Exposure of iron foundry workers to polycyclic aromatic hydrocarbons: benzo(a)pyrene-albumin adducts and 1-hydroxypyrene as biomarkers for exposure

Ø Omland, D Sherson, Å M Hansen, T Sigsgaard, H Autrup, E Overgaard

Abstract
Exposure to polycyclic aromatic hydrocarbons (PAHs) in foundry workers has been evaluated by determination of benzo(a)pyrene-serum albumin adducts and urinary 1-hydroxypyrene. Benzo(a)pyrene binding to albumin and 1-hydroxypyrene were quantitatively measured by enzyme linked immunosorbent assay (ELISA) and reverse phase high performance liquid chromatography (HPLC), respectively. 70 male foundry workers and 68 matched controls were investigated. High and low exposure groups were defined from breathing zone hygiene samples, consisting of 16 PAH compounds in particulate and gaseous phase. Mean total PAH was 10.40 µg/m³ in the breathing zone, and mean dust adsorbed PAH was 0.15 µg/m³. All carcinogenic PAH was adsorbed to dust. Median benzo(a)pyrene-albumin adduct concentrations (10-90% percentiles) were similar in foundry workers (smokers 0.55 (0.27-1.00) and non-smokers 0.58 (0.17-1.15)) pmol/mg albumin and age matched controls (smokers 0.57 (0.16-1.45) and non-smokers 0.70 (0.19-1.55) pmol/mg albumin). Median 1-hydroxypyrene concentrations were significantly higher (P < 0.0001) in smoking and non-smoking foundry workers (0.022 (0.006-0.075) and 0.027 (0.006-0.164) µmol/mg creatinine) than in smoking and non-smoking controls (0 (0.0-0.22) and 0 (0.0-0.10) µmol/mg creatinine). Dose-response relations between total PAH, pyrene, carcinogenic PAHs, and 1-hydroxypyrene for smokers, and polycyclic aromatic hydrocarbons adsorbed to dust for non-smokers are suggested. Exposure to PAHs adsorbed to dust showed an additive effect. There was no correlation between the concentrations of 1-hydroxypyrene and benzo(a)pyrene-albumin adducts. The change in 1-hydroxypyrene over a weekend was also studied. Friday morning median 1-hydroxypyrene concentrations were significantly higher in both smokers and non-smokers (0.021 (0.0-0.075) and 0.027 (0.006-0.164) µmol/mg creatinine than Monday morning median concentrations (0.007 (0.0-0.021) and 0.008 (0.0-0.021) µmol/mg creatinine). Smoking did not affect the concentrations of 1-hydroxypyrene or benzo(a)pyrene-albumin adducts. These data suggest that 1-hydroxypyrene is a sensitive biomarker for low dose PAH exposure. Exposure to PAHs may be aetiological related to increased risk of lung cancer in foundry workers.


Increased risk of lung cancer has been shown to occur in several studies of iron foundry workers. Polycyclic aromatic hydrocarbons (PAHs) may be an aetiological factor. Exposure to carcinogenic chemicals (for example PAHs) can be evaluated by measuring covalent binding with macromolecular targets, DNA, and proteins. As target tissue is not usually available several surrogate materials have been studied—namely, white blood cells, lymphocytes, and serum proteins. 1-15 1-Hydroxypyrene, a urinary metabolite of pyrene, has recently been used as to monitor PAH exposure in humans. 12-15

In iron foundries PAHs are present in both the gaseous phase and adsorbed on to dust. Benzo(a)pyrene adsorbed to particles in the lungs may augment carcinogenicity due to increased long term retention.17-18 These findings suggest that inhaled PAHs adsorbed to particles covalently interact with macromolecules to a greater extent than gaseous PAHs. 19

The purpose of this study was to: (a) quantify the concentrations of serum benzo(a)pyrene-albumin adducts and urinary 1-hydroxypyrene in healthy iron foundry workers, (b) investigate possible positive correlations between these biomarkers and PAH exposure variables (total PAHs, particulate PAHs, carcinogenic PAHs, and pyrene), (c) determine whether positive correlations exist between the two biomarkers, and (d) compare Friday and Monday morning 1-hydroxypyrene concentrations.

