Polycyclic Aromatic Hydrocarbon-DNA Adducts in White Blood Cell DNA and 1-Hydroxypyrene in the Urine from Aluminum Workers: Relation with Job Category and Synergistic Effect of Smoking

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Abstract

We examined a group of 105 workers from a primary aluminum plant for the presence of polycyclic aromatic hydrocarbon (PAH)-DNA adducts in their WBC and 1-hydroxypyrene in their urine. Workers were recruited from five job categories with different PAH exposure: the anode factory; the bake oven; and the electrolysis and the pot-relining departments. Unexposed workers from the foundry department served as the control group. The exposure to PAH was measured by personal monitoring, and the average PAH concentrations in the work atmosphere ranged from 0.4 μg/m³ in the foundry to 150 μg/m³ in the pot-relining department. The average exposure to benzo(a)pyrene was under the Swedish exposure limit of 5 μg/m³.

The internal dose of pyrene was measured utilizing the 1-hydroxypyrene concentration in pre- and postshift urine samples. High exposure to PAH in the work atmosphere was associated with increased concentrations of 1-hydroxypyrene in the urine. The average increase in concentration of 1-hydroxypyrene ranged from 0.2 nmol/mol creatinine in the control group to 5.9 nmol/mol creatinine in the pot-relining department; an accumulation of 1-hydroxypyrene over a 5-day working period was observed. A good correlation was found between PAH exposure and the concentration of 1-hydroxypyrene in the urine on a group level (r = 0.90; P = 0.02).

PAH-DNA adducts were determined by 32p-postlabeling analysis (nuclease P1 enrichment procedure). In 93% of the workers, PAH-DNA adducts were detected and workers with a high PAH exposure had significantly higher PAH-DNA adduct values than did those with a low PAH exposure. A good correlation was found between PAH exposure and the average PAH-DNA adduct values in blood on a group level (r = 0.90; P = 0.02), but not on the level of individual workers. A highly significant correlation was found between the average PAH-DNA adduct values and the concentration of 1-hydroxypyrene in the urine at the end of day 5 for smokers (r = 0.66; P < 0.001; n = 23), but for nonsmokers this relationship was only marginally significant (r = 0.25; P = 0.089; n = 31).

Multiple regression analysis clearly showed the effect of smoking on DNA adduct values in WBCs (P = 0.10), as well as on 1-hydroxypyrene concentration in the urine (P < 0.001). Higher DNA adduct values in smokers were found only in those job groups with high PAH exposure. Higher concentrations of 1-hydroxypyrene in smokers were found in all groups, but the difference between smokers and nonsmokers was most pronounced in those groups with the highest PAH exposure. The strong effect of smoking in the exposed groups suggests a synergistic effect of smoking in combination with occupational PAH exposure on the formation of PAH-DNA adducts and 1-hydroxypyrene in the urine.

Introduction

Aluminum workers are exposed to coal tar fumes and dusts during manufacturing of the anodes, which are made from a mixture of coke and coal tar pitch. Epidemiological surveys in occupationally exposed populations, such as coke oven and iron foundry workers, roofers, and primary aluminum plant workers, indicate that long-term exposure to coal tar-derived products is associated with certain cancers (1, 2). Coal tar pitch fumes and dusts contain PAHs,1 many of which have shown carcinogenic activity in experimental animals and are suspected carcinogens in humans. PAHs form a well-studied class of compounds, and extensive knowledge exists about their metabolism (3). The metabo-

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1 The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; Bl(a)P, benzo[a]pyrene; BPDE, 1,2-trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene; r, Spearman’s rank correlation coefficient.

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lism of PAHs results mainly in the formation of more water-soluble derivatives that are excreted in the urine, but sometimes a part of the parent compound becomes activated into biochemically reactive forms. For example, metabolic activation of BaP results in the formation of dihydrodiol epoxides, which are the ultimate reactive forms capable of binding to nucleic acids (4). Noncarcinogenic PAHs such as pyrene do not yield metabolites that form DNA adducts (5), but they are excreted in the urine as conjugated metabolites. DNA adduct formation is thought to be involved in mutation and neoplastic transformation of target organs (6, 7).

