CYP1A1 and GSTM1 polymorphisms affect urinary 1-hydroxypyrene levels after PAH exposure

Anna-Karin Alexandrie1,2, Margareta Warholm1,2, Ulrika Carstensen3, Anna Axmon4, Lars Hagmar4, Jan Olof Levin5, Conny Östman2,6 and Agneta Rannug1,2,7

1Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
2National Institute for Working Life, Stockholm, Sweden
3Department of Occupational and Environmental Medicine, University Hospital, Umeå, Sweden
4Department of Occupational and Environmental Medicine, University Hospital, Lund, Sweden
5National Institute for Working Life, Department of Chemistry, Umeå and 6Department of Analytical Chemistry, Stockholm University, Stockholm, Sweden
7To whom correspondence should be addressed at: Institute of Environmental Medicine, Karolinska Institutet, S-171 77 Stockholm, Sweden
Email: agneta.rannug@imm.ki.se

Certain human biotransformation enzymes have been implicated in the formation and scavenging of the ultimate reactive metabolites, the diolepoxides, from polycyclic aromatic hydrocarbons (PAHs). In the present study, performed on aluminum smelter workers, we have analyzed airborne PAH, the pyrene metabolite 1-hydroxypyrene (1-OHP) in urine, and genotypes for biotransformation enzymes involved in PAH metabolism. The aim was to evaluate the correlation between external exposure and biomarkers of exposure and to investigate to what extent genetic polymorphism in metabolic enzymes can explain interindividual variation in urinary 1-OHP levels. DNA was prepared from blood samples from 98 potroom workers and 55 controls and altogether eight polymorphisms in the CYP1A1, mEH, GSTM1, GSTP1 and GSTT1 genes were analyzed. The 1-OHP excretion was found to correlate significantly (P ≤ 0.005) to the exposure. The interindividual difference in excretion of 1-OHP was vast (>100-fold) and univariate and multivariate regression analyses were used to find the variables that could determine differences in excretion. The variation could, to some degree, be explained by differences in exposure to airborne particulate-associated PAHs, the use of personal respiratory protection devices, smoking habits and genetic polymorphisms in the cytochrome P450 1A1, GSTM1 and GSTT1 enzymes. The part of the variance that could be explained by differences in biotransformation genotypes seemed to be of the same order of magnitude as the variance explained by differences in exposure. In the control group as well as in the occupationally exposed group, the highest 1-OHP levels were observed in individuals carrying the CYP1A1 Ile/Val genotype who were also of the GSTM1 null genotype. The results show that urinary 1-OHP is a sensitive indicator of recent human exposure to PAHs and that it may also to some extent reflect the interindividual variation in susceptibility to PAHs.

Introduction

Better exposure measures are needed for improving the quantitative risk assessment of polycyclic aromatic hydrocarbons (PAHs). The present study is part of a comprehensive evaluation of biological markers for PAH exposure. The study was performed in a modern Swedish aluminum production plant. During the electrolytic process of aluminum smelting, coal tar pitch volatiles are emitted into the work environment and workers are heavily exposed to PAHs via inhalation and skin absorption. In order to find relevant indicators for human exposure and possible genotoxic effects several biomarkers were applied but in this study only 1-hydroxypyrene (1-OHP) excretion in urine turned out to be a sensitive biomarker for PAH exposure (1,2). The 1-OHP excretion was found to significantly correlate to the exposure, although a wide interindividual variation was observed.