Subjects and methods
STUDY POPULATION AND CONTROL GROUP
The study population consisted of 70 male iron foundry workers. They were employed in melting, machine moulding, casting, or sand preparation. All were occupationally exposed to PAHs. The foundry was located in a small Danish town. The control group consisted of 68 male blue collar workers from a drinking water supply plant near Copenhagen, all
Table 1  Comparison of control and exposed groups

<table>
<thead>
<tr>
<th></th>
<th>Control group (tobacco supply workers) (n = 68)</th>
<th>Exposed group (foundry workers) (n = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>43</td>
<td>40</td>
</tr>
<tr>
<td>Age range (y)</td>
<td>24–66</td>
<td>24–63</td>
</tr>
<tr>
<td>Pack-years</td>
<td>18 (11)</td>
<td>16 (13)</td>
</tr>
<tr>
<td>Urinary creatinine (mmol/l)</td>
<td>11·3 (4·3)</td>
<td>17·8 (6·9)</td>
</tr>
<tr>
<td>Non-smokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>Age range (y)</td>
<td>26–64</td>
<td>24–66</td>
</tr>
<tr>
<td>Urinary creatinine (mmol/l)</td>
<td>13·1 (6·2)</td>
<td>19·6 (6·8)</td>
</tr>
</tbody>
</table>

All workers were men. Values are mean (SD) unless stated otherwise.

occupationally unexposed to PAHs. These groups were matched for age and smoking habits (table 1). Information about employment, smoking, alcohol consumption, respiratory and urogenital symptoms, and medications including the use of coal tar salves were obtained from questionnaires and checked by personal interviews. Tobacco, cigars, and cheroots smoked were translated to cigarettes when calculating pack-years. The research protocol was accepted by the local ethics committee.

EXPOSURE GROUP AND AIR SAMPLING

Foundry workers were subdivided into low and high PAH exposure groups based on breathing zone measurements (n = 17): (1) total PAH: low <10·00 µg/m³; high ≥10·00 µg/m³; (2) particulate PAH: low <0·21 µg/m³; high ≥0·21 µg/m³; (3) carcinogenic PAH: low <0·10 µg/m³; high ≥0·10 µg/m³ and (4) pyrene: low <0·10 µg/m³; high ≥0·10 µg/m³.

The sample preparation and steps of reversed phase high performance liquid chromatography (HPLC) have been previously described in detail21–22 and are only summarised here. Air samples (600–800 l) were collected at a flow rate of 1·9 l/min during the work shift (about seven hours). Particulates were collected on a 37 mm glass fibre filter (Millipore AP 4003–705) in a standard cassette of polystyrene, and gaseous PAH compounds on a PVC tube in series with a styrene-divinylbenzene adsorption tube. The concentration of 16 selected PAH compounds24 was measured by comparison with the chromatogram of standard PAH preparations. Total PAH concentration was calculated as the sum of the particulate and gaseous concentrations. Total particulate concentration was calculated as the sum of the compounds attached to the glass fibre filter. The total concentration of carcinogenic compounds was calculated as the sum of the concentrations of benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, and indeno(1,2,3-cd)pyrene.25

BLOOD COLLECTION

Blood samples (10 ml) were collected from exposed subjects on a Friday morning in April 1989. The blood was allowed to coagulate overnight at 4°C. The coagulum was removed after centrifugation at 3000 rpm. The serum samples were frozen at −20°C until analysed.

ANALYSIS OF BENZO[a]PYRENE-ALBUMIN ADDUCTS IN BLOOD SAMPLES

The level of benzo(a)pyrene-albumin adducts was detected by competitive enzyme linked immunosorbsent assay (ELISA) as previously described.10 26 Serum (2 ml) was isolated from the blood samples by centrifugation, and albumin was precipitated by addition of saturated ammonium sulphate (final concentration 50%). After removal of globulins by centrifugation, albumin was precipitated from the supernatant by acidification with acetic acid.