To improve safety at the workplace and to identify subpopulations of workers who are at an increased risk for cancer, it is essential to monitor human exposure to carcinogenic and mutagenic agents. Monitoring of human exposure in workplaces traditionally consisted mainly of measuring the concentration of certain chemicals in ambient air. Biological monitoring of workers is likely to lead to a much more reliable risk assessment. Monitoring human urine for 1-hydroxypyrene, a metabolite of pyrene, is one way of biological monitoring and was shown to be a reliable indicator for PAH exposure in several occupational settings (8, 9). Furthermore, it has been suggested that determination of the extent of binding of PAH to DNA in humans may serve as a biomarker of exposure to genotoxic PAH. Therefore, sensitive detection methods have been optimized [enzyme immunoassays (10, 11); 12P-postlabeling assays (12); and fluorometric assays (13, 14)]. Several studies have shown that levels of PAH-DNA adducts are significantly increased in peripheral WBC of occupationally exposed smoke-exposed workers, foundry workers, and roofers (15). A recent study of workers employed in two aluminum production plants showed increased WBC adduct levels in one plant, but not in the other, compared with those of control subjects (16, 17). Although the effect of smoking on adduct levels in WBC from healthy non-occupationally exposed individuals has not been clarified yet (18–20), aromatic-DNA adduct levels in other human tissues, such as lung (21–23) and placenta (24), have been associated with smoking. These uncertainties on the effect of smoking on adduct levels in WBC are also encountered in studies with occupationally exposed workers (25, 26).

In this investigation we examined a group of 105 aluminum workers from five job categories as a model population for testing the applicability of two biomonitoring techniques to assess occupational exposure to PAH. The workers were examined for 1-hydroxypyrene in urine and for PAH-DNA adducts in WBC by 12P-postlabeling assay for the following purposes: (a) to determine whether 1-hydroxypyrene in urine and PAH-DNA adduct levels in WBC are related to external PAH exposure in different job categories; (b) to examine whether urinary 1-hydroxypyrene excretion and PAH-DNA adduct levels depend on other factors, e.g., smoking and the use of protective devices; and (c) to see if PAH-DNA adduct levels in WBC correlate with the urinary excretion of 1-hydroxypyrene.

**Methods**

**Choice of Subjects, Sample Collection, and Questionnaire Data.** Aluminum is produced by electrolytic reduction of alumina. Differences between the electrolysis processes essentially depend on the type of anodes used. These anodes can be either self-baking anodes in the Soderberg process, or prebaked in ovens before being used. Our study population consisted of 105 workers employed in an aluminum production plant operating with the prebake anode process. In this type of aluminum plant, anodes are produced in the anode production departments of a paste-manufacturing plant and a bake oven. Significant amounts of PAH are released in these departments. The studied workers were recruited from four job categories with different PAH exposure: the anode factory (mixing and shaping of the anode) (n = 16); the bake oven (baking of the anode) (n = 20); the pot-relining department (n = 13); and the electrolysis department (n = 24). As a control group, 32 workers were chosen from the foundry department of the same aluminum plant. The average ± SD age of all participants was 41.0 ± 8.3 years, and their ages ranged from 24 to 58 years. A trained interviewer conducted inquiries about smoking habits, occupational history, alcohol consumption, medication, and use of protective equipment (gloves and face masks).

Sampling of blood, urine, and ambient air took place from May to October 1989. Blood samples were collected at days 1, 3, and 5 of a 5-day working period. Samples were coded, placed on ice, and transported immediately to the Netherlands Cancer Institute. Total WBC were isolated on the same day. Urine samples were collected from each worker; 1 sample at the beginning and 1 sample at the end of an 8-h working period on 5 consecutive working days. The urine samples were immediately frozen at −20°C. On the same 5 days, exposure levels of PAHs in the breathing zone were determined by personal air sampling. During working hours, the particulate PAHs were collected on a teflon filter using a personal air sampling pump (Dupont de Nemours Inc., models P4000 and S2500) with a constant flow of 2.0 liters/min through a teflon membrane filter (diameter, 25 mm; pore size, 0.5 μm; Millipore FHLP). The PAHs were designed according to International Standards Organization TC146 standard. The PAHs with three or more aromatic rings are mainly retained on the filter absorbed to particulate matter, while PAHs of lower molecular weight in the vapor phase are not retained. After sampling, the filters were stored in the dark at −20°C until analysis.

