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Influence of genetic polymorphisms of biotransformation enzymes on gene mutations, strand breaks of deoxyribonucleic acid, and micronuclei in mononuclear blood cells and urinary 8-hydroxydeoxyguanosine in potroom workers exposed to polyaromatic hydrocarbