Urinary 8-Hydroxydeoxyguanosine as a Biomarker of Oxidative DNA Damage in Workers Exposed to Ethylbenzene

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This study assessed the relationships between ethylbenzene exposure and levels of 8-hydroxydeoxyguanosine (8-OHdG) among spray painters. Sixty-four male workers employed at a large shipyard were recruited for this investigation. Fifteen spray painters exposed to paint, together with two non-exposed groups, namely 19 sandblasting workers and 30 office staffs were selected as the subjects. Personal exposure to xylene and ethylbenzene in air were collected using diffusive samplers. Urine samples of the spray painters were collected after a month-long holiday leave and during the pre- and post-workshifts. Urine samples of sandblasting workers and office staffs were gathered after their shift. Urinary mandelic acid and methyl hippuric acid were used as biological indices of dose of ethylbenzene and xylene, respectively. Urinary 8-OHdG was used as biomarker of oxidative DNA damage. The post-workshift concentration of urinary 8-OHdG for 10 spray painters (30.3 ± 9.28 μg g⁻¹ creatinine) significantly exceeded that of holiday leave (7.20 ± 1.08 μg g⁻¹ creatinine; P = 0.001). The post-workshift concentration of urinary 8-OHdG was higher among 15 spray painters (29.0 ± 6.52 μg g⁻¹ creatinine) than sandblasting workers (9.14 ± 2.05 μg g⁻¹ creatinine; P = 0.01) and office staffs (8.35 ± 0.84 μg g⁻¹ creatinine; P = 0.007). A stepwise regression model revealed an 8.11 μg g⁻¹ creatinine increase per 1 p.p.m. increase in ethylbenzene [95% confidence interval (CI) 4.13–12.1]. A stepwise regression model revealed an increase of 6.04 μg g⁻¹ creatinine (95% CI 2.23–9.84) per 1 p.p.m. in ethylbenzene after adjustment of age (95% CI 2.23–9.84). This pilot study suggests that occupational exposure to paint increases oxidative DNA injury. Moreover, urinary 8-OHdG levels displayed greater DNA damage in spray painters compared to other unexposed groups and their holiday leave samples. A significant correlation was found between urinary 8-OHdG and the exposure to ethylbenzene. The ethylbenzene exposure could not explain all urinary 8-OHdG measured. Other components of paint deserve further investigation.

Keywords: 8-hydroxydeoxyguanosine; DNA oxidative damage; ethylbenzene; mandelic acid

INTRODUCTION

Xylene is one of the most used solvents in the industry. Commercial xylene typically contains ethylbenzene. Both chemicals are chronically toxic to the central
nervous system and can cause acute irritation of the eyes, skin, and mucous membrane (Savolainen et al., 1984; Baker et al., 1985; ACGIH, 1996). Over 90% of xylene is metabolized to methyl hippuric acid (MHA) in human; 64% of ethylbenzene is metabolized to mandelic acid (MA) and excreted into urine (Engstrom et al., 1984). In animal experiments, neoplasm in the kidney and testis of rats and in the lung and liver of mice was induced via inhalation of ethylbenzene (Chan et al., 1998). Additionally, ethylbenzene has been classified as possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC).

Reactive oxygen species (ROS) attack DNA, generating intermediates, which can react with DNA and form adducts, including single-strand break and the formation of modified bases such as 8-hydroxydeoxyguanosine (8-OHdG) (Kawanishi et al., 2001). Thus, the measurement of 8-OHdG may be useful as a marker of oxidative DNA damage. Ethylbenzene has been manifested to generate ROS in liver microsomes from exposed rats (Serron et al., 2000). Ethylbenzene hydroperoxide (a sunlight-irradiation product of ethylbenzene) increased 8-OHdG adduct formation in calf thymus DNA in vitro (Toda et al., 2003). Midorikawa et al. (2004) indicated oxidative DNA damage induced by the metabolites of ethylbenzene, namely ethylhydroquinone and 4-ethylcatechol.

This pilot study examines the relation between exposure to ethylbenzene and the occurrence of oxidative DNA damage in field workers. Urinary MA and MHA were used as biological exposure indices of ethylbenzene and xylene, respectively. In addition, 8-OHdG was used to measure oxidative DNA damage.