**Measurement of PAH in Air Samples.** The particulate PAH collected on the teflon filters were extracted ultrasonically for 30 min with acetonitrile (1.5 ml). Clear supernatants of the extracts were used to analyze PAHs by HPLC equipped with a Vydac C18 column and a fluorescence detector. Elution was performed at a flow rate of 1.0 ml/min with a gradient of water/acetonitrile, starting with a 1:1 mixture which changed to pure acetonitrile. Standard PAH mixtures (EEC Bureau of Standards, Brussels, Belgium) were used as reference materials. The air samples were analyzed for 12 different PAHs: phenanthrene; anthracene; fluoranthene; pyrene; benzo[a]anthracene; chrysene; benz(b)fluoranthene; benz(k)fluoranthene; benzo(a)pyrene; dibenz(a,h)anthracene; indeno(1,2,3-c,d)pyrene; and benz[g,h,i]perylene.

**Determination of 1-Hydroxypyrene in Urine.** The measurement of urinary 1-hydroxypyrene excretion was as performed by the method of Jongeneelen et al. (8) and will only be summarized here. The method includes enzymatic hydrolysis of the conjugated metabolites by a mixture of glucuronidase and sulfatase and clean up of the samples by...
DNA Isolation. Peripheral blood samples (50 ml) were withdrawn by venapuncture into tubes containing EDTA. WBC were collected by centrifugation after incubation with 0.12 M NH₄Cl to lyse the erythrocytes. DNA was isolated by treatment with pancreatic RNase, proteinase K, and extraction with phenol, chloroform:isoamyl alcohol (24:1), and diethyl ether. DNA was precipitated from the aqueous phase with cold ethanol and dissolved in 2 mM Tris-HCl, pH 7.4 (2.0 mg/ml), for ³²P-postlabeling analysis. Coded DNA samples were stored at −20°C.

RNA Adduct Determination by ³²P-Postlabeling Assay. DNA samples were analyzed essentially as described by Reddy and Randerath (27). Five µg DNA was digested for 3.5 h at 37°C with 250-mU micrococcal nuclease (Sigma Chemical Co., St. Louis, MO) and 8-mU spleen phosphodiesterase (Cooper Biomedical Corp., Malvern, UK) in a total volume of 5 µl containing 10 mM sodium succinate and 10 mM CaCl₂, pH 6. Samples were then digested further with 2 µg nuclease P1 (Boehnnger, Mannheim, Germany) in a total volume of 7.5 µl, containing 0.1 mM ZnCl₂ and 0.06 M sodium acetate (pH 5) for 1 h at 37°C. After the addition of 2.5 µl 0.5 M Tris base, the DNA digest was labeled with [γ-³²P]ATP (50 µCi) using 2.5 units of T4 polynucleotide kinase (Boehringer). The reaction was terminated with 40 mM potassium pyrophosphate (Sigma) after 30 min. The [γ-³²P]ATP was synthesized in our laboratory using carrier-free ³²P-orthophosphate (Amersham, Buckinghamshire, UK). The specific activity was determined as described and was generally around 3000 Ci/mmol. Purification and resolution of ³²P-labeled adducts were carried out by TLC on polyethyleneimine-cellulose sheets (Macherey-Nagel, Düren, Germany). Chromatography was done according to a published method (27) using the following solvents: D1, 1.0 M sodium phosphate (pH 6.0); D2, 3.5 M lithium formate-8.5 M urea (pH 3.5); D3, 0.8 M LiCl-0.5 M Tris-HCl-8.5 M urea, (pH 8.0); and D4, 1.7 M sodium phosphate, (pH 6.0).

The chromatograms were visualized by autoradiography at −80°C using intensifying screens. Adducts were excised from the chromatograms and radioactivity was measured by Cerenkov counting. Carcinogen-DNA adduct levels were calculated by determining the relative adduct labeling, which is the ratio of count rates of adduct nucleotides to count rates of total (adduct plus normal) nucleotides. The specific radioactivity of [γ-³²P]ATP, determined by labeling a known amount of dAp (deoxyadenosine 3'-phosphate), was used for relative adduct-labeling calculations (27). In each experiment a [³H]BPDE-DNA standard with a modification level of 1 adduct/10² nucleotides was included and recoveries between 80 and 100% were usually obtained. The reproducibility (coefficient of variation = 11.6%) of the assay was determined by repeated analysis of 30 randomly selected urine samples. Concentrations of 1-hydroxypyrene were expressed as µmol/mol creatinine to correct for volume differences. Since correction with extremely low or high creatinine values was considered unreliable, urine samples with creatinine values beyond the range of 4–34 mmol/liter were excluded from analysis.