In humans a substantial variability in biologic response to PAHs is to be expected because of interindividual differences in the activity of the enzyme systems involved in the formation of reactive intermediates and their detoxification through conjugation and excretion. Enzyme polymorphisms have been suggested to explain interindividual differences in the rate of activation of PAH-derived carcinogens. The general mechanism of metabolic activation of PAHs, such as benzo[a]pyrene (B[a]P), is via the formation of bay-region dihydrodiol epoxides e.g. benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE), via cytochrome P450 (CYP) and epoxide hydrolase. Metabolic activation of PAH in human lung has mainly been ascribed to CYP1A1, an inducible extrahepatic enzyme with aryl hydrocarbon hydroxylase (AHH) activity. In addition to CYP1A1, other CYP isozymes, such as 1A2, 1B1 and 3A4 may also participate in the metabolic activation of PAHs (3–5). Further metabolism results in deactivation of PAH derives through glutathione conjugation by glutathione S-transferases (GSTs). GSTP1, the most abundant GST in lung, and GSTM1, have the ability to detoxify epoxides such as BPDE, whereas GSTT1 preferentially conjugates smaller compounds. Other detoxifying enzymes are UDP-glucuronosyl transferase and sulfotransferase, which conjugate PAH metabolites that possess hydroxyl groups.

Increased risk of lung cancer in smokers has been associated with high AHH inducibility and a positive correlation between AHH activity, and the level of BPDE adducts has been observed in lung (6–8). Among several polymorphisms identified in the CYP1A1 gene two closely linked mutations have been extensively studied in relation to AHH inducibility and cancer risk, one that results in a new restriction site (MspI) in the 3′-untranslated region of the gene and another located in exon 7 which results in an amino acid exchange (Ile462Val). Conflicting results regarding the importance of CYP1A1 poly-

Abbreviations: AHH, aryl hydrocarbon hydroxylase; B[a]P, benzo[a]pyrene; anti-BPDE, anti-benzo[a]pyrene-trans-7,8-dihydrodiol-9,10-epoxide; CYP, cytochrome P450; GST, glutathione transferase; mEH, microsomal epoxide hydrolase; PAH, polycyclic aromatic hydrocarbon; 1-OHP, 1-hydroxypyrene.
morism for susceptibility to lung cancer have been presented from studies carried out in Asian populations and in European and North American populations (9,10). There is also a growing number of studies that link the mutations in the CYP1A1 gene to increased susceptibility to DNA damage. The alleles carrying the MspI mutation or the Ile→Val mutation, the CYP1A1*2A or CYP1A1*2B alleles, were more commonly found in PAH-exposed individuals with high levels of DNA adducts derived from anti-BPDE (11). These alleles were also associated with increased occurrence of p53 mutations in tumor tissue (12) and have, in some studies but not all, been associated with increases in the catalytic activity of the enzyme and with higher AHH inducibility (13).

A number of polymorphisms within the microsomal epoxide hydrolase (mEH) gene have been identified but only two have been found to alter the enzymatic activity (14). In vitro experiments suggest that the Tyr113→His exchange in exon 3 is associated with decreased mEH activity, whereas the substitution of His139 with Arg in exon 4 is associated with increased mEH activity. Whether these mEH polymorphisms contribute to interindividual differences in susceptibility to carcinogens, such as PAHs, has not yet been clearly established.

The most studied human GST, GSTM1, is lacking in ~50% of all individuals in Caucasian populations. Absence of the enzyme is caused by a deletion of the GSTM1 gene (15). From a combined analysis of 12 case-control studies of GSTM1 status and lung cancer it was concluded that GSTM1 deficiency is a moderate risk factor for lung cancer development with an odds ratio of 1.41 (95% CI = 1.23–1.61; P < 0.0001) (16). The GSTT1 polymorphism is also caused by a deletion that results in total lack of gene product. In a Swedish population ~11% were found to lack GSTT1 activity (17,18), whereas slightly higher frequencies have been reported in other Caucasian populations (19). The GSTP1 gene was recently found to be polymorphic in amino acid positions 104 and 113 (20). Residue 104 lies in close proximity to the active site and it has been shown that the Val variant has altered specific activity and affinity for electrophilic substrates (21,22). With respect to lung cancer in humans and exposure to PAH-containing material, the results from studies on GSTP1 and GSTT1 polymorphisms are too limited and variable to allow any definite conclusion.

