that specified by the U.S. Occupational Health and Safety Administration (OSHA, 5 mg m⁻³) and the American Council of Governmental Industrial Hygienists (ACGIH, 3 mg m⁻³) (30), it far exceeded the CEPA 24 h standard by at least 300% during work hours. The high levels of PM2.5 in this study may pose potential health risks to highway toll station workers as the above-mentioned standards were set for the sake of protecting public health with an adequate safety margin. The measured mean p-PAHs exposed concentrations was 319.90 ng m⁻³ for the workers, which was at least 15 times higher than that detected in campus of Tianjin (9), indicating a massive contribution of vehicle emission to ambient PAHs. Similar results have been reported in previous studies. For example, the p-PAH concentrations in air sample from motor vehicle emission were 7.5 times higher than those observed at campus; in the occupational exposure research for traffic policeman, Hu et al. (9) suggested the p-PAH concentration at road intersections was 40 times as great as that detected in campus. For individual PAHs, BaP is the only compound that is regulated by environmental standards. CEPA recommended the annual BaP exposure standard at 1.0 ng m−³ for and the 24 h exposure standard at 2.5 ng m−³. The annual standard of CEPA is consistent with the WHO standard. For highway toll station workers, the average BaP exposure concentration was 21.60 ng m⁻³ during observation period, which far exceeded its CEPA standard (8.64 times higher for 24 h standard and 21.60 times higher for annual standard). The carcinogenic potency of PAH exposure could be estimated by calculating the BaP equivalent concentration (BaPeq) based on toxic equivalent factors (TEFs, Table 1). BaPeq concentration for the particulate phases examined in this study was 41.31 ng m⁻³, which was over 16 times higher than the CEPA 24 h standard, indicating potential health risk for highway toll station workers.

The occupational exposure levels of toll station workers were further compared with those of other workers, including vehicle inspection workers (4), fastener manufacturing industry workers (8), traffic policemen (9), carbon black manufacturing industry workers and cooks (Table 4). The total PAH concentrations (PAHs) at the fastener manufacturing industry were higher than other occupational environments, and the highest BaP concentrations were found in carbon black manufacturing industry sectors. Considering the carcinogenic effects of the PAH mixtures in terms of BaPeq concentration, the BaPeq exposure level of highway toll station workers was significantly higher than vehicle inspection workers, cooks of Indian and Chinese stall but much lower than workers in other occupational environments.

In the external exposure assessment study, the selection of sampling sites and the relative location of the study population to the sampling sites can have great influences on the results. Assessing exposure using personal sampling technique provided more reliable, accurate and quantititative than fixed-point sampling. However, differences in physiology and behaviour between different individuals can affect the uptake and metabolism of pollutants such as PAHs, which may in turn lead to huge fluctuations on the results of exposure assessment. To avoid such limitations, biomonitoring, measuring subject’s urinary 8-OHdG concentration to assess OS level was conducted in the present study. In previous studies, OS has been identified as one potential mechanism underlying the toxic effect of air pollutants, and a hierarchical OS model has been proposed to explain the dose-dependent response. ROS formed at lower exposure levels could activate an antioxidant response such as the synthesis of enzymes related to cyto protective, detoxification. Unlike the lower exposure levels, higher exposure levels can activate the transcription NF-KB and activator protein-1 response, alter the function of mitochondria and increase expression of pro inflammatory cytokine. Oxidative DNA
damage in human caused by environmental factors was specified as one of the factors contributing to the formation of malignant tumours.

Table 4. Comparison of PAH exposure concentrations between highway toll station workers and other studies. (ng m\(^{-3}\))

| Study Population     | PAHs  | BaP   | BaPeq | City    |
|----------------------|-------|-------|-------|---------|
| Highway toll station | 330   | 21.6  | 41.31 | Tianjin |
| Fastener             | 2239  | 105   | 126   | Taiwan  |
| Traffic policemen    | 867.5 | 26.2  | 82.4  | Tianjin |
| Carbon black         | 1953  | 341   | 566   | Taiwan  |
| Palletizing          | 1449  | 285   | 314   |         |
| Chinese stall        | 109.2 | 5.6   | 31.9  | Singapore |
| Malay stall          | 439   | 16    | 170   |         |
| Indian stall         | 36.0  | 0.9   | 2.1   |         |
| Bus line             | 111.7 | 1.4   | 4.4   |         |
| Gasoline line        | 56    | 1.3   | 3.3   |         |
| Diesel line          | 199.8 | 4.4   | 12.4  |         |

In occupational exposure study for workers in rubber factories, azo-dye industries, coke oven plants and roofers, exposure to PM with large quantities of PAHs can lead to elevation of urinary 8-OHdG concentration. However, the results for workers related with emission from traffic sources were in contradiction to each other. In contrary, the results from the study for garage and waste collection workers showed that there was no association between airborne PAHs concentrations and urinary 8-OHdG concentration. In the present study, although the participants were asked not to eat roasted or fried meats and to avoid smoking and alcoholic drinks, some other factors may contribute to the variation of OS as well. For example, moderate physical exercise could reduce the urinary 8-OHdG level, while coffee consumption may lead to a significant increase in excretion of urinary 8-OHdG. However, the effects of these factors on the urinary 8-OHdG levels were not investigated in this study. In addition, the work time of highway toll station workers is day-night shift. We only do the measurement during their day shift working time; the obtained results may be different with those of night shift. Because all participants were male adults, the results of this study cannot be generalized to female or other populations that may be sensitive. Besides, though the exposure risk for highway toll station workers caused by particulate PAHs was presented; however, the exposure risk level attributed to gas phase PAHs was not analysed. The above-mentioned limitations in this study should be considered in future research study.

4. Conclusions

The measured mean exposure concentrations of PM2.5 were 230.73 μg m\(^{-3}\), and the mean p-PAHs exposure concentrations were 319.90 ng m\(^{-3}\) during their day shift working period. Urinary concentrations of 8-OHdG increased by two times following an 8-h work shift in participants. Significant positive associations were found between most of the individual PAH compounds and post-work shift urinary 8-OHdG levels. The results of this study supported that p-PAHs from traffic-related emission can lead to oxidative DNA damages in the human body.
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