Results

Air Monitoring. The average exposure to total PAHs, pyrene, and B(a)P of individual aluminum workers is presented in Table 1 for each of the four job categories and the control group. Large differences in exposure to PAH were observed between the various categories. The highest concentrations of PAH and pyrene were measured in the pot-refining department. The highest concentration of B(a)P was detected in the anode factory.

The Spearman’s rank correlation coefficient between pyrene and total PAH concentration in the work atmosphere of 20 workers was rₛ = 0.98 (average of 5 consecutive working days). This highly significant correlation was found at three work sites: the bake oven (rₛ = 0.90; n = 5); the anode factory (rₛ = 0.96; n = 8); and the pot-refining department (rₛ = 0.96; n = 7). A good correlation was also found between B(a)P and total PAHs in the anode factory (rₛ = 0.97; n = 8) and for the bake oven (rₛ = 0.69; P < 0.001; n = 5). The correlation between B(a)P and total PAH in the pot-refining department (rₛ = 0.43; P = 0.01; n = 7) was lower than that for pyrene and total PAH but still significant.

A typical PAH profile found in the anode factory is

<table>
<thead>
<tr>
<th>Department</th>
<th>Pyrene</th>
<th>Benzo(a)pyrene</th>
<th>Total PAH$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bake oven</td>
<td>22</td>
<td>1.5 (0.5–22.7)</td>
<td>0.35 (0.1–14.4)</td>
</tr>
<tr>
<td>Anode factory</td>
<td>50</td>
<td>6.7 (0.3–31.8)</td>
<td>1.51 (0.1–11.6)</td>
</tr>
<tr>
<td>Pot-refining</td>
<td>41</td>
<td>32.3 (0.15–223)</td>
<td>1.05 (&lt;0.02–9)</td>
</tr>
<tr>
<td>Electrosysis</td>
<td>23</td>
<td>0.12 (0.03–0.7)</td>
<td>0.03 (&lt;0.02–0.2)</td>
</tr>
<tr>
<td>Foundry</td>
<td>16</td>
<td>0.04 (&lt;0.02–0.2)</td>
<td>0.02 (&lt;0.02–0.06)</td>
</tr>
</tbody>
</table>

$^a$GM, geometric mean.
$^{b}$Sum of 12 PAHs.

(27). In each experiment a $[^{3}H]$BPDE-DNA standard with a modification level of 1 adduct/10² nucleotides was included and recoveries between 80 and 100% were usually obtained. The reproducibility (coefficient of variation = 11.6%) of the assay was determined by repeated assays of the $[^{3}H]$BPDE-DNA standard.

Statistical Methods. Correlation coefficients were calculated with the use of Spearman’s rank correlation analysis. For these analyses the urinary excretion of 1-hydroxypyrene of a worker was defined as the difference between the post- and the preshift samples (the increase over 5 working days). For blood samples the average value of 3 days was used; for air measurements the average of the exposures of 5 working days was used. Differences in 1-hydroxypyrene concentration in the urine and average DNA adduct levels in blood samples among groups were compared by Student’s t test. Multiple regression analysis was carried out with the level of DNA adducts or 1-hydroxypyrene as the dependent variable with the SPSS/PC+ computer software package. Independent variables included: smoking habits (yes or no), number of cigarettes smoked per day during the last week, job category (5 levels), age, number of years employed by the plant, and use of protective equipment (yes or no). The number of workers engaged in each analysis varies somewhat due to missing data.
polyaromatic hydrocarbon. Similar profiles were illustrated in Fig. 1, showing that pyrene is the most abundant polyaromatic hydrocarbon. Similar profiles were found at other worksites with high PAH exposure. Although large fluctuations were found in the absolute total PAH concentration on different days, the relative amounts of various PAHs remained fairly constant.