Blood was redissolved in 500 µl of 0·1M sodium phosphate buffer, pH 7·4, and digested by pronase. The concentration of albumin was determined by Biuret reagent assay. The digest was applied to a prewet C18 Sepak column. The column was washed with 5 ml of 5% methanol to remove small peptides and amino acids. The PAH metabolites were eluted with 80% methanol (5 ml) and the eluate was evaporated to 500 µl in a vacuum.

COMPETITIVE ELISA

Polystyrene 96 microwell plates (NUNC immunoplates, Nunc, Roskilde, Denmark) were coated with 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDE) modified dextran. The competitive antigen was diluted 1:10 and 1:100 in phosphate buffered saline (PBS), and 100 µl/well (five wells for each dilution) was added, followed by the primary antibody 8E11 (kindly provided by Dr R Santella, Columbia University, NY, USA) diluted 1:250 000 in PBS (100 µl/well). A series of different concentrations of hydrolysed BPDE (0·1 fm to 1·0 pm) were included in each plate. The level of modification was estimated from the standard curve with the 1:1000 dilution of the competitive antigen, and was expressed as pmol BPDE/mg albumin. The competitive assay has been described in detail elsewhere.27

Antibody 8E11, which detects BPDEs, released by acid hydrolysis of proteins, also recognises benzo(a)pyrene diols, triols, and tetrals and cross reacts with several other PAHs, with different affinities.10 11 Consequently results are expressed as benzo(a)pyrene equivalents that correspond to the amount of BPDE necessary to give the same level of inhibition.

URINE COLLECTION AND DETERMINATION OF 1-HYDROXYPYRENE AND CREATININE

The urine samples from foundry workers were collected as the second urinary voids on Friday morning and the following Monday morning in April 1989, and frozen at −20°C until analysed. Urinary creatinine was measured to standardise the results.28

The sample preparation and chromatography have been previously described in
Exposure of iron foundry workers to PAHs biomarkers for exposure

515
detail.21 The standard plot was linear for the concentration range of the standard curve. Urine samples (10-0 ml) were buffered with 10-0 ml 0-2 N sodium acetate buffer (pH 5-0) and hydrolysed enzymatically with 200 µl β-glucuronidase/sulphatase (26 400 U β-glucuronidase and 440 U sulphatase) for 20 hours at 37-5°C in a shaking water bath. After hydrolysis the sample was centrifuged for 10 minutes at 3000 rpm (928 g) at ambient temperature. Samples of the standard solution and the centrifuged sample were applied to a Bond Elute sample enrichment and purification cartridge packed with C-18 (Varian, Harbor City, CA, USA). The cartridge was primed with 2-5 ml acetonitrile, followed by 10 ml water, and the hydrolysed sample was passed through the cartridge at a flow rate of 2-5 ml/min. Subsequently, the cartridge was washed with 5 ml 0-2 N sodium acetate buffer (pH 5-0). The retained solutes were eluted with 1-5 ml acetonitrile. Samples of 25 µl of the final extracts were injected into the HPLC column.

STATISTICAL ANALYSIS

Data were analysed with the statistical package for social sciences (SPSS). Statistical methods comprised Student's t-test and non-parametric tests for paired and non-paired data.31 All tests were two tailed.

Results

The mean (SD) total PAH concentration was 10-40 (4-04) µg/m.3 The mean dust adsorbed PAH concentration was 0-15 (0-21) representing 1-44% of the total PAH concentration. Table 2 shows the mean concentrations of the 16 standard PAHs in gaseous and particulate forms. Four of the six carcinogenic PAHs were measurable in the dust adsorbed form only. Pyrene was found in both phases.