As a biomarker of human PAH exposure, 1-OHP has gained a strong position (23–25). Pyrene is one of the most abundant PAHs in coal tar, and the highly fluorescent pyrene metabolite 1-OHP can be quantified in urine by HPLC. Levels of particulate-associated pyrene correlated well with levels of total PAH in breathing zone air. Urinary 1-OHP gives a more accurate assessment of total PAH exposure from all exposure sources, including dermal absorption, than PAH levels in air (26). CYP1A1 and other PAH-metabolizing CYPs metabolize pyrene to 1-OHP, which is excreted in the urine as the corresponding glucuronide. It has been estimated that ~30% of retained pyrene after PAH inhalation is excreted in urine in the form of 1-OHP (27).

The aim of the present investigation was to evaluate the influence of biotransformation polymorphisms on the excretion of 1-OHP in urine in PAH exposed workers and in unexposed controls. Other factors that may influence the excretion of 1-OHP such as age, tobacco smoking and use of protection devices were also analyzed in detail.
The amount of pyrene found in particulate-associated form accounted for 42% of the total amount of pyrene collected. The concentration of B[a]P was within the same range as pyrene with a median of 0.97 µg/m³ (range 0.02–23.5). Exposure data for total particulate PAHs and pyrene is missing from four exposed and two control subjects. Data for B[a]P is missing from two more subjects, one exposed and one control. Exposure data for the gaseous phase is missing from three exposed subjects (Table II).

The Spearman correlation coefficients for pyrene and total PAH concentration in air were 0.79 (P < 0.001) and 0.93 (P < 0.001) for gaseous and particulate-associated phases, respectively (Table III). A strong positive correlation (r = 0.87, P < 0.001) was also found between pyrene and B[a]P. An extensive presentation of the work environment analyses is given elsewhere (2).

Table II. Concentration of PAH congeners (µg/m³) in air and 1-OHP (µmol/mol creatinine) in urine of potroom workers and control subjects

<table>
<thead>
<tr>
<th>Polymorphic site</th>
<th>Forward primers</th>
<th>Reverse primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1, MspI at T6235C</td>
<td>5'-CAGTGAAAGGAGTGTAGCGGCT</td>
<td>5'-TGGAGGCTTGTCTCCTGTC</td>
</tr>
<tr>
<td>CYP1A1, exon 7 Ile462Val</td>
<td>5'-GAAGTCCCTACCTGACGCT</td>
<td>5'-AAAGCTTCCCCGCGGCAAAT</td>
</tr>
<tr>
<td>mEH, exon 3 Tyr131His</td>
<td>5'-GGFTGTGTCTGTGTTTCTG</td>
<td>5'-AGTCCTAAGGTTAGGTTTTG</td>
</tr>
<tr>
<td>mEH, exon 4 His139Arg</td>
<td>5'-CAGAGCCGTACAGCCGTAC</td>
<td>5'-GTGGATGTTACGAGATCATGC</td>
</tr>
<tr>
<td>GSTM1 deletion</td>
<td>5'-CTCCTTCTACTTCTCCCATCC</td>
<td>5'-CCTACTTCTACTTCTCCCATCCGCC</td>
</tr>
<tr>
<td>GSTP1, exon 5 Ile104Val</td>
<td>5'-AGTCTCTCATCCCTCCCA</td>
<td>5'-CACATTGCTATCCTTTTCGCCG</td>
</tr>
<tr>
<td>GSTP1, exon 6 Ala113Val</td>
<td>5'-AGTCTCTCATCCCTCCCA</td>
<td>5'-CAGTGAAGAGGTGTAGCCGCT</td>
</tr>
<tr>
<td>GSTT1 deletion</td>
<td>5'-TTTCTTACTAGTGTCCTACATCT</td>
<td>5'-TCACCGGATCATGCGCCAGCA</td>
</tr>
<tr>
<td>β-Actin</td>
<td>5'-TGACGGGTTACCCCACTGTCGCCATCTA</td>
<td>5'-CTAGAGCATTGCGGACGATGAGGG</td>
</tr>
</tbody>
</table>