MATERIALS AND METHODS

Study population

Sixty-four male workers at a large shipyard were recruited for this study: the exposed group comprised of 15 spray painters and 19 sandblasting workers and 30 office staffs who did not use paint in their work were selected as two unexposed groups. Written informed consent was obtained from each subject and study procedures were approved by the Institutional Review Board of the Taipei Veterans General Hospital. Subjects provided information via questionnaires regarding demographics (e.g. age, degree of education, and years of work experience) and lifestyle factors (e.g. smoking and drinking habits). Spray painters wore air purifying half-face respirators to apply solvent-based paint to block units of assembled ships using airless spray guns. The major solvents of paints were xylene and ethylbenzene. Other constituents of paint include epoxy resin, chromates, etc. Sandblasting workers spray steel grits to remove sludge of blocks, and their workload is similar to that of spray painters. The office staffs were in charge of ship design and worked in an office of the shipyard.

Air sampling

Personal exposure to ethylbenzene and xylene inside the respirator was collected using 3M 3500 organic vapor monitor. Each worker was asked to clip the sampler to their collar. When workers wore respirators during spray painting, the sampler was mounted inside the respirator using a fastener. When the respirator was not in use during rest periods, the sampler was detached from the respirator and then attached to the collar.

Urine sample collection

Urine samples of spray painters were collected before and after work on the last of a five consecutive workdays and after a month-long holiday leave. Urine samples of sandblasting workers were gathered before and after their work. Meanwhile, urine samples of office staff were collected at the end of their work. Each urine sample was stored in a plastic bottle and frozen at −20°C until analyzed.

Urine analysis for MA, MHA, and creatinine

Urinary creatinine, MA, and MHA were measured by high performance liquid chromatography (HPLC) (Waters 2695 Separation Module; Waters, Milford, MA, USA) with ultraviolet (UV) detector (Waters 2478 Dual λ. Absorbance). The urine sample was centrifuged at 3000 r.p.m. for 15 min. The supernatant was diluted 100 times with 990 μl of water/methanol 1:1 (v/v) and 10 μl of aliquot was injected into the HPLC system. The UV detector was set at 225 nm. The analysis was performed in a Gemini™ C18 stainless steel column (4.6 × 250 mm, 5 μm; Phenomenex, Torrance, CA, USA). The mobile phase consisted of 20 mM potassium dihydrogen phosphate buffer (pH 3.0 adjustment with phosphoric acid)/acetonitrile 85:15 (v/v) and the flow rate was 0.8 ml min⁻¹. Total MHA concentration was calculated by summing the concentrations of the ortho-, meta-, and para-isomers. The intra- and inter-assay coefficients of variation (CVs) were <8% in the urine assays. Additionally, creatinine
was used to correct the concentration of metabolite in the urine.

**Urine analysis for 8-OHdG**

Urinary 8-OHdG concentrations were measured using a competitive enzyme-linked immunosorbent assay (BIOXYTECH® 8-OHdG-EIA Kit; OXIS International Inc., Portland, OR, USA). Frozen urine samples were thawed and centrifuged at 2000 r.p.m. for 10 min. Aliquots (50 μl) of urine samples and standards were added to microtiter plates precoated with 8-OHdG, followed by 50 μl of the primary antibody, anti–8-OHdG monoclonal antibody solution, and incubated for 1 h at 37°C with continuous mixing at 100 r.p.m. The plates were washed twice using 250 μl of phosphate buffered saline. The enzyme-labeled horseradish peroxidase-conjugated secondary antibody (100 μl) was added to each well for 1 h at 37°C and incubated for 1 h at 37°C with continuous mixing at 100 r.p.m. The plates were washed twice using 250 μl of phosphate buffered saline. The enzyme-labeled horseradish peroxidase-conjugated secondary antibody (100 μl) was added to each well and allowed to react in the dark at room temperature for 15 min; 100 μl of 1.0 M phosphoric acid was added to terminate the reaction. The intensity of the color produced for each sample was measured at 450 nm using a computer-controlled microplate reader (EL 340; Bio Tek Instruments, Highland Park, WV, USA). Duplicate or triplicate measurements were performed for each sample and the CV was within 10% during analysis period. The standard curve was generated by plotting absorbance versus the logarithmic of concentration. Finally, urinary 8-OHdG concentration was expressed as micro grams per gram of creatinine.

**Statistical analysis**

The data were analyzed using the SAS 9.13 statistical package. Descriptive data were obtained for age, years of work experience, and degree of education. A linear mixed model was used to compare 8-OHdG levels in urine with or without occupational exposure to paint in the exposed group. Student’s t-test was used to evaluate the differences of urinary 8-OHdG levels between the exposed and unexposed groups. Linear regression analyses were performed to compare the 8-OHdG levels among spray painters, sandblasting workers, and office staff, adjusting for potential confounders such as age, smoking, and drinking. A stepwise regression procedure was performed to evaluate the relations of urinary 8-OHdG levels to the concentrations of ethylbenzene, xylene, MA, and MHA. The level of significance for all analyses was set to 0.05.

