Urinary 1-hydroxypyrene and 8-hydroxydeoxyguanosine Levels among Coke-oven Workers for 2 Consecutive Days

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Abstract: Urinary 1-hydroxypyrene and 8-hydroxydeoxyguanosine Levels among Coke-oven Workers for 2 Consecutive Days: Thi-To-Uyen NGUYEN, et al. Department of Health Policy and Management, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Japan—Objectives: This study evaluated the levels of exposure to polycyclic aromatic hydrocarbons (PAHs) and their relationship with oxidative DNA damage among Vietnamese coke-oven workers. Methods: We collected urine from 36 coke-oven workers (exposed group) at the beginning and end of the shift on 2 consecutive days. We also collected urine from 78 medical staff (control group). Information was collected by questionnaire about smoking status, drinking habit, and working position. Urinary 1-hydroxypyrene (1-OHP) and 8-hydroxydeoxyguanosine (8-OH-dG) were measured using HPLC. All statistical analyses were performed with SPSS version 19. Results: Urinary 1-OHP was significantly higher in the coke-oven workers than in the control group (p<0.05). Top-oven workers had the highest levels of internal exposure to PAHs, followed by side-oven and then bottom-oven workers (5.41, 4.41 and 1.35 ng/mg creatinine, respectively, at the end of the shift on day 2). Urinary 8-OH-dG was significantly higher in top- and side-oven workers at the end of the shift on day 2 (4.63 and 5.88 ng/mg creatinine, respectively) than in the control group (3.85 ng/mg creatinine). Based on a multi-regression analysis, smoking status had a significant effect on urinary 8-OH-dG (p=0.049). Urinary 1-OHP tended to have a positive correlation with urinary 8-OH-dG (p=0.070). Conclusions: Vietnamese coke-oven workers were exposed to PAHs during their work shift. Urinary 1-OHP exceeded the recommended limit, and elevated oxidative DNA damage occurred in top- and side-oven workers on the second day of work. A tendency for positive correlation was found between urinary 1-OHP and urinary 8-OH-dG.

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Key words: 1-Hydroxypyrene, 8-Hydroxydeoxyguanosine, Coke oven, Polycyclic aromatic hydrocarbons, Vietnam

In the coke-making process, the destructive distillation of coal in a coke oven creates several thousand compounds, many of which are polycyclic aromatic hydrocarbons (PAHs)1,2. PAHs are complex mixtures of more than 100 different chemicals that contain fused benzene rings. PAHs with four or more benzene rings such as benzo(a)pyrene are considered to be human carcinogens3. The International Agency for Research on Cancer (IARC) also classifies coke-oven emissions as Group I human carcinogens2. The main routes of occupational exposure to PAHs are inhalation and skin contact4,5.

Pyrene is a PAH that is formed by four benzene rings and is always present in PAH mixtures6,7. Pyrene is mostly metabolized to 1-hydroxypyrene (1-OHP) and then eliminated in urine and feces4,8. Urinary 1-OHP levels are highly correlated with total PAH levels in ambient air4,6,7 and with skin contamination9. Thus, urinary 1-OHP is widely used as a biomarker for occupational exposure to PAHs in coke-oven workers6,9,10.

An increased risk of cancer among coke-oven workers exposed to PAHs has been reported8. PAHs...
undergo metabolic activation, bind covalently to DNA and form adducts, and induce oxidative damage to DNA\(^{11}\). 8-Hydroxydeoxyguanosine (8-OH-dG) is a biomarker of oxidative DNA damage with relevance to mutagenesis and carcinogenesis\(^{12-14}\). Therefore, many previous studies have investigated the effect of occupational exposure to PAHs on oxidative DNA damage and cancer risk in coke-oven workers. Some of these studies found positive relationships between occupational exposure to PAHs and urinary 8-OH-dG or 8-OH-dG in white blood cells\(^{15-17}\), whereas others did not\(^{18-20}\). One of the problems is that most of these studies measured urinary 1-OHP and urinary 8-OH-dG or 8-OH-dG in white blood cells only once\(^{15, 16, 19-21}\) and did not follow the time course of occupational exposure to PAHs, despite most workers worldwide working consecutively for more than 2 days. It is already known that urinary 1-OHP reveals levels of PAH exposure with a half-life of 6–35 hours\(^{21}\), whereas the half-life of urinary 8-OH-dG is about 6 hours\(^{22}\). As a result, we hypothesized that workers exposed to PAHs would experience an increase in 1-OHP during 8 hours of work each day and daily accumulation during the work week, whereas the increase in 8-OH-dG level should be similar every day. To prove this hypothesis, it was necessary to follow both indices continuously. However, to the best of our knowledge, no study to date has measured both indices for 2 consecutive days among coke-oven workers.