Figure 1 shows the median values for 1-hydroxypyrene concentrations from exposed workers and controls. Smoking and non-smoking foundry workers had significantly increased 1-hydroxypyrene concentrations compared with their control counterparts. The median values (10-90% percentiles) for the four groups were 0-022 (0-006-0-075), 0-027 (0-006-0-164), 0 (0-0-022), and 0 (0-0-010) µmol/mol creatinine respectively. There was no significant difference in 1-hydroxypyrene concentration between smokers and non-smokers in the exposed or the control groups (P exposed = 0-53, P unexposed = 0-06).

Figure 2 illustrates the median benzo(a)pyrene-albumin adduct concentrations in blood from foundry workers and controls. The adduct concentrations were not increased in either smoking or non-smoking foundry workers, compared with smoking and non-smoking controls (P smokers = 0-65, P non-smokers = 0-20). The median values for the four groups were 0-55 (0-27-1-00), 0-58 (0-17-1-15), 0-57 (0-16-1-45), and 0-70 (0-19-1-55) pmol/mg albumin respectively. No difference in benzo(a)pyrene-albumin adduct concentrations was found between smokers and non-smokers in either the exposed or the control group (P exposed = 0-99, P unexposed = 0-22).

No correlations were found between any of the PAH exposure variables (total PAHs, particulate PAHs, carcinogenic PAHs, and pyrene) and median benzo(a)pyrene-albumin adduct concentrations in the blood, either for smokers or for non-smokers. Regression analyses showed no correlations between concentration of 1-hydroxypyrene and albumin adducts (R² = 5 × 10⁻²).

Figure 3 shows median 1-hydroxypyrene concentrations according to total PAH exposure for smokers and non-smokers. Smoking

Table 2 Mean (SD) concentrations of 16 gaseous and particulate PAHs

<table>
<thead>
<tr>
<th>PAH</th>
<th>Particulate PAHs (µg/m³)</th>
<th>Gaseous PAHs (µg/m³)</th>
<th>Total PAHs (µg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>0 (0)</td>
<td>9-68 (3-85)</td>
<td>9-68 (3-85)</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0 (0)</td>
<td>0-03 (0-17)</td>
<td>0-03 (0-17)</td>
</tr>
<tr>
<td>Fluorene</td>
<td>0-04 (0-01)</td>
<td>0-08 (0-05)</td>
<td>0-08 (0-05)</td>
</tr>
<tr>
<td>Phenantrene</td>
<td>0-01 (0-04)</td>
<td>0-31 (0-16)</td>
<td>0-32 (0-17)</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0-02 (0-02)</td>
<td>0-04 (0-03)</td>
<td>0-05 (0-03)</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>0-02 (0-07)</td>
<td>0-11 (0-15)</td>
<td>0-13 (0-20)</td>
</tr>
<tr>
<td>Pyrene</td>
<td>0-004 (0-01)</td>
<td>0-01 (0-02)</td>
<td>0-01 (0-02)</td>
</tr>
<tr>
<td>Benzo(a)anthracene*</td>
<td>0-01 (0-04)</td>
<td>0-01 (0-02)</td>
<td>0-02 (0-03)</td>
</tr>
<tr>
<td>Chrysene</td>
<td>0-02 (0-03)</td>
<td>0 (0)</td>
<td>0-02 (0-03)</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene*</td>
<td>0-03 (0-01)</td>
<td>0 (0)</td>
<td>0-03 (0-04)</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene*</td>
<td>0-02 (0-04)</td>
<td>0 (0)</td>
<td>0-02 (0-04)</td>
</tr>
<tr>
<td>Benzo(a)pyrene*</td>
<td>0-02 (0-04)</td>
<td>0 (0)</td>
<td>0-02 (0-04)</td>
</tr>
<tr>
<td>Dibenz(a,h)anthracene</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Benzo(g,h,j)perylene</td>
<td>0-02 (0-08)</td>
<td>0 (0)</td>
<td>0-05 (0-08)</td>
</tr>
<tr>
<td>Indeno(1,2,3-cd)pyrene</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total (16 PAHs)</td>
<td>0-15 (0-21)</td>
<td>10-24 (4-03)</td>
<td>10-40 (4-04)</td>
</tr>
</tbody>
</table>