Table I. PCR primer pairs used in this study

### Results

#### Air monitoring

The time-weighted average exposures to pyrene, B[a]P and total PAH in potroom workers and control subjects are presented in Table II. The concentration of total particulate-associated PAHs among the potroom workers ranged from 1.42 to 270 µg/m³ (median 15.8) as compared with 0.01–0.36 (median 0.11) for control subjects. Corresponding figures for total PAHs in the gaseous phase were 0.13–131 µg/m³ (median 16.3) in workers and <0.01–0.41 (median 0.20) in control subjects. Irrespective of gaseous or particulate-associated form, the median concentrations of each PAH and total PAH were significantly higher in potroom samples than in control samples. The median concentration of pyrene in potroom samples was 1.56 µg/m³ (range 0.01–9.52) in the gaseous phase and 11 (range 0.07–34.4) in the particulate-associated phase. Thus, the amount of pyrene found in particulate-associated form accounted for 42% of the total amount of pyrene collected. The concentration of B[a]P was within the same range as pyrene with a median of 0.97 µg/m³ (range 0.02–23.5). Exposure data for total particulate PAHs and pyrene is missing from four exposed and two control subjects. Data for B[a]P is missing from two more subjects, one exposed and one control. Exposure data for the gaseous phase is missing from three exposed subjects (Table II).

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#### 1-OHP in urine

The median concentration of urinary 1-OHP sampled before work was 3.43 µmol/mol creatinine (range 0.07–26.6) in potroom workers and 0.11 (range 0.02–0.99, P < 0.001) in the controls (Table II). In the potroom workers, the level of 1-OHP was increased to a median of 4.31 µmol/mol creatinine (range 0.09–17.7) immediately after work. The interindividual change in excretion over the workday was, however, substantial. The majority of the workers belonged to the afternoon shift (14:00–22:00). The morning urinary 1-OHP level seemed to be influenced to a large extent by exposure during the
previous evening shift. Thus, all further statistical analyses in potroom workers are based on measurements of urinary 1-OHP sampled after work. For controls, the statistical analyses on 1-OHP levels derived from urine sampled before work (n = 54) due to the low number of after-work samples collected (n = 5). The results from the analyses for 1-OHP in urine in samples collected before work are missing in four exposed subjects and in one control subject and, in the samples collected after work, data are missing for one exposed subject.

All individual PAHs, except for particulate-associated methylphenanthrenes and anthracene, were weakly but significantly correlated to urinary 1-OHP collected after work. Moderate but significant correlations were found between both gaseous (r = 0.25, P = 0.016) and particulate-associated (r = 0.34, P = 0.001) pyrene and 1-OHP (Table III). Similar correlations were found for total PAH in gaseous phase and particulate-associated form (r = 0.28, P < 0.005 and r = 0.37, P < 0.001, respectively).

Influence of smoking, use of protection devices and genotypes on 1-OHP excretion

In the control subjects there was a significant difference in the median concentration of urinary 1-OHP in samples taken before work between smokers (0.17 µmol/mol creatinine, range 0.09–0.99) and non-smokers (0.10, range 0.02–0.50, P < 0.001). There was no significant difference in the median 1-OHP levels between smokers and non-smokers among the exposed (P > 0.5).

A lower median concentration of after-work urinary 1-OHP was observed among potroom workers who used respiratory protection devices (filter or airstream) at least 90% of the time (3.16 µmol/mol creatinine, range 0.09–7.84) compared with workers who used the protection equipment less frequently (1–89% of time, 4.71, range 0.12–10.9) or not at all (4.40, range 0.09–17.7). The effect of respiratory protection on 1-OHP, stratified by PAH exposure (particular phase), is shown in Table IV. At lower than median PAH exposures the most pronounced protection was observed.