Vietnam has achieved economic growth in recent decades through rapid industrialization. This rapid industrialization and urbanization has caused serious air pollution with PAHs\(^{23-28}\). Some studies have evaluated PAH pollution in large cities such as Hanoi and Ho Chi Minh City, which have large populations and heavy traffic\(^{24, 27, 28}\). However, assessment of occupational exposure to PAHs and its adverse effects among industrial workers has never been conducted in Vietnam. In this study, we focused on coke-oven workers, who are known to be highly exposed to PAHs\(^{\text{a}}\). The aim of this study was to evaluate occupational exposure to PAHs and its relationship with oxidative DNA damage. For accurate evaluation, we measured both urinary 1-OHP and 8-OH-dG at the beginning of the shift and at the end of the shift on 2 consecutive days.

### Methods

#### Study population

The exposed group was selected from coke-oven workers who had been working for at least 1 year in the coke factory (Thai Nguyen Iron and Steel Corporation). The company is located 80 km north of Hanoi and has around 600 workers. The control group was selected from medical staff living in the same city. We explained the objectives of our study to all subjects and how their personal information would be protected. Participants had the right to opt out of the study at any stage. Only those who agreed to participate submitted a completed questionnaire and urine samples. The study was approved by the Ethics Committee of Medicine and Medical Care, University of Occupational and Environmental Health, Japan.

#### Study design

The exposed group consisted of 36 workers directly engaged in coke-making operations. They were grouped by their work positions: 20 worked on the top of the oven (top-oven workers); eight worked at the side of the oven (side-oven workers); and eight worked at the bottom of the oven (bottom-oven workers). A questionnaire was used to collect information about each worker’s age, smoking status, drinking habit and working position.

Urine samples were collected at the beginning of the shift (pre-shift) and at the end of the shift (post-shift) for 2 consecutive working days after a day off. We designated urine samples as follows: pre- and post-shift samples collected on day 1 were designated as pre- and post-day 1 samples, respectively, and pre- and post-shift samples collected on day 2 were designated as pre- and post-day 2 samples, respectively.

The control group consisted of 56 male and 22 female medical staff. They were invited to respond to a similar questionnaire as completed by the exposed group, except for questions regarding working position. A spot urine sample was provided by each control participant.

All urine samples of the control and exposed groups were kept frozen at –80°C until measurement of 1-OHP and 8-OH-dG.

#### Analysis of urinary 1-OHP

The urine samples were analyzed for 1-OHP by HPLC. One milliliter of urine was mixed with 600 µl acetate buffer, pH 5, and 20 µl β-glucuronidase (143,911 U). The mixture was incubated for 2 hours at 37°C with shaking. After incubation, the mixture was applied to a Sep-Pak C18 Cartridge that was primed with 5 ml methanol and 10 ml water. The cartridge was washed with 2.5 ml 40% methanol. The retained solutes were eluted with 5 ml pure methanol. Fifty microliters of the eluted solution was injected into the HPLC system (L-2485 fluorescence detector, L-2300 column oven, L-2200 autosampler, L-2130 pump; Hitachi, Tokyo, Japan). The column was a TSK gel ODS-80TM (Tosoh, Tokyo, Japan). The wavelengths of excitation and emission were 242 nm and 388 nm, respectively. The temperature of the column was maintained at 30°C. The urinary 1-OHP concentra-
tions were adjusted by urinary creatinine excretion (ng/mg creatinine). The mobile phase was 57:43 acetonitrile: phosphate buffer (pH 7.0) at a flow rate of 0.8 ml/min.