*Carcinogenic PAHs. Seventeen measurements were taken.
foundry workers in both low and high total PAH exposure groups had significantly higher concentrations than smoking controls. The median values for the three groups were 0.020 (0.004–0.062), 0.022 (0.012–0.180), and 0 (0–0.022) μmol/mol creatinine respectively. Non-smoking foundry workers also had significantly higher 1-hydroxypyrene concentrations than non-smoking controls. High exposure foundry workers had slightly lower 1-hydroxypyrene concentrations than low exposure workers. The median values were 0.026 (0.008–0.083), 0.036 (0.006–0.174), and 0 (0–0.018) μmol/mol creatinine respectively.

Figure 4 shows the median 1-hydroxypyrene concentrations and exposure to particulate PAH for smokers and non-smokers. Non-smoking foundry workers with low or high exposures to dust adsorbed PAH had significantly higher concentrations of 1-hydroxypyrene than non-smoking controls. The median values for the three groups were 0.026 (0.004–0.164), 0.036 (0.006–0.062), and 0 (0–0.010) μmol/mol creatinine respectively. Both low and high exposure foundry workers who smoked had significantly higher concentrations of 1-hydroxypyrene than smoking controls. High exposure workers had slightly lower 1-hydroxypyrene concentrations than low exposure workers. The median values were 0.020 (0–0.057), 0.023 (0.001–0.178), and 0 (0–0.022) μmol/mol creatinine.

The median 1-hydroxypyrene concentrations according to exposure to carcinogenic PAHs and pyrene showed the same relation as for total PAHs. Both smoking and non-smoking foundry workers had significantly higher concentrations of 1-hydroxypyrene than their control counterparts (P low < 0.0001, P high < 0.0001). For both exposure variables smokers showed dose-response relations whereas non-smokers did not.

Figure 5 shows Friday and Monday morning median 1-hydroxypyrene concentrations.

Concentrations of 1-hydroxypyrene on Friday morning were significantly higher for both smokers and non-smokers than on Monday morning. The median Friday values were 0.021 (0–0.075) and 0.027 (0.006–0.164) μmol/mol creatinine and Monday values were 0.007 (0–0.021) and 0.008 (0–0.021) μmol/mol creatinine.

Discussion

The mean total PAH exposure in this foundry, 10–40 μg/m³, is higher than the mean exposure recently described in another Danish foundry, but lower than the mean exposure in German foundries (17–54 μg/m³). Similar exposure levels, (8–32 μg/m³) have been found in roofers.

The mean concentration in our study of both benzo(a)pyrene (0.01 μg/m³) and pyrene (0.01 μg/m³) was lower than previously described in another Danish foundry (0.04 and 0.28 μg/m³) and considerably lower than the mean concentration found in the German study (0.47 and 1.47 μg/m³). The range of concentrations in 10 Canadian foundries for benzo(a)pyrene (0.004–8.15 μg/m³) and pyrene (up to 12.93 μg/m³) reflects a much higher exposure. Recent Finnish studies have described a high exposure group with benzo(a)pyrene five times greater than ours (≥0.04 μg/m³), thus indicating a substantially higher exposure in Finnish foundries.

The mean PAH adsorbed on dust was 1–4% of the measured total PAH concentration. This ratio is considerably lower than in German foundries, where the PAH adsorbed on dust was 23–20% of the total PAH concentration.

The reasons for these differences are uncertain, but may reflect different sampling methods, different binding materials, heterogeneous hygienic conditions, and seasonal differences in air sampling.

As reported by others we found that all carcinogenic PAHs were adsorbed on dust. This might be important considering the prolonged retention of particulate PAHs found in animal studies.

In our study, the level of albumin adducts was similar in the exposed and the control
Exposure of iron foundry workers to PAHs: biomarkers for exposure

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