The impact of genetic polymorphisms on the concentration of urinary 1-OHP is shown in Table V. Except for GSTT1, where subjects lacking this gene had lower 1-OHP levels (3.60 versus 4.47 µmol/mol creatinine), no statistically significant genotype-related differences in 1-OHP levels in potroom workers were found when the exposure levels were not taken into consideration. However, a non-significant elevation in the level of 1-OHP was seen in the subjects carrying the CYP1A1 (exon 7) Val allele. The median concentration of 1-OHP in urine was 7.47 µmol/mol creatinine (range 2.36–17.7) in the seven subjects with the Ile/Val genotype compared with 4.28 (range 0.99–13.9) in subjects with the Ile/Ile genotype. Also, the control subjects, the influence of the CYP1A1 Val allele was observed. Subjects carrying the CYP1A1 Ile/Val genotype had significantly increased 1-OHP levels in urine. The median concentration in the seven subjects with the Ile/Val genotype was 0.17 µmol/mol creatinine (range 0.08–0.50), and in those with the Ile/Ile genotype it was 0.10 (range 0.02–0.99), P = 0.04. In the control group, GSTT1 did not influence the 1-OHP levels.

In the potroom workers, when divided into a low exposure group (less than or equal to the median total particulate PAH) and a high exposure group (greater than median total particulate PAH), some significant differences in 1-OHP excretion between different CYP1A1 genotypes were detected (data not shown). In the low exposure group, 1-OHP levels were significantly higher in the eight subjects with CYP1A1 (MspI) T/C or C/C genotypes (median 4.60 µmol/mol creatinine, range 3.44–9.09) compared with 39 subjects with the C/C genotype (median 3.55, range 0.99–8.70, P = 0.02). The three subjects in the high exposure group with the rare CYP1A1 Ile/Val genotype had significantly higher 1-OHP levels (median 10.5 µmol/mol creatinine, range 9.77–17.7) than those with the common Ile/Ile genotype (n = 43, median 5.08, range 0.12–13.9, P = 0.02). An increase in the 1-OHP level was found also for the two subjects with the Ile/Val genotype in the low exposure group compared with the carriers of the Ile/Ile genotype.

Univariate regression analyses with urinary 1-OHP sampled after work as the dependent variable, and smoking, age, airborne PAH exposure, usage of respiratory protection devices and metabolic genotypes for CYP1A1, mEH, GSTM1, GSTTI and GSTP1 as the determining variables, were performed. These analyses showed that the total particulate-associated PAH level, use of protection devices and genotype for the CYP1A1 (exon 7) gene significantly influenced the concentration of urinary 1-OHP (P < 0.05 for all). In addition, the current number of cigarettes smoked per day and genotypes for the GSTM1 and GSTTI genes also had a possible influence on the level of 1-OHP in urine (P < 0.1 for all). The CYP1A1 Ile/Val genotype, the GSTM1 (−/−) genotype and the GSTTI (+/+ or +/−) genotypes were associated with increased

### Table III. Correlations between pyrene, B[α]P and total PAH in air and correlations between urinary 1-OHP and pyrene, B[α]P and total PAH exposure in potroom workers

<table>
<thead>
<tr>
<th></th>
<th>Pyrene</th>
<th>1-OHPa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>r_s</td>
</tr>
<tr>
<td>Airborne gaseous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrene</td>
<td>95</td>
<td>0.79</td>
</tr>
<tr>
<td>Total PAH</td>
<td>95</td>
<td>0.79</td>
</tr>
<tr>
<td>Airborne particulate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B[α]P</td>
<td>93</td>
<td>0.87</td>
</tr>
<tr>
<td>Pyrene</td>
<td>93</td>
<td>0.34</td>
</tr>
<tr>
<td>Total PAH</td>
<td>94</td>
<td>0.93</td>
</tr>
</tbody>
</table>

a Urinary 1-OHP sampled after work.

b The sum of seven gaseous phase PAH congeners during a full workday.

b B[α]P versus total PAH in particulate associated form, P = 0.005.

The sum of 22 particulate associated PAH congeners during a full workday.
levels of urinary 1-OHP. All the determining variables with a P-value < 0.1 in the univariate analysis were entered into a multivariate regression analysis. The effect of smoking did not remain significant and this variable was therefore removed from the model. The final multivariate analysis showed that total particulate-associated PAH exposure, use of respiratory protection devices and metabolic genotype for the CYP1A1 and GSTM1 genes significantly determined urinary 1-OHP excretion (Table VI). The GSTT1 genotype failed to contribute significantly to the prediction of urinary 1-OHP (P = 0.06).