### PAH-DNA Adducts in WBC

Fifty-four (95%) of the 57 blood samples tested showed PAH-DNA adduct levels above the detection limit (Table 2). All 43 workers of the bake oven, anode factory, pot-relining, and electrolysis departments, and 11 (79%) of 14 workers from the control group (foundry) showed adduct levels above the detection limit. Large intra- and interindividual differences between adduct values were found and it was not possible to detect an increase in DNA adduct levels over a 5-day working period. Therefore, the average adduct levels per worker of 2 or 3 days of a 5-day working period were calculated. The average adduct level of the highly exposed groups, the bake oven, anode factory, and the pot-relining department, 40.8 ± 41.6 adducts/10⁸ nucleotides (n = 29), was significantly enhanced compared with that of the control group, 7.3 ± 7.8 adducts/10⁸ nucleotides (n = 14; P < 0.0001) (Table 2). No significant differences in DNA adduct levels were found between workers from the three highly exposed groups. Adduct levels of workers from the electrolysis department and from the control group did not differ significantly.

Among the 102 workers questioned, 47 (46%) were current smokers, whereas 55 (54%) were non-smokers. Appreciable differences in smoking habits between the various departments were noted. In the bake oven and in the anode factory, 35 and 25%, respectively, of the workers were smokers, whereas the pot-relining workers had 60%, the electrolysis workers had 54%, and the control group had 53% smokers. The number of cigarettes smoked was comparable; the average number of cigarettes smoked/day was 17.

In the total exposed population, the average PAH-DNA adduct value of smokers (29.2 adducts/10⁸ nucleotides; n = 24) was 86% higher than that of non-smokers (15.7 adducts/10⁸ nucleotides; n = 32; P = 0.08). Although a difference was found, it was not statistically significant, which may be due to the large SD and the small size of the groups. No relationship was noted between the PAH-DNA adduct levels and the number of cigarettes smoked during the last week, and there was also no association with the amount smoked during the last 24 h. Adduct levels in smokers were increased only in the exposed groups (Table 2).

### 1-Hydroxypyrene in the Urine

The 1-hydroxypyrene concentration in the urine ranged between 0.17 and 26.9 μmol/mol creatinine. To illustrate the time course of 1-hydroxypyrene excretion in urine over a 5-day working period, the average 1-hydroxypyrene concentrations in preshift urine samples and in postshift urine samples of five bake oven workers are presented in Fig. 2. An increase in urinary 1-hydroxypyrene concentration was found during one shift (from 8 am to 4 pm), as well as in the course of a 5-day working period. A decrease was observed between two shifts, but high values did not drop to the preshift level of the day before. This is in agreement with the reported elimination half-life of 1-hydroxypyrene in the range of 6–35 h (8).

A significant effect of smoking on the excretion of 1-hydroxypyrene was observed for all job groups, but the difference in 1-hydroxypyrene excretion between smokers and non-smokers was the largest in the three departments with high PAH exposure (Table 3). This difference in 1-hydroxypyrene concentration was found in preshift urine sam-

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**Table 2.** Average (± SD) assay values of PAH-DNA adducts in WBC from smoking and nonsmoking aluminum workers (n = number of workers assayed).

<table>
<thead>
<tr>
<th>Department</th>
<th>N'P-postlabeling analysis (adducts/10⁸ nucleotides)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smokers n</td>
</tr>
<tr>
<td>High exposure</td>
<td></td>
</tr>
<tr>
<td>Bake oven</td>
<td>6</td>
</tr>
<tr>
<td>Anode factory</td>
<td>1</td>
</tr>
<tr>
<td>Pot-relining</td>
<td>5</td>
</tr>
<tr>
<td>All</td>
<td>12</td>
</tr>
<tr>
<td>Low exposure</td>
<td></td>
</tr>
<tr>
<td>Electrolysis</td>
<td>5</td>
</tr>
<tr>
<td>Foundry</td>
<td>7</td>
</tr>
<tr>
<td>All</td>
<td>12</td>
</tr>
</tbody>
</table>

*Value was compared with nonsmokers (P = 0.233).
ples, as well as in postshift urine samples. In preshift urine samples of day 1, the average 1-hydroxypyrene concentration of smokers (0.66 μmol/mol creatinine; n = 44) was 89% higher than that of nonsmokers (0.35 μmol/mol creatinine; n = 51; P = 0.0002). The increase of 1-hydroxypyrene concentration over a 5-day working period in workers employed in the anode production and bake oven departments was higher in smokers than in nonsmokers.

