Seasonal Effect on Airborne Pyrene, Urinary 1-Hydroxypyrene, and Benzo(a)pyrene Diol Epoxide-Hemoglobin Adducts in the General Population

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Abstract

Exposure to airborne polycyclic aromatic hydrocarbons (PAHs) in 65 employees (40 sampled both in summer and winter, 15 sampled in summer only, and 10 sampled in winter only) with no occupational exposure to PAHs was assessed by measuring: personal exposure to pyrene, urinary excretion of 1-hydroxypyrene (1-OHP), and benzo(a)pyrene diol epoxide adducts to hemoglobin (BPDE-Hb). Overall, office employees were exposed to significantly higher levels of pyrene in winter (4.54 ± 2.35 ng/m³, mean ± SD) than in summer (1.67 ± 1.92 ng/m³, mean ± SD; P < 0.001), but no such seasonal variability was observed in 1-OHP excretion. Tobacco smoking was the major determinant of 1-OHP excretion. BPDE-Hb adducts were measured by gas chromatography-mass spectrometry as benzo(a)pyrene tetrals (BPT) released from adducted hemoglobin. In the 65 employees analyzed, mean BPT levels ± SD were higher in winter (0.14 ± 0.38 fmol/mg Hb) than summer (0.031 ± 0.022 fmol/mg Hb). This difference was not statistically significant, probably because of the small proportion of subjects with detectable adducts (11% in summer and 16% in winter). BPDE-Hb adducts were not significantly associated with sex, age, diet, smoking habits, or with pyrene levels and 1-OHP excretion. This is the first report providing reference BPDE-Hb adduct values for the general population not occupationally exposed to environmental PAHs and shows a tendency to seasonal variability, with higher BPT levels in winter when environmental PAHs are also high.

Introduction

Ambient air pollution contributes to the overall cancer risk, especially lung cancer, in urban areas. Although the proportion of human cancer attributable to air pollution is difficult to quantify, epidemiological studies indicate that urban residents may have a smoking-adjusted increase in lung cancer risk up to 1.5 times that of country dwellers (1, 2). Urban pollutant levels are mainly related to the intensity of traffic, amount, and sources of residential heating, industry, and meteorological conditions, particularly temperature inversion, which slows down the dispersion of air pollutants (3).

One of the major carcinogenic agents in polluted atmosphere is the class of PAHs, which have been associated with an increased risk of respiratory and neoplastic diseases (4). To understand better the health hazards connected with PAH air pollution, PAH biological markers have been monitored in relation to exposure.

High levels of PAH-related DNA and proteins adducts have been found to be related to ambient levels of PAHs, including BaP, a representative of PAHs (5–9). BaP is metabolically activated to BPDE, the ultimate carcinogenic metabolite known to bind covalently to DNA and blood proteins in humans (10–12).

Assessment of PAH-Hb adducts has been proposed as an alternative marker of exposure to PAHs, mainly because these adducts reflect exposure and metabolic activation over the 4-month life span of erythrocytes (12). We showed recently that Hb adducts of BPDE (BPDE-Hb) can be related to traffic exhaust exposure among newspaper vendors in the city of Milan, Italy (13).

To assess the value of BPDE-Hb adducts as biological markers for low levels of PAH exposure in the general urban population, we conducted a study among office employees living in Milan, investigating correlations between Hb adducts and PAH exposure variables. Three markers were analyzed: (a) individual exposure to pyrene, as an indicator of PAH exposure; (b) urinary 1-OHP excretion, representing individual metabolism of pyrene; and (c) BPDE-Hb adduct levels as a measure of the biologically active dose of BaP.

Because seasonal changes in PAH-related DNA adducts have been reported (7, 14–16), we collected samples twice, in

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3 The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; BaP, benzo(a)pyrene; BPDE, (+) 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene; Hb, hemoglobin; 1-OHP, 1-hydroxypyrene; PEM, personal exposure monitoring; BPT, benzo(a)pyrene tetrox.
summer 1995 and in winter 1996, to investigate whether Hb adducts have a seasonal pattern.