The explained variance, r², of the model with the five remaining determinants was, however, only 0.28. In a corresponding univariate regression analysis for controls with urinary 1-OHP sampled after work, the independent variables smoking and number of cigarettes smoked per day were the only determinants that significantly influenced the 1-OHP level (P ≤ 0.002 for both).

The influence of combined genotypes for CYP1A1 (exon 7) and GSTM1, both significant predictors in the multivariate analysis, on the 1-OHP levels are presented in Figure 1a and b. Slight differences in the median concentration of 1-OHP were observed in control subjects with the combined CYP1A1 and GSTM1 genotypes; Ile/Ile, +/+ or +/− (0.10 µmol/mol creatinine, range 0.02–0.18); Ile/Ile, −/− (0.11 µmol/mol creatinine, range 0.05–0.99); Ile/Val, +/+ or −/− (0.15 µmol/mol creatinine, range 0.13–0.17) and Ile/Val, −/− (0.17 µmol/mol creatinine, range 0.08–0.50). The difference was statistically significant between subjects with the Ile/Ile +/+ or +/− genotypes and the Ile/Val −/− genotype, P = 0.04 (Figure 1a).

### Table IV. Concentration of 1-OHP (µmol/mol creatinine) in urine in different exposure groups with respect to use of respiratory protection devices

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>Low exposure group</th>
<th>High exposure group</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Use of respiratory protection devices</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never/sometimes</td>
<td></td>
<td>39</td>
<td>4.09</td>
<td>0.09–9.09</td>
</tr>
<tr>
<td>Always</td>
<td></td>
<td>8</td>
<td>2.46</td>
<td>0.09–4.34</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td></td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

**Table IV.** Concentration of 1-OHP (µmol/mol creatinine) in urine in different exposure groups with respect to use of respiratory protection devices.

- **P-value:** The predictor variables that determine the concentration of urinary 1-OHP, after work, in potroom workers are shown.
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**Table V.** Concentration of 1-OHP (µmol/mol creatinine) in urine with respect to metabolic genotypes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>1-OHP in potroom workers</th>
<th>P-value</th>
<th>1-OHP in controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>Median</td>
<td>Range</td>
<td></td>
</tr>
<tr>
<td>Use of protection devices</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTM1</td>
<td></td>
<td>47</td>
<td>4.31</td>
<td>0.09–17.7</td>
<td>0.18</td>
</tr>
<tr>
<td>T6253C</td>
<td></td>
<td>79</td>
<td>4.18</td>
<td>0.09–13.9</td>
<td>0.16</td>
</tr>
<tr>
<td>CYP1A1, exon 7</td>
<td></td>
<td>18</td>
<td>4.69</td>
<td>0.55–17.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Ile/Ile</td>
<td></td>
<td>90</td>
<td>4.28</td>
<td>0.09–13.9</td>
<td>0.18</td>
</tr>
<tr>
<td>Tyr/Val</td>
<td></td>
<td>51</td>
<td>4.07</td>
<td>0.23–17.7</td>
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<tr>
<td>Tyr/Ile/Ile</td>
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<td>46</td>
<td>4.44</td>
<td>0.12–17.7</td>
<td>0.38</td>
</tr>
<tr>
<td>His/Ile</td>
<td></td>
<td>60</td>
<td>4.21</td>
<td>0.12–13.2</td>
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<tr>
<td>His/Ile/Ile</td>
<td></td>
<td>37</td>
<td>4.82</td>
<td>0.09–17.7</td>
<td>0.18</td>
</tr>
<tr>
<td>GSTD1, exon 5</td>
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<td>45</td>
<td>4.22</td>
<td>0.09–10.6</td>
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<td>Ile/Ile</td>
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<td>52</td>
<td>4.51</td>
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<td>GSTD1, exon 6</td>
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<td>54</td>
<td>4.08</td>
<td>0.09–17.7</td>
<td>0.28</td>
</tr>
<tr>
<td>Ala/Ala/Ile/Ile</td>
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<td>85</td>
<td>4.22</td>
<td>0.09–17.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Ala/Val/Val/Val</td>
<td></td>
<td>12</td>
<td>5.11</td>
<td>2.92–13.2</td>
<td>0.05</td>
</tr>
<tr>
<td>GSTT1</td>
<td></td>
<td>16</td>
<td>3.60</td>
<td>0.47–10.9</td>
<td>0.05</td>
</tr>
</tbody>
</table>