A significant relationship was noted between the number of cigarettes smoked during the last week and the 1-hydroxypyrene concentrations in preshift, as well as in postshift urine samples (P = 0.005). The association with the number of cigarettes smoked during the last week was much stronger than the one with the amount smoked during the past 24 h. Urine samples of workers in the control group did not show a significant increase in 1-hydroxypyrene concentration over a 5-day working period.

**Relationships between the Various Parameters.** The relationship between the air concentration of PAH and the average PAH-DNA adduct values of days 1, 3, and 5 was examined for the total exposed population. At the level of the individual workers no significant correlations (all are <0.20) were found between PAH-DNA adducts in blood and the air concentrations of PAH, pyrene, or B(a)P separately, and those PAHs that are known to form diol epoxide-DNA adducts. However, good correlations (r = 0.90; P = 0.02) were found between PAH exposure and PAH-DNA adducts in blood, and 1-hydroxypyrene in the urine at the level of job category (Fig. 3).

A highly significant correlation was found between the average PAH-DNA adduct values (days 1, 3, and 5) and the concentration of 1-hydroxypyrene in the urine at the end of day 5 for smokers (r = 0.66; P < 0.001; n = 23) (Fig. 4), but for nonsmokers this relationship was not significant (r = 0.25; P = 0.089; n = 31).

Table 4 shows the results of multiple regression models predicting DNA adduct levels in WBC and 1-hydroxypyrene in the urine in the whole study population and the exposed workers. Only smoking and job category were both significantly associated with PAH-DNA adduct levels and excretion of 1-hydroxypyrene in the urine. Table 2 also shows that within the highly exposed groups (anode factory, bake oven, and pot reline) job category is not significantly associated with adduct levels.

No significant relationship was found between

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**Table 3** Average (± SD) assay values of 1-hydroxypyrene (μmol/mol creatinine) in the urine of smoking and nonsmoking aluminum workers at the beginning and at the end of a 5-day working period (number of workers assayed is given between parentheses)

<table>
<thead>
<tr>
<th>Department</th>
<th>Smokers</th>
<th>Nonsmokers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preshift</td>
<td>Postshift</td>
</tr>
<tr>
<td>High exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bake oven</td>
<td>1.00 ± 0.72 (6)</td>
<td>8.43 ± 4.08 (6)</td>
</tr>
<tr>
<td>Anode factory</td>
<td>1.34 ± 0.52 (2)</td>
<td>4.84 ± 3.64 (2)</td>
</tr>
<tr>
<td>Pot-relining</td>
<td>0.81 ± 0.48 (6)</td>
<td>6.72 ± 4.25 (6)</td>
</tr>
<tr>
<td>All</td>
<td>0.97 ± 0.47 (14)*</td>
<td>7.19 ± 4.00 (14)*</td>
</tr>
<tr>
<td>Low exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrolysis</td>
<td>0.58 ± 0.31 (13)</td>
<td>0.88 ± 0.42 (13)</td>
</tr>
<tr>
<td>Foundry</td>
<td>0.47 ± 0.37 (16)</td>
<td>0.62 ± 0.31 (17)</td>
</tr>
<tr>
<td>All</td>
<td>0.52 ± 0.34 (29)*</td>
<td>0.74 ± 0.38 (30)*</td>
</tr>
</tbody>
</table>

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* Value was compared with preshift nonsmokers (P = 0.009).
* Value was compared with end of shift nonsmokers (P = 0.017).
* Value was compared with preshift nonsmokers (P = 0.019).
* Value was compared with end of shift nonsmokers (P = 0.003).
During the past 8 years several studies have been reported in which PAH-DNA adducts in WBCs from occupationally exposed workers in coke oven plants (25, 26, 33–35) and foundries (36–38) have been determined by \(^{32}\)P-postlabeling analysis and/or immunoassays, but very few studies in aluminum plants have been described.

In this extensive study of a primary aluminum production plant, we analyzed DNA from WBC from 57 workers, including 14 workers from the foundry department of the same plant as controls. We found a high correlation between PAH-DNA adduct levels and exposure groups, defined as job categories (\(r = 0.90; P = 0.02\)), as illustrated in Fig. 3. When comparing our results with those of another recent aluminum plant study (16, 17), we found 15–20 times higher adduct values. Schoket et al. (17) found a higher average DNA adduct level in peripheral blood lymphocyte samples from only one of the factories, compared with a local control group, but job categories, PAH concentrations in air, and excretion of 1-hydroxypyrene were not reported.