**RESULTS**

**Study population**

The study population characteristics were compared among spray painters, sandblasting workers, and office staffs (Table 1). The average age was 39.6 ± 8.34 years (mean ± SD) for the spray painters, 37.6 ± 11.0 years for the sandblasting workers, and 47.0 ± 5.38 for the office staffs. The office staffs had higher educational degree than the spray painters and sandblasting workers, respectively ($P < 0.001; P < 0.001$). Prevalence of current smoking and drinking was lower among the office staffs than the spray painters and sandblasting workers ($P < 0.001; P < 0.001$). On average, the work experience was 5.91 ± 2.90 years in spray painters, 8.45 ± 9.96 years in sandblasting workers, and 20.5 ± 7.83 in office staffs.

**Air ethylbenzene and xylene concentrations in exposed group**

Air sampling results showed that ethylbenzene and xylene were the major solvents used on site. Median ethylbenzene concentration was 2.25 p.p.m. with an inter-quartile range of 1.31–4.06 p.p.m. Xylene was 1.25 p.p.m. with an inter-quartile range of 0.74–2.9 p.p.m. (data are not shown).

**Urinary MA and MHA concentrations in exposed group**

The pre-workshift MA and MHA concentrations were 89.3 ± 12.0 (mean ± SE) and 55.0 ± 9.45 mg g⁻¹ creatinine, respectively. The post-workshift MA and MHA concentrations were 116.9 ± 20.0 and 105.0 ± 23.4 mg g⁻¹ creatinine, respectively. The cross-shift changes were 27.6 ± 16.5 and 50.1 ± 23.0 mg g⁻¹ creatinine, respectively. The post-workshift MHA concentrations were found to be significantly different compared to the pre-workshift samples ($P = 0.047$) (data are not shown).

**Comparison of urinary 8-hydroxyguanosine levels among holiday leave, pre-workshift, and post-workshift in exposed group**

The mean concentration of urinary 8-OHdG for spray painters in the holiday leave, pre-workshift, and post-workshift is shown in Table 2. In the holiday leave, the concentration of urinary 8-OHdG was 7.20 ± 1.08 μg g⁻¹ creatinine (mean ± SE). The pre-workshift and post-workshift 8-OHdG concentrations were 17.59 ± 3.94 and 30.3 ± 9.28 μg g⁻¹ creatinine, respectively. A linear mixed model was used to compare the concentration of urinary
Table 1. Characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Spray painter (exposed group), n = 15</th>
<th>Sandblasting worker (unexposed group A), n = 19</th>
<th>$P^a$ between sandblasting worker and spray painter</th>
<th>Office staff (unexposed group B), n = 30</th>
<th>$P^a$ between office staff and spray painter</th>
<th>$P^a$ between sandblasting worker and office staff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Education</td>
<td>15 (100%) 18 (94.7%) 1.000 2 (6.70%)</td>
<td>18 (94.7%) 1 (5.3%) 1.000 28 (93.3)</td>
<td></td>
<td>2 (6.70%) 28 (93.3)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td>11 (73.3) 12 (63.2) 1.000 3 (10.0)</td>
<td>12 (63.2) 1 (5.3%) 1.000 28 (93.3)</td>
<td></td>
<td>3 (10.0) 28 (93.3)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Drinking</td>
<td>10 (66.7) 15 (78.9) 0.832 1 (3.3)</td>
<td>15 (78.9) 1 (5.3%) 0.832 28 (93.3)</td>
<td></td>
<td>1 (3.3) 28 (93.3)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (year)</td>
<td>39.6 ± 8.34$^b$ 37.6 ± 11.0 0.559 47.0 ± 5.38</td>
<td>37.6 ± 11.0 0.559 47.0 ± 5.38</td>
<td></td>
<td>47.0 ± 5.38 0.559</td>
<td>0.005</td>
<td>0.002</td>
</tr>
<tr>
<td>Work experience (year)</td>
<td>5.91 ± 2.90 8.45 ± 9.96 0.303 20.5 ± 7.83</td>
<td>8.45 ± 9.96 0.303 20.5 ± 7.83</td>
<td></td>
<td>20.5 ± 7.83 0.303</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^a$Chi-square test.