This study is part of an ongoing larger coordinated project involving Research Institutes and Universities in the Lombardia Region, Northern Italy, aimed at evaluating exposure of the general population to air pollution in the city of Milan and identifying specific measures to prevent harmful effects.

Materials and Methods

Subjects and Sampling. Sixty-five people (33 females and 32 males) were enrolled in this study. Eligible individuals were clerks, ages 18–60 years and living in the urban area of Milan. They were employees of the Milan Municipality or the Occupational Medicine Institute, both located in central Milan. Eighteen were smokers, and 47 were nonsmokers.

A standardized questionnaire was administered to each subject to record age, current smoking habits, passive smoking exposure, selected medical treatments, and dietary factors, including grilled or smoked food. Each person, assisted by a trained interviewer, completed a time-activity diary and a home-office commuting environment questionnaire to obtain information about activities and microenvironments during the monitoring period. Personal exposure to respirable particulate matter was monitored for 24 h, and measurements were made during the working day. Informed consent was obtained, and a blood sample and spot urine sample were collected when the environmental monitoring was completed.

Of the 65 subjects, 40 were sampled in both summer and winter (June-July 1995 and February-March 1996), 15 were sampled only in summer, then withdrew from the second sampling. Thus, 10 new employees were sampled only in winter.

Demographic characteristics of the study population are summarized in Table 1. Recruitment, air, urine, and blood sampling were done at the Department of Occupational and Environmental Health, University of Milan.

PEM. Environmental samplings and air sample analyses were done as reported in Minoia et al. (17). Individual exposure was monitored using personal samplers (Moc. EGO or 2 L/E Zambelli Srl) equipped with glass wool filters (Ø, 20 mm; Micro Filtration System, Dublin, CA) and ORBO-43 PAH absorption cartridges (Supelco, Bellefonte, PA). The sampler operated continuously for a 24-h period (from 9:00 a.m. to 9:00 a.m. the next day) and was fitted on the individuals near the breathing zone and kept near their bed overnight. Sampling flow was calibrated at 1.2 l/min (1728 l/day).

Glass wool filters and PAH absorption cartridges were extracted three times with methylene chloride in an ultrasonic bath for 5 min. Extracted pyrene was then quantified using high-pressure liquid chromatography coupled with a fluorescence detector (17). The detection limit was ≤0.05 ng/m³.

Urinary 1-OHP. Urine samples were analyzed at the “S. Maugeri” Foundation, Laboratory of Environmental Hygiene and Industrial Toxicology, Pavia, according to the method proposed by Jongeneelen et al. (18). Ten ml of urine underwent enzymatic hydrolysis and 1-OHP was separated on LC-DIOL solid phase extraction columns (Supelco). Elution was with methanol. The organic phase was concentrated under a gentle stream of nitrogen and analyzed by reverse-phase high-pressure liquid chromatography (19). The detection limit was ≤0.05 ng 1-OHP/ml urine.

BPDE-Hb Adducts. Adducts were analyzed at the “Mario Negri” Institute for Pharmacological Research, Department of Environmental Health Sciences, Milan. BPDE adducts were analyzed as BPTs released from Hb after acid hydrolysis and quantitated by high-resolution gas chromatography-negative ion chemical ionization-mass spectrometry with selected ion recording after Extrelut extraction and immunoaffinity purification, as described previously (13). The detection limit was ≤0.05 fmol BPT/mg Hb.

Statistical Analysis. Correlations were checked by the Spearman rank-order correlation test. To compute mean adduct levels, persons with unmeasurable levels were considered as having half the minimum detectable value. The Mann-Whitney two-tailed U test was used to compare summer and winter samples. A difference was considered significant at P < 0.05.

Results

Personal Exposure to Pyrene. Fig. 1 shows the seasonal personal pyrene levels in all employees. All samples had detectable levels of pyrene. The winter average was 4.54 ± 2.35 ng/m³ (mean ± SD) and the summer average 1.67 ± 1.92 ng/m³ (mean ± SD). The difference was significant (P < 0.001, Mann-Whitney two-tailed U test).