The results of two recent coke oven studies (25, 35) also showed a significant relationship between PAH-DNA adduct values and PAH exposure by job category.

In our study smokers had consistently higher PAH-DNA adduct values than nonsmokers in the highly exposed groups (\(P = 0.08\)), but no effect of smoking was found in the electrolysis department and in the control group (Table 2). No correlation at the individual level was found between PAH-DNA adducts in blood and the concentration of PAHs or B(a)P in the air, but a good correlation was found at the group level (Fig. 3). There was no relationship between adducts and the number of cigarettes smoked per day. These results differ only slightly from those of our previous study in coke oven workers (26). In that study, smoking controls had higher adduct levels than nonsmoking controls, but this difference was not statistically significant (\(P = 0.07\)). Only in the control group was a significant relationship noted between adduct levels and the number of cigarettes smoked/day (\(r = 0.33; P = 0.03\)).

In several previous studies on occupational PAH exposure the effect of smoking has been examined, but a clear relationship was not always found. In their study on coke oven workers, Övrebo et al. (25) found a significant correlation between smoking and adduct levels only in the control group, when measured by \(^{32}\)P-postlabeling (\(P = 0.02\)), but not by ultrasensitive enzyme radioimmunoassay. Schoket et al. (17) reported that neither \(^{32}\)P-postlabeling nor ELISA gave detectable differences in adduct levels between smokers and nonsmokers. Two other studies of foundry workers showed no differences in adduct levels between smokers and nonsmokers (36, 37).

It must be emphasized, however, that direct comparison of DNA adduct values between different studies should be done with caution because many variables may influence the results. First, the PAH profile varies among aluminum plants depending on the process used. Secondly, the bioavailability of PAH, their absorption to particulate matter, and the size of the particles will influence the uptake. The relative amounts of the various subpopulations of WBC and differences in their capacity to metabolize PAH will be an important factor in the formation of DNA adducts in individuals. Together with individual differences in DNA repair capacity, these parameters will determine the ultimate level of DNA adducts measured in a particular tissue at a certain time. The \(^{32}\)P-postlabeling analysis itself may be
subject to appreciable variation, as was demonstrated in a recent interlaboratory trial (39).

The concentrations of urinary 1-hydroxypyrene in the same groups of workers showed an increase of 1-hydroxypyrene during the day and a decrease overnight. A general increase was observed during a 5-day working period, at the end of which a difference in 1-hydroxypyrene concentration was found of more than 15 times over that seen in controls. This is in good agreement with a 17-fold difference reported by Tolos et al. (31). A strong correlation was found between 1-hydroxypyrene in the urine at day 5 and the average PAH concentration of the five job categories (r = 0.90; P = 0.02) (Fig. 3). On an individual basis, however, correlations between 1-hydroxypyrene in the urine and PAH in the work atmosphere of the three highly exposed groups were low (<0.20).

Smokers had significantly higher 1-hydroxypyrene concentrations in the urine than did nonsmokers at the beginning as well as at the end of a 5-day working period (P < 0.001). This difference was found in all groups, but it was most pronounced in those departments with high PAH exposure. A clear relationship was found between 1-hydroxypyrene in the urine and the number of cigarettes smoked per day (P = 0.005). No relationship was found between adduct levels or 1-hydroxypyrene concentrations and the use of protective materials for hands and/or face. It is quite likely, however, that protective materials were mostly used by workers with the highest PAH exposure, which renders it impossible to evaluate their effectiveness in a group of workers with a large variation in degree of exposure. Other factors such as the number of years employed at the plant or alcohol consumption did not have any influence on either PAH-DNA adduct values or on 1-hydroxypyrene concentrations.