Analysis of urinary 8-OH-dG
The urinary 8-OH-dG level was determined according to the method of Kasai\textsuperscript{29}, with slight modification. The urine samples were defrosted at room temperature for 1 hour and centrifuged at 13,000 rpm for 5 minutes at room temperature. Then, a 25-µl aliquot of urine supernatant was mixed with an equal volume of 8-hydroxyguanosine solution. The prepared solution (20 µl) was fractionated using an 8-OH-dG analyzer system with an analysis kit (Shiseido Co. Ltd., Tokyo, Japan). Urinary 8-OH-dG concentrations were adjusted by urinary creatinine excretion (ng/mg creatinine).

Statistical analysis
Differences in gender, age, body mass index (BMI), smoking status, and drinking habit between the control and exposure groups were evaluated by ANOVA and \( \chi^2 \) test. The differences in urinary 1-OHP concentration and urinary 8-OH-dG among bottom-oven, side-oven and top-oven workers and the control group were compared by median value and tested by Mann-Whitney U test. The Wilcoxon signed-rank test was used for comparison of urinary 1-OHP and 8-OH-dG between pre-shift and post-shift or between the first and second day. Multiple regression analysis was performed, with the predictive properties of independent variables (gender, age, BMI, smoking status, and drinking habit) and dependent variables (8-OH-dG and 1-OHP). To analyze the correlation between urinary 1-OHP and 8-OH-dG, we adopted the maximum value of urinary 1-OHP and that of 8-OH-dG as representative values for each coke-oven worker during the 2 consecutive days. All the statistical analyses were performed with SPSS version 19.

Results

Participant characteristics
The characteristics of the exposed and control groups are shown in Table 1. There were no significant differences in gender, age, BMI, smoking status or drinking habit between the control and exposed groups (\( p > 0.05 \)). Among the exposed group, all the bottom-oven workers were female, and none of them were smokers or drinkers.

Comparison of urinary 1-OHP and 8-OH-dG before the shift
The pre-day 1 levels of urinary 1-OHP and 8-OH-dG are shown in Fig. 1. With regard to urinary 1-OHP, top-oven workers showed the highest level (2.23 ng/mg creatinine), followed by side-oven workers (1.38 ng/mg creatinine), bottom-oven workers (0.80 ng/mg creatinine), and the control group (0.39 ng/mg creatinine). Urinary 1-OHP was significantly higher in all the exposed subgroups than in the control group (\( p < 0.05 \)). Among the control group, urinary 1-OHP was significantly higher in smokers (0.49 ng/mg creatinine) than in nonsmokers (0.34 ng/mg creatinine) (\( p = 0.001 \)).

With regard to urinary 8-OH-dG, there were no significant differences among any of the subgroups and control group. The urinary 8-OH-dG levels in the top-oven, side-oven and bottom-oven workers and control group were 3.94, 4.01, 3.36 and 3.85 ng/mg creatinine, respectively.

Trends in urinary 1-OHP and 8-OH-dG during work days
The urinary 1-OHP and 8-OH-dG levels of the exposed group on the 2 consecutive working days are presented in Fig. 2. The post-day 1 and post-day 2 urinary 1-OHP levels were both significantly higher than pre-day 1 and pre-day 2 levels in all the subgroups (\( p < 0.05 \)). Urinary 1-OHP was increased in the post-day 1 samples, decreased in the pre-day 2 samples and then increased again in the post-day 2 samples.