$^b$Mean ± SD.

Table 2. Comparison of urinary 8-OHdG among the holiday leave, pre-workshift, and post-workshift samples in spray painters

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Holiday leave (n = 10)</th>
<th>Pre-workshift (n = 10)</th>
<th>$P^a$ between holiday leave and pre-workshift</th>
<th>Post-workshift (n = 10)</th>
<th>$P^a$ between holiday leave and post-workshift</th>
<th>$P^a$ between pre-workshift and post-workshift</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OHdG (μg g$^{-1}$ creatinine)</td>
<td>7.20 ± 1.08 (4.44–15.66)$^b$</td>
<td>17.59 ± 3.94 (3.70–42.1)</td>
<td>0.0011</td>
<td>30.3 ± 9.28 (7.30–100.51)</td>
<td>0.0038</td>
<td>0.1622</td>
</tr>
</tbody>
</table>

$^a$Linear mixed model

$^b$Mean ± SE. (Min.–Max.)
8-OHdG among the holiday leave, pre-workshift, and post-workshift groups. The mean pre-workshift 8-OHdG concentration was significantly higher than in the holiday leave sample and the difference was 10.39 ± 4.05 µg g⁻¹ creatinine (P = 0.001). Moreover, the mean post-workshift 8-OHdG concentration was significantly higher than in the holiday leave sample and the difference was 23.07 ± 9.21 µg g⁻¹ creatinine (P = 0.004).

Comparison of urinary 8-hydroxyguanosine between exposed and unexposed groups

The mean concentration of urinary 8-OHdG for the spray painter, sandblasting worker, and office staff groups is listed in Table 3. The mean post-workshift concentration of urinary 8-OHdG was higher among spray painters (29.0 ± 6.52 µg g⁻¹ creatinine) than sandblasting workers (9.14 ± 2.05 µg g⁻¹ creatinine; difference 19.86, 95% confidence interval (CI) 5.41–34.30) and office staff (8.35 ± 0.84 µg g⁻¹ creatinine, difference 20.65, 95% CI 6.581–34.712).

To exclude age, smoking, and drinking effects, a linear regression model was fitted for 8-OHdG, adjusting for the covariates shown in Table 4, and the adjusted difference in the mean post-workshift 8-OHdG concentration between spray painters and office staff was 32.13 µg g⁻¹ creatinine (95% CI 21.1–43.15). Moreover, the adjusted difference in the mean 8-OHdG concentration between spray painters and sandblasting workers was 19.30 µg g⁻¹ creatinine (95% CI 10.79–27.81). Additionally, the mean post-workshift 8-OHdG concentration was significantly higher among sandblasting workers than office staff, with a difference of 12.83 µg g⁻¹ creatinine (95% CI 2.70–22.96). A moderate relationship was found between age and 8-OHdG (β = 0.358, 95% CI –0.034 to 0.756).

Relations of urinary 8-OHdG to ethylbenzene, xylene, MA, and MHA

A significant correlation existed between urinary 8-OHdG and ethylbenzene (r = 0.820, P = 0.001).

Urinary 8-OHdG concentration was moderately related to the level of xylene (r = 0.553, P = 0.062). A moderate correlation existed between urinary 8-OHdG and MA (r = 0.495, P = 0.06). No significant association existed between urinary 8-OHdG and MHA (r = 0.266, P = 0.338) (data are not shown). A stepwise regression model revealed an increase of 8.11 µg g⁻¹ creatinine (95% CI 4.13–12.1) per 1 p.p.m. in ethylbenzene (Table 5).

DISCUSSION

Previous studies indicated that 8-OHdG levels were affected by age, body mass index, smoking, drinking, and nutrition (Kaneko et al., 1996; Mecocci et al., 1999; Kasai et al., 2001). To control for influences of the potential confounders on 8-OHdG, urine samples of spray painters were collected during the holiday leave, pre-workshift, and post-workshift, respectively. Individual comparisons were performed to ascertain the changes of 8-OHdG in urine samples of spray painters were due to occupational exposure.

### Table 3. Comparison of urinary 8-OHdG between the exposed and reference groups

<table>
<thead>
<tr>
<th></th>
<th>Spray painter (exposed group), n = 15</th>
<th>Sandblasting worker (unexposed group A), n = 19</th>
<th>Office staff (unexposed group B), n = 30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-workshift</td>
<td>Post-workshift</td>
<td>Pre-workshift</td>
</tr>
<tr>
<td></td>
<td>8-OHdG (µg g⁻¹ creatinine)</td>
<td>8-OHdG (µg g⁻¹ creatinine)</td>
<td>8-OHdG (µg g⁻¹ creatinine)</td>
</tr>
<tr>
<td>Sprayer painter</td>
<td>19.29 ± 2.89a</td>
<td>28.99 ± 6.52b,c</td>
<td>8.59 ± 0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.14 ± 2.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.35 ± 0.84</td>
</tr>
</tbody>
</table>

*aMean ± SE.