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### Table VI. Multivariate regression analysis based on variables with P < 0.1 in the univariate regression analyses followed by a stepwise procedure

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>n</th>
<th>β</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PAH (p, In-transformed)</td>
<td>93</td>
<td>1.12</td>
<td>0.34</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Use of protection devices</td>
<td>97</td>
<td>−0.81</td>
<td>0.36</td>
<td>0.03</td>
</tr>
<tr>
<td>CYP1A1, exon 7 Ile462Val</td>
<td>97</td>
<td>2.53</td>
<td>1.11</td>
<td>0.03</td>
</tr>
<tr>
<td>GSTM1</td>
<td>97</td>
<td>1.18</td>
<td>0.57</td>
<td>0.04</td>
</tr>
<tr>
<td>GSTT1</td>
<td>97</td>
<td>−1.44</td>
<td>0.76</td>
<td>0.06</td>
</tr>
</tbody>
</table>

- **P-value:** The predictor variables that determine the concentration of urinary 1-OHP, after work, in potroom workers are shown.
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**Table VI.** Multivariate regression analysis based on variables with P < 0.1 in the univariate regression analyses followed by a stepwise procedure.
The observed differences were not explained by smoking as none of the subjects with the CYP1A1 Ile/Val genotype were smokers.

Aluminum smelter workers with the combined CYP1A1 Ile/Val and GSTM1 (−/−) risk genotypes had substantially higher urinary 1-OHP levels compared with all other genotype combinations (P < 0.05 for all, Figure 1b). The median concentration was 10.1 μmol/mol creatinine (range 7.47–17.7) in the four workers with the Ile/Val −/− genotype, whereas the median concentrations were between 3.16 and 4.36 (range 0.09–13.9) in subjects with the other genotype combinations. Further analyses of the impact of combined genotypes on the 1-OHP levels stratified by external PAH exposure and use of protection devices were not possible due to the limited number of workers carrying the Val allele. It should be noted that the four subjects with the Ile/Val −/− genotype had a higher PAH exposure compared with subjects with other genotype combinations but the differences between median values for total PAHs as well as up to 17.7 μmol/mol creatinine in the showed a median 1-OHP value of 0.17 μmol/mol creatinine.

Canadian residents, living <500 m from a Söderberg aluminum reduction plant, showed significantly increased excretion of 1-OHP compared with control subjects living in another industrial town (37). In occupational settings the 1-OHP content in urine may be increased 10–100-fold. Coke ovens, carbon electrode production plants, tar distilleries, aluminum smelters and creosote impregnation plants represent workplaces where high urinary 1-OHP levels are often observed (24). In this study the concentration of urinary 1-OHP sampled after work ranged up to 17.7 μmol/mol creatinine (median 4.31) in the potroom workers. Earlier biological monitoring of nine potroom workers within the same plant showed that 1-OHP ranged up to 3.57 μmol/mol creatinine (median 2.1) (23). Thus, the decreasing airborne PAH concentrations in this plant during the last years have not resulted in decreased excretion of urinary 1-OHP.