Table 1. Characteristics of the coke-oven workers and control group

<table>
<thead>
<tr>
<th></th>
<th>Number of subjects</th>
<th>Gender</th>
<th>Age mean ± SD</th>
<th>BMI mean ± SD</th>
<th>Smoker n (%)</th>
<th>Drinker* n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed group</td>
<td>36</td>
<td>28 / 8</td>
<td>39.6 ± 9.5</td>
<td>21.7 ± 1.9</td>
<td>16 (44.0)</td>
<td>17 (47.2)</td>
</tr>
<tr>
<td>Top-oven workers</td>
<td>20</td>
<td>20 / 0</td>
<td>37.7 ± 10.2</td>
<td>21.7 ± 1.9</td>
<td>10 (50.0)</td>
<td>14 (70.0)</td>
</tr>
<tr>
<td>Side-oven workers</td>
<td>8</td>
<td>8 / 0</td>
<td>44.8 ± 9.5</td>
<td>21.2 ± 2.2</td>
<td>6 (75.0)</td>
<td>3 (37.5)</td>
</tr>
<tr>
<td>Bottom-oven workers</td>
<td>8</td>
<td>0 / 8</td>
<td>39.3 ± 6.2</td>
<td>22.2 ± 2.1</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Control group</td>
<td>78</td>
<td>56 / 22</td>
<td>39.3 ± 4.0</td>
<td>22.6 ± 2.6</td>
<td>31 (39.7)</td>
<td>32 (41.0)</td>
</tr>
</tbody>
</table>

*Drinker: ≥2 drinks a week.
2 samples. The highest levels detected in post-day 2 samples for bottom-oven, side-oven and top-oven workers were 1.35, 4.41 and 5.41 ng/mg creatinine, respectively. Regarding urinary 8-OH-dG, only top-oven workers showed a significant increase in post-day 1 and post-day 2 levels ($p=0.048$ and $p=0.005$, respectively). Urinary 8-OH-dG in side-oven workers increased continuously during the 2 consecutive days; however, no significant change was seen in the bottom-oven workers. Comparing the subgroups, the top-oven and side-oven workers (4.63 and 5.88 ng/mg creatinine, respectively) showed significantly higher post-day 2 concentrations than the bottom-oven workers and control group (3.58 and 3.85 ng/mg creatinine, respectively) ($p<0.05$).

Multiple regression analysis of urinary 1-OHP, 8-OH-dG and lifestyle

Table 2 shows the results of multiple regression analysis for several variables such as gender, age, BMI, smoking status and drinking habit in relation to urinary 1-OHP and 8-OH-dG. Only smoking status had a significant effect on urinary 8-OH-dG ($p=0.049$), and BMI tended to have a negative effect on urinary 8-OH-dG ($p=0.079$), while other factors such as gender, age, or drinking habit did not ($p>0.1$).

Correlation of urinary 1-OHP and 8-OH-dG

Table 3 shows that urinary 1-OHP tended to have positive effect on urinary 8-OH-dG after adjusting for BMI and smoking status ($p=0.084$), and the tendency for correlation was also observed after adding gender, age and drinking habit to the multiple regression model ($p=0.070$).

Discussion

This is believed to be the first study to measure both urinary 1-OHP and 8-OH-dG before and after the shift for 2 consecutive days to differentiate the time course of both indices among coke-oven workers. We demonstrated the following clear findings about PAH exposure in coke-oven workers. First, we found that the pre-day 1 urinary 1-OHP level was significantly higher in all the exposed groups than in the control group. Jongeneelen et al.\textsuperscript{30} reported that excretion of 1-OHP consists of fast- and slow-excreting components. Fast excretion of 1-OHP occurs with a half-life of 1–2 days, whereas slow excretion occurs with a...
The half-life of 16 days. This means that even after 1 day off, a certain amount of 1-OHP is still accumulated in the body, which explains the difference in pre-shift concentrations of urinary 1-OHP among each group.

Second, the pre-shift level of urinary 1-OHP was significantly higher than pre-shift level in all the subgroups, on both day 1 and day 2. This clearly indicates that there was a significant level of occupational exposure to PAHs during each day in coke-oven workers, regardless of workplace location. Moreover, the trend for the post-day 2 level of urinary 1-OHP to be higher than the post-day 1 level among all subgroups demonstrated exposure on each work day as well as accumulation from previous days.