*bComparison of the post-workshift 8-OHdG level between spray painter and sandblasting worker, P = 0.010, student t test.

*cComparison of the post-workshift 8-OHdG level between spray painter and office staff, P = 0.007, student t test.

### Table 4. Simple linear regression of 8-OHdG by group and a general linear regression with potential confounders as covariates (n = 69)

<table>
<thead>
<tr>
<th></th>
<th>Increase in 8-OHdG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td>Sandblasting worker versus office staff</td>
<td>0.792</td>
</tr>
</tbody>
</table>

*aAdjusted for age, drink, and smoke.
Table 5. Stepwise regression models of urinary 8-OHdG for ethylbenzene, xylene, MA, and MHA (n = 12)*

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylbezene</td>
<td>8.114</td>
<td>4.129–12.100</td>
</tr>
<tr>
<td>Ethylbezene</td>
<td>6.036</td>
<td>2.230–9.841</td>
</tr>
<tr>
<td>Age</td>
<td>1.323</td>
<td>0.110–2.537</td>
</tr>
</tbody>
</table>

*Excluded variables: xylene, MA, MHA, smoke, and drink; three air samples were missing.

Results showed that the post-workshift 8-OHdG levels of spray painters were an average 75% higher compared with the holiday leave samples. Furthermore, this study selected sandblasting workers and office staff as the unexposed groups, respectively, to control the potential influence of workload on 8-OHdG. Spray painters exposed to ethylbenzene had average 69 and 71% higher levels of urinary 8-OHdG compared to sandblasting workers and office staffs, respectively. After adjustment of age, smoking, and drinking behavior, the post-workshift 8-OHdG levels of spray painters were also significantly higher than those of office staff and sandblasting workers, respectively. In addition, shot blasting workers are exposed to surface coatings or debris from the workpiece during their workshifts. This may be the reason why the shot blasting workers have higher 8-OHdG than the office workers.

Qualitative and quantitative analysis of air samples revealed that spray painters were primarily exposed to ethylbenzene and xylene. Urinary 8-OHdG was positively related to atmospheric ethylbenzene and was moderately correlated with urinary MA. Although the ethylbenzene concentrations inside respirators were low, previous studies indicated that dermal contact is the other major source of exposure for shipyard spray painters (Chang et al., 2007a,b). Thus, Urinary MA has the potential to assess worker exposure to ethylbenzene by routes of inhalation and skin absorption. The findings of this pilot study indicated that increased DNA damage was measured using urinary 8-OHdG in spray painters owing to exposure to paint. A stepwise regression model revealed an increase of 6.04 μg g⁻¹ creatinine (95% CI 2.23–9.84) per 1 p.p.m. in ethylbenzene after adjustment of age. Recently, a novel pathway that oxidative DNA damage induced by the peroxide and H₂O₂ derived from ethylbenzene hydroperoxide, a sunlight-irradiated product of ethylbenzene may lead to expression of the genotoxicity (Toda et al., 2003).

Recently, increasing numbers of studies have suggested that 8-OHdG might be a useful biomarker of DNA damage from occupational exposure to toxic chemicals. Workers exposed to polycyclic aromatic hydrocarbons, chromium (VI), residual oil fly ash, and benzene, respectively, displayed increased urinary 8-OHdG concentrations (Lagorio et al., 1994; Kuo et al., 2003; Kim et al., 2004; Lai et al., 2005). In this study, urinary 8-OHdG levels were elevated in workers exposed to paint and that the post-workshift 8-OHdG levels of spray painters were on average 75% higher compared with the holiday leave samples. Spray painters exposed to paint suffered oxidative damage to DNA is consistent with IARC evaluation that occupational exposure as a painter is carcinogenic (Group 1).

Taken together, these studies show that 8-OHDG is a sensitive indicator for exposure to the material that causes DNA oxidative damages.

A significant correlation was found between urinary 8-OHdG and the exposure to ethylbenzene. The ethylbenzene exposure could not explain all urinary 8-OHdG measured. Other components of paint deserve further investigation.

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