The effect of smoking in occupationally exposed aluminum workers may be explained by a synergistic effect between the ambient PAH and those in cigarette smoke. One explanation for the apparent higher PAH-DNA adduction formation in smokers is that the intake of a PAH mixture can result in enhanced DNA adduct formation from specific carcinogens within the mixture, e.g., other components of the mixture may serve to enhance the metabolic pathway involved in forming specific reactive intermediates. Results of in vitro experiments with hamster embryo cell cultures by Smolarek et al. (40) suggested that pretreatment of B(a)P induces a specific isozyme of cytochrome P-450, CYP1A1, increasing the proportion of B(a)P metabolized to the most reactive metabolite, anti-BPDE. In a similar fashion, the induction of CYP1A1 may also increase the proportion of PAH converted to phenolic metabolites. Culp and Beland (41) showed that total-DNA binding was greater in coal tarfed mice than in mice fed B(a)P alone, and the adduct levels decreased in the following order: lung > liver > forestomach. Hughes and Phillips (42) found that a mixture of dibenzo[a]pyrene and B(a)P resulted in DNA binding 65% higher than that expected from the binding levels of the PAH when applied separately. On the other hand, when dibenzo[a]pyrene, dibenzo[a]pyrene, and B(a)P were applied together to mouse skin, the total binding was 35% lower than expected (42). These results indicate that there are tissue-specific differences in the activation of PAH components in a mixture when compared to a specific compound contained within the mixture.

We also studied skin contamination in a small group of workers from the anode paste factory (n = 8), the bake oven (n = 5), and the pot-relining department (n = 7) and the

### Table 4

<table>
<thead>
<tr>
<th>Variable</th>
<th>Log(PAH-DNA adducts)$^a$</th>
<th>Log(1-Hydroxypyrene)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficient (± SE)</td>
<td>P-value</td>
</tr>
<tr>
<td>Bake oven$^d$</td>
<td>0.84 (± 0.24)</td>
<td>0.001</td>
</tr>
<tr>
<td>Anode factory$^d$</td>
<td>0.71 (± 0.32)</td>
<td>0.03</td>
</tr>
<tr>
<td>Pot relining$^d$</td>
<td>0.93 (± 0.25)</td>
<td>0.0005</td>
</tr>
<tr>
<td>No. of cigarettes smoked last week$^e$</td>
<td>-0.02 (± 0.02)</td>
<td>0.20</td>
</tr>
<tr>
<td>Smoked cigarettes last week (yes)$^e$</td>
<td>0.39 (± 0.3)</td>
<td>0.20</td>
</tr>
<tr>
<td>Face unprotected$^h$</td>
<td>-0.28 (± 0.25)</td>
<td>0.26</td>
</tr>
<tr>
<td>Always</td>
<td>0.08 (± 0.44)</td>
<td>0.85</td>
</tr>
<tr>
<td>Often</td>
<td>0.03 (± 0.22)</td>
<td>0.90</td>
</tr>
<tr>
<td>Hands unprotected$^b$</td>
<td>-0.12 (± 0.32)</td>
<td>0.71</td>
</tr>
<tr>
<td>Always</td>
<td>0.20 (± 0.35)</td>
<td>0.58</td>
</tr>
<tr>
<td>Often</td>
<td>0.19 (± 0.25)</td>
<td>0.45</td>
</tr>
<tr>
<td>Sometimes</td>
<td>0.07 (± 0.22)</td>
<td>0.74</td>
</tr>
<tr>
<td>Lunch in restaurant$^f$</td>
<td>0.34 (± 0.39)</td>
<td>0.40</td>
</tr>
</tbody>
</table>

$^a$ R² = 0.45 (experimental variability).

$^b$ Adducts/10⁸ nucleotides.

$^c$ μmol/mol creatinine.

$^d$ Reference category: electrolysis and foundry departments.

$^e$ Reference category: no cigarettes smoked.

$^f$ Reference category: not smoked during last week.

$^g$ Reference category: face never unprotected.

$^h$ Reference category: hands never unprotected.

$^i$ Reference category: never lunch in restaurant.
results are reported elsewhere (43). The dermal exposure of pyrene and B(a)P was monitored at three sites, forearm, neck, and upper leg, using exposure pads as pseudoabsorbing skin. The daily dermal dose was almost 5 times higher than the daily inhaled dose, 395 and 83 pg pyrene, respectively, per 8-h working period. The correlation between urinary 1-hydroxypyrene and pyrene in air was equal to or lower than the correlation between 1-hydroxypyrene and pyrene on skin pads. We concluded that dermal exposure contributes substantially to the body burden of pyrene and that dermal absorption may be an important modifying factor of the relation between air concentration and biomarkers of PAH. The scale of sampling of dermal exposure was too small to be a factor in the statistical analysis of this study.

References


Polycyclic aromatic hydrocarbon-DNA adducts in white blood cell DNA and 1-hydroxypyrene in the urine from aluminum workers: relation with job category and synergistic effect of smoking.


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