The group that was most exposed to PAHs was the top-oven workers, followed the side-oven workers and then bottom-oven workers, which was similar to previous studies. This result agrees with the previous studies by Jongeneelen et al. and Buchet et al. showing that the PAH concentrations in the air at the top of the oven were higher than those at the side and bottom of the oven. Comparing the urinary 1-OHP level of the coke-oven workers with those in other countries, the exposure level in Vietnam seemed to be lower than those in Taiwan and China. However, a recommended level of urinary 1-OHP for occupational exposure has been proposed by Jongeneelen, that is $<2.3\, \text{µmol/mol creatinine}$ (equivalence $4.42\, \text{ng/mg creatinine}$). In this study, the urinary 1-OHP levels of the top-oven and side-oven workers exceeded this occupational exposure limit after 2 days of work. Therefore, countermeasures should be implemented to reduce their exposure, such as improvement of the manufacturing facility and use of personal protective equipment. Simultaneously, annual monitoring for exposure to PAHs, especially for top- and side-oven workers, should be conducted.

Regarding urinary 8-OH-dG, there was no significant difference in pre-day 1 levels between the exposed and control groups. This may be because the half-life of excreted urinary 8-OH-dG is about 6 hours, which is shorter than that of urinary 1-OHP. During the 2 working days, the trend in urinary 8-OH-dG did not clearly indicate accumulation, which was seen for urinary 1-OHP. Only the post-day 2 levels of urinary 8-OH-dG of the top- and side-oven

<table>
<thead>
<tr>
<th>Variables</th>
<th>1-OHP Coefficient</th>
<th>1-OHP p-value</th>
<th>8-OH-dG Coefficient</th>
<th>8-OH-dG p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>0.309</td>
<td>0.229</td>
<td>0.146</td>
<td>0.764</td>
</tr>
<tr>
<td>Age</td>
<td>−0.001</td>
<td>0.957</td>
<td>0.035</td>
<td>0.218</td>
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<tr>
<td>BMI</td>
<td>−0.030</td>
<td>0.419</td>
<td>−0.125</td>
<td>0.079</td>
</tr>
<tr>
<td>Smoking status</td>
<td>0.131</td>
<td>0.542</td>
<td>0.809</td>
<td>0.049</td>
</tr>
<tr>
<td>Drinking habit</td>
<td>0.214</td>
<td>0.292</td>
<td>−0.202</td>
<td>0.599</td>
</tr>
</tbody>
</table>

Gender: 1 male, 0 female. Smoking status: 1 yes, 0 no. Drinking habit: 1≥2 drinks a week, 0<2 drinks a week.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Model 1* Coefficient</th>
<th>Model 1* p-value</th>
<th>Model 2* Coefficient</th>
<th>Model 2* p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>0.372</td>
<td>0.522</td>
<td>0.372</td>
<td>0.522</td>
</tr>
<tr>
<td>Age</td>
<td>0.038</td>
<td>0.260</td>
<td>0.038</td>
<td>0.260</td>
</tr>
<tr>
<td>BMI</td>
<td>−0.192</td>
<td>0.019</td>
<td>−0.215</td>
<td>0.011</td>
</tr>
<tr>
<td>Smoking status</td>
<td>0.901</td>
<td>0.026</td>
<td>0.877</td>
<td>0.069</td>
</tr>
<tr>
<td>Drinking habit</td>
<td>0.113</td>
<td>0.084</td>
<td>0.124</td>
<td>0.070</td>
</tr>
</tbody>
</table>

Gender: 1 male, 0 female. Smoking status: 1 yes, 0 no. Drinking habit: 1≥2 drinks a week, 0<2 drinks a week. *Adjusted for BMI and smoking status. **Adjusted for gender, age, BMI, smoking status and drinking habit.
workers were significantly higher than that of the control group. We consider that this was possibly related to the degree of exposure to PAHs. In our study, bottom-oven workers were exposed to PAHs to some extent, but their exposure may not have been enough to cause detectable oxidative DNA damage. However, the exposures of the top- and side-oven workers were sufficient to cause oxidative DNA damage that was detectable based on the increase in urinary 8-OH-dG. Taking account of the fact that the urinary 1-OHP levels of the top- and side-oven workers exceeded the recommended PAH exposure limit, this seems reasonable.