In an in vitro study with human lung microsomes, the rate of oxidative metabolism of pyrene showed wide interindividual variation with the highest activity observed in smokers (38). The pyrene 1-hydroxylase activity was 70% inhibited by monoclonal antibodies raised against rat CYP1A1, which indicates the importance of the CYP1A1 enzyme for pyrene metabolism in the lung. Gene–environment interactions on the excretion of 1-OHP were confirmed in the present study, where certain CYP1A1 (exon 7), and GSTM1 genotypes were found to have a limited, but significant, influence on 1-OHP excretion. The CYP1A1 (MspI) polymorphism was also analyzed in a study of 80 coke-oven workers from Taiwan (39). It was found that the subjects with the homozygous variant genotype had a 2-fold higher post-shift 1-OHP level than the grouped wild-type and heterozygous genotypes. Pan et al. (40) reported, on the other hand, no influence of GSTM1 or CYP1A1 (exon 7)
Genetic polymorphisms and urinary 1-OHP

Polymorphisms on 1-OHP excretion in a study on 99 Chinese coke-oven workers. They observed no correlations between DNA adducts and either total PAH exposure or urinary 1-OHP, but demonstrated a significant correlation between DNA adduct levels and urinary 1-OHP in workers with the CYP1A1 Ile/Val or Val/Val genotypes (40). The PAH levels were in general higher in that study compared with the PAH levels in the present study. In a study on police officers in Italy exposed to urban air levels of PAHs Merlo et al. (41) also reported analyses of CYP1A1 (MspI), GSTM1 and GSTT1 polymorphisms in relation to 1-OHP excretion. They observed an influence of the CYP1A1 genotype on 1-OHP levels but only in light smokers. Another study, on environmentally PAH-exposed women in Bohemia, indicated an influence of the enzyme N-acetyltransferase 2 in addition to GSTM1 on biomarkers of PAH exposure (42).

In this study only ~30% of the interindividual variation in 1-OHP excretion could be accounted for by factors related to external exposure levels, tobacco smoking and genotype. Two major contributing factors to the large interindividual variation in 1-OHP excretion are probably differences in the retention of inhaled pyrene and in the efficiency to metabolize pyrene to the 1-hydroxylated product. In a recent well controlled study on 10 human PAH-exposed volunteers, the retention and metabolic efficiency were estimated to be ~60% (range 36–81%) and ~30% (range 13–54%), respectively (27). In our study, the part of the variance that could be explained by differences in biotransformation genotypes seemed to be of the same order of magnitude as the variance explained by differences in exposure. The extrahepatic CYP1A1 is primarily expressed in the lung. In the liver, where most of the PAH metabolism takes place, the pyrene hydroxylase activity is probably achieved through several other enzymes (see Introduction). Metabolism via CYP1A1 may represent only a part of the 1-OHP observed in urine and the influence of CYP1A1 polymorphisms could thus be of different importance depending on inhalation or dermal routes of exposure. This could be the explanation for the relatively limited influence of the biotransformation polymorphisms observed in this and other studies on urine metabolites or adducts to white blood cell DNA. Polymorphisms in the important PAH metabolizing enzymes in lung, such as CYP1A1 may, however, be of critical importance for lung cancer from airborne PAH. Therefore, a continued investigation of the functional aspects of the CYP1A1 gene polymorphisms is warranted.

In the present study an effect of GSTM1 polymorphism on urinary 1-OHP levels was found although the GSTM1 activity is not directly linked to the metabolism of 1-OHP, which is mainly excreted as glucuronide conjugate. Deficiency in GSTM1 may increase the glucuronidation pathway as a result of accumulation of PAH derivatives that are otherwise conjugated to glutathione. Deficiency in glutathione conjugation may also increase the induction of CYP1A1 as described by Vaury et al. (43) and thereby increase formation of the 1-OHP glucuronide. Urinary excretion of 1-OHP was found to correlate in a highly significant manner to the exposure levels of the carcinogen B(a)P and to total particulate-associated PAHs. In this study we have in addition observed that this hydroxylated metabolite of pyrene was found in higher levels in individuals that carry the CYP1A1 Ile/Val genotype who were also of the GSTM1 null genotype. The CYP1A1 polymorphisms have earlier been associated with increased formation of the ultimate DNA-binding diol epoxides of B[a]P (11) and with increased risk for lung cancer in GSTM1-deficient subjects (31,44,45).

In summary it can be concluded that the urinary level of 1-OHP is a sensitive indicator of human exposure to PAHs and that in addition to being a marker of exposure it may also to some extent reflect the interindividual variation in susceptibility to PAHs.

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References


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