Average pyrene levels detected in all individuals according to their smoking habits and then dichotomized for seasonal sampling are reported in Table 2. Overall, the average level of
Table 2  Levels of pyrene (ng/m³) detected by 24-h personal monitoring in office employees, grouped according to their smoking habits and then dichotomized for seasonal sampling.

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Summer (n = 55)</th>
<th>Winter (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsmokers</td>
<td>2.97 ± 2.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.67 ± 1.64</td>
<td>4.18 ± 2.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Median, 2.6</td>
<td>(n = 37; median, 1.02)</td>
<td>(n = 35; median, 4.1)</td>
</tr>
<tr>
<td>All Matched&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.70 ± 1.36</td>
<td>4.97 ± 2.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 25; median, 1.08)</td>
<td>(n = 25; median, 4.55)</td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>3.4 ± 2.96</td>
<td>5.48 ± 2.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median, 3.6</td>
<td>(n = 15; median, 0.55)</td>
<td>(n = 15; median, 5.0)</td>
</tr>
<tr>
<td>All Matched&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.69 ± 2.58</td>
<td>5.48 ± 2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 15; median, 0.52)</td>
<td>(n = 15; median, 5.0)</td>
<td></td>
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</table>

<sup>a</sup> Mean ± SD.  
<sup>b</sup> Winter nonsmokers/summer nonsmokers: P < 0.001, Mann-Whitney two-tailed U test.  
<sup>c</sup> Forty subjects sampled both in summer and winter.

Table 3  Urinary levels of 1-hydroxypyrene (ng/ml urine) in office employees, grouped according to their smoking habits and then dichotomized for seasonal sampling.

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Summer (n = 55)</th>
<th>Winter (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsmokers</td>
<td>0.24 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21 ± 0.28</td>
<td>0.26 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Median, 0.17</td>
<td>(n = 37; median, 0.13)</td>
<td>(n = 35; median, 0.20)</td>
</tr>
<tr>
<td>All Matched&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.24 ± 0.35</td>
<td>0.24 ± 0.26</td>
<td></td>
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<tr>
<td></td>
<td>(n = 25; median, 0.12)</td>
<td>(n = 25; median, 0.16)</td>
<td></td>
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<tr>
<td>Smokers</td>
<td>0.54 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.51 ± 0.27</td>
<td>0.58 ± 0.38&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Median, 0.52</td>
<td>(n = 18; median, 0.58)</td>
<td>(n = 15; median, 0.46)</td>
</tr>
<tr>
<td>All Matched&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.48 ± 0.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.58 ± 0.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
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<tr>
<td></td>
<td>(n = 15; median, 0.47)</td>
<td>(n = 15; median, 0.46)</td>
<td></td>
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</table>

<sup>a</sup> Mean ± SD.  
<sup>b</sup> Winter nonsmokers/summer nonsmokers: P < 0.013, Mann-Whitney two-tailed U test.  
<sup>c</sup> Forty subjects sampled both in summer and winter.  
<sup>d</sup> Overall smokers/nonsmokers: P < 0.001, Mann-Whitney two-tailed U test.  
<sup>e</sup> Winter smokers/nonsmokers: P < 0.001, Mann-Whitney two-tailed U test.  
<sup>f</sup> Winter smokers/nonsmokers: P < 0.001, Mann-Whitney two-tailed U test.

Pyrene was not different between smokers and nonsmokers. In winter samples, pyrene levels were 1.3 times significantly higher in smokers than nonsmokers. This difference did not reach statistical significance, when the subset of subjects sampled both in summer and winter were compared. No difference was observed in summer samples between smokers and nonsmokers. Winter levels of pyrene were 2.5–3 times higher than summer samples, both in smokers and nonsmokers.

**Urinary 1-OHP.** Fig. 2 compares the seasonal pattern of 1-OHP concentrations in all winter and summer urines. All samples had detectable levels of 1-OHP. No significant difference was found in samples collected in summer and winter. No correlation was found between the 1-OHP excretion pattern and pyrene levels.