Only a few studies have estimated both urinary 1-OHP and 8-OH-dG repeatedly among coke-oven workers. These repeated consecutive measurements revealed the trend in urinary 1-OHP and 8-OH-dG separately and in combination. A repeated measurement study by Chao et al. found that the pattern of urinary 1-OHP was opposite to that of urinary 8-OH-dG. Urinary 1-OHP gradually increased during the work week, but urinary 8-OH-dG was highest at the beginning of the week and then gradually decreased at the end. Their measurements were conducted intermittently and skipped some work days, so it is difficult to explain why this discrepancy occurred. Our study measured both urinary 1-OHP and 8-OH-dG four times at before and after the work shift for 2 consecutive days; therefore, we could clearly follow the trend in urinary 1-OHP and 8-OH-dG. There were increasing trends in urinary 1-OHP and 8-OH-dG after the work day, and the highest levels were found in the post-day 2 samples from top- and side-oven workers. Therefore, it is appropriate to implement biological monitoring of urinary 1-OHP and 8-OH-dG at the end of the shift to evaluate exposure to PAH among coke-oven workers.

The influence of occupational and environmental exposure to PAHs on the formation of 8-OH-dG has been investigated in many studies. Some studies have confirmed that urinary 8-OH-dG is significantly increased in workers exposed to PAHs. Moreover, they have also found a positive relationship between urinary 1-OHP and 8-OH-dG. We found a tendency for positive correlation between urinary 1-OHP and 8-OH-dG after adjusting for gender, age, BMI, smoking status and drinking habit. However, some studies reported that there was no relationship between urinary 1-OHP and 8-OH-dG. The first possible reason for these conflicting results is the different levels of internal exposure to PAHs. Another possibility is that coke-oven emissions contain several other carcinogens, such as nitrosamine, arsenic compounds and benzene. These toxic substances can also affect the amount of oxidative DNA damage.

Finally, we would like to discuss the effect of lifestyle on urinary 1-OHP and 8-OH-dG. Smoking at work may increase urinary 1-OHP among smokers. We detected an increase in urinary 1-OHP in smokers among the control group. However, we could not detect the effect of smoking on urinary 1-OHP among coke-oven workers. The reason for this may be that high exposure to PAHs in the workplace masked the effect of smoking. This result was consistent with several previous studies. In addition, we observed that smokers and nonsmokers among top- and side-oven workers often stayed in the same room during break times. Thus, nonsmokers may have been exposed to passive smoke. Tobacco smoke contains several toxic and cancer-causing chemicals. Our results showed that smoking status was positively correlated with oxidative DNA damage. These findings were consistent with previous studies. Therefore, urinary 1-OHP is considered to be a good biomarker of internal exposure to PAHs. Use of personal air samplers and skin pads would enable us to differentiate between PAH exposure through inhalation and dermal contact. Second, the top- and side-oven workers comprised men, including smokers, while bottom-oven workers were all women who were nonsmokers. However, we could eliminate these factors by multiple regression analysis.

In conclusion, analysis of urinary 1-OHP among coke-oven workers in Vietnam showed occupational exposure to PAHs, with accumulation during the work shift. The urinary 1-OHP exceeded the recommended limit, and the post-day 2 results indicated that increased oxidative DNA damage occurred in top- and side-oven workers. Therefore, it is strongly recommended that some countermeasures should be instituted to improve the working conditions. A tendency for positive correlation was found between urinary 1-OHP and 8-OH-dG. In addition, confounding factors such as smoking status may influence the levels of oxidative DNA damage.

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References


