Urinary Concentrations of five Hydroxy-PAHs in 598 Subjects Living in Central Italy Determined by HPLC/MS/MS

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Abstract
In this study we performed the simultaneous quantitation of 1-OHPy, 1-OHNAP, 2-OHNAP, 3-OHBaPy and 6-OHNPy in human urine by means of HPLC-MS/MS, and of cotinine (metabolite of nicotine) on the same samples. Urine samples were from 445 non-smoking and 153 smoking subjects living in the same area of Central Italy. These can give important information about the exposure levels of the general population, on the different exposure sources and on the influence of smoking on these biomarkers.

Introduction
Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic pollutants both of outdoor and indoor air: outdoor sources of PAHs include traffic emissions and power sources such as oil, and biomass, whereas indoor sources include cooking, residential heating. Tobacco smoke is also a significant source of exposure to PAHs.

In this study, as a part of the “ABC Human Biomonitoring study”, the simultaneous analytical quantitation of five urinary monohydroxylated metabolites of four PAHs is reported, selected on the basis of their source and significance in 598 residents in an industrial area of Central Italy: 1-hydroxypyrene (1-OHPy), the metabolite of pyrene, is the biomarker proposed by ACGIH for the occupational exposure to PAHs mixtures; 1-hydroxynaphthalene (1-OHNAP) and 2-hydroxynaphthalene (2-OHNAP), the metabolites of naphthalene, are biomarkers mostly used to assess the exposure of the general population to airborne PAHs; 3-hydroxybenzo[a]pyrene (3-OHBaPy), is the metabolite of benzo[a]pyrene, classified as class 1 “human carcinogens” by IARC; 6-hydroxynitropyrene (6-OHNPy) metabolite of 1-Nitropyrene, is used as a molecular marker for diesel exhaust. The cotinine, urinary metabolite of nicotine was also measured in these samples in order to classify the subjects as smokers or not: the cut off value for the definition of smokers is set at urinary cotinine > 100 µg/g of creatinine [1]. All concentrations of metabolites were expressed in µg/g of creatinine as normalization factor.

Materials & Methods
The studied subjects were aged 35-69 years, 153 (26%) are smokers, 445 (74%) non-smokers. The analyses were performed using a validated and published isotopic dilution HPLC/MS/MS method [2]. Limits of detection were 24 ng/l for 1-hydroxypyrene, 24 and 120 ng/l for 1 and 2-hydroxynaphthalene, 1.3 ng/l for the 6-hydroxynitropyrene and 48 ng/l for 3-hydroxybenzo[a]pyrene in the 70%; accuracy was higher than 90% and variability lower than 19%.

Urine samples were spiked with the deuterium labelled internal standards, adjusted to pH 5.0, mixed with ammonium acetate buffer and incubated with 25 µL of β-Glucuronidase/aryl sulfatase at 38°C for 16 h in order to deconjugate the metabolites from glucuronic acid. All samples were purified on SPE. Separation was performed on reverse phase C8 column at 40°C using tandem mass spectrometric detection in the negative ion, ESI-MRM mode. For each analyte, the area of the peak of a blank urine sample was subtracted from the areas of the corresponding urine calibration standards.
Each sample was tested in duplicate and the arithmetic mean value of the peak areas of two replicate injections is used.

**Results**

The mean results obtained in the studied population, expressed in µg/g of creatinine, are the following:

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Mean Concentration (µg/g)</th>
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<tbody>
<tr>
<td>1-OHPy</td>
<td>70.37</td>
</tr>
<tr>
<td>6-OHNPy</td>
<td>58.75</td>
</tr>
<tr>
<td>3-OHBAPy</td>
<td>9.13</td>
</tr>
<tr>
<td>1-OHNAP</td>
<td>1626.55</td>
</tr>
<tr>
<td>2-OHNAP</td>
<td>4801.53</td>
</tr>
</tbody>
</table>

Data stratified both by gender (M for males and F for females) and smoking habit are graphically reported in Figure 1 and Figure 2. Metabolites have been separated in two groups on the basis of the different abundance.

Results indicate that 1 and 2 naphthol and 1OH-pyrene are significantly higher in smokers (t test, p<0.001); in fact there is also a significant correlation with the urinary cotinine concentration of the same samples (r >0.50). On the other side 3-OHBAP and 6-OHNPy do not correlate with the cotinine. Besides 6-OHNPy is significantly higher in males than in females (t test, p<0.001).

**Conclusions**

The result can give important information about the exposure levels of the general population and on the different exposure sources, and also provides reference values for targeted occupational studies. As smoking is a typical confounding factor for environmental and occupational exposure assessment to PAHs, its influence on the levels of the analytes was investigated. The fact that 6-OHNPy and 3-OHBAPy are not affected by the smoking status renders these biomarkers particularly suitable for studying exposure to environmental pollution. Besides we found a significant difference in the exposure to diesel exhaust between males and females, whose causes have to be further investigated. A further elaboration of these data using Chemometrics will improve the interpretation of results.

**References**