Smokers had ~2 times higher levels of urinary 1-OHP than nonsmokers (Table 3). This difference was seen in both summer and winter samples, whether all subjects or the subset analyzed in both seasons were compared. In addition, there was a positive correlation between the number of cigarettes smoked per day and 1-OHP levels. This relationship was similar in summer and winter (r = 0.6, P < 0.001; r = 0.5, P < 0.001, respectively; Spearman correlation test).

Sampling time did not influence 1-OHP excretion among smokers, but among nonsmokers, winter levels of urinary 1-OHP were significantly higher than summer ones (Table 3).

**BPDE-Hb Adducts.** Levels of BPDE-Hb adducts (fmol/mg Hb) in all employees are shown in Table 4. Overall, Hb adducts were 4.5 times higher in winter than summer, but the difference did not reach statistical significance. In most samples, BPDE-Hb adducts were below the detection limit of the method (5 ± 0.05 fmol/mg Hb), and only 6 of 55 (11%) summer samples, and 8 of 50 (16%) winter samples had detectable adducts. Restricting the analysis to those individuals with measurable adducts (8 of 50 in winter and 6 of 55 in summer), mean values were 11 times significantly higher in winter (0.945 ± 0.72 fmol BPT/mg Hb, mean ± SD) than summer (0.083 ± 0.04 fmol BPT/mg Hb, mean ± SD; P = < 0.001, Mann-Whitney two-tailed U test).

In the 40 people sampled both in summer and winter, 30 subjects had adducts below the limit of detection of the method. Although the difference between seasons was not significant, mean values were four times higher in winter than summer. Again, when individuals with detectable adducts either in winter (5 of 40) or in summer (5 of 40) were considered, mean adduct level was 10-fold enhanced in winter (0.86 ± 0.79 fmol BPT/mg Hb, mean ± SD) compared with summer (0.083 ± 0.04; P = 0.02, Mann-Whitney two-tailed U test).

BPDE-Hb adduct levels were not associated with sex, age, smoking habits, frequency of consumption of selected food items, or with personal pyrene exposure monitored for 24 h. The concentrations of BPDE-Hb adducts and urinary 1-OHP did not show any correlation.

**Discussion**

Very few studies have monitored BPDE-Hb adducts in people exposed to low levels of PAH, such as those experienced by most people living in Western European and North American...
cities. We reported recently that BPDE-Hb adducts could be detected in newspaper vendors in Milan, Italy, and differences in adduct concentrations might be related to traffic exhaust exposure (13).

In this study, we investigated whether BPDE-Hb adducts were a useful marker of PAH exposure in office employees, as a prototype of a general population with no occupational exposure to PAH. Individual exposure to PAH was measured as levels of pyrene, which is considered an appropriate indicator of the air PAH mixture (20). Twenty-four-h PEM data showed a significant 3-fold increase in pyrene levels in winter compared with summer, consistent with the winter-related increase in PAH air pollution reported by many authors (3, 21). The average PEM pyrene level detected in winter (4.54 ± 2.35 ng/m², mean ± SD) is at least three times lower than that reported by Øvrebø et al. (21) for populations living in a highly polluted industrialized area and is essentially similar to ambient air pyrene concentrations measured recently in Italian cities, such as Milan (22), Genoa (23), and Rome (24).

Pyrene levels were 2.5–3 times higher in winter than in summer, both in smokers and nonsmokers. Interestingly, in overall winter samples from smokers, pyrene levels were significantly 31% higher than from nonsmokers. No such difference was seen in overall summer samples. A possible explanation could be the way low ambient temperatures apparently influence the distribution and concentration of PAH on particulate matter (24). Thus, the interaction between smoking habits and winter temperatures might enrich the PAH content on air respirable particles among smokers. Furthermore, tobacco smoke-derived pyrene is more likely to be a major contributor to indoor pyrene pollution in winter than in summer, when rooms are ventilated better.

Pyrene is almost exclusively metabolized to 1-OHP, which accounts for ~90% of the total urinary excretion of pyrene (25). High exposure to pyrene in workplaces and in the atmosphere of highly industrialized regions (18, 21, 26) has been associated with increased concentrations of 1-OHP in the urine. Our study found no correlation between pyrene PEM data and 1-OHP excretion. Winter levels of pyrene among these employees are at least 1000 times lower than reported for occupationally exposed groups (18, 26). At low environmental levels, other well-known pyrene sources, such as cigarette smoke and diet, may be major contributors to 1-OHP excretion (27). Indeed, we found mean concentrations of 1-OHP in urine of smokers were double those of nonsmokers. The mean urinary concentrations of 1-OHP adjusted for creatinine among nonsmoking (0.1 μmol/mol creatinine) and smoking (0.23 μmol/mol creatinine) office workers were comparable with 1-OHP concentrations of nonsmoking and smoking controls reported in several studies (18, 28).

The dose relationship between the number of cigarettes smoked per day and 1-OHP urinary levels reported previously by us (19) and other investigators (21, 23, 27) was confirmed in our study, where smokers were moderate (≤15 cigarettes/day).

We observed a significant seasonal difference in 1-OHP excretion only among nonsmokers, with higher excretion in winter than in summer. This might reflect the reported winter seasonal peak in airborne pyrene that is not overshadowed by inhalation of tobacco smoke-derived pyrene. However, the lack of seasonal difference among smokers might also be the result of the small group.

Neither pyrene levels nor 1-OHP excretion were correlated with BPDE-Hb adducts. This might reflect differences in the three markers, Hb adducts being an internal dose marker of cumulative exposure, 1-OHP a metabolic marker of recent exposure, and pyrene PEM levels being indicators of the external dose.

Overall, Hb adducts were higher in winter than in summer. To our knowledge, this is the first evidence that adducts other than PAH-DNA adducts might have a seasonal pattern. Data concerning seasonal variations in PAH-related biomarkers refer mainly to DNA adducts and are conflicting. High PAH-DNA adduct levels during summer, when aryl hydrocarbon hydroxylase inducibility is greatest, have been reported in lung cancer patients, but not in controls (7), and in police officers in Genoa, but not in their referents (16).

On the other hand, DNA adduct levels were higher in winter than summer in people from highly polluted cities, in accordance with the winter-related peak of air pollution (14, 15). This difference might be related to high environmental PAH levels that easily mask seasonal patterns of aryl hydrocarbon hydroxylase activity. Inducibility of enzyme systems after exposure to PAH has been reported (29, 30).

As regards the seasonal effect on BPDE-Hb adducts, our results are in apparent contrast with previous ones (7, 14–16), because our subjects are exposed to very low levels of PAH but have higher levels of BPDE-Hb adducts in winter than summer. This might depend on differences in the specificity of adduct detection. Using gas chromatography–mass spectrometry, we were able to measure BPTs as the exact chemical species responsible for Hb adduction, whereas methods like the 32P-
post labeling assay used by other authors lack chemical specificity, measuring several types of PAH adducts.

We are therefore led to suggest that airborne PAH concentrations in winter in Milan might be high enough to enhance metabolic activation of BaP, increasing its metabolic conversion to the reactive BPDE. Thus, more Hb adducts might be formed in winter, even allowing for genetic differences in induction and ability to activate BaP (31). It is also reasonable to assume that the enhanced BPDE-Hb adduct levels observed in winter might simply be due to the fact that people spend most of their time indoors, and opening windows less frequently limited the ventilation of air-polluted rooms.

The summer BPDE-Hb adduct measurements from the present study were compared with our previous data on newspaper vendors, also sampled in summer (13). The average amount of BPDE-Hb adducts in clerks was from 10 to 20 times lower than for newspaper vendors (Fig. 3). This comes as no surprise, because newspaper vendors in Italy have a long daily outdoor exposure to air pollution, being in the stand at least 10–12 h, often located at crossroads in streets with heavy traffic. Short daily transport time appears to be the main source of outdoor exposure among the clerks in this study. Therefore, measurements of BPDE-Hb adducts differentiate between different exposure levels in the urban population.

Hb adducts were below the sensitivity of the method in most samples, but there was a tendency to seasonal variation, with higher BPDE-Hb levels in winter, when environmental PAH are also high.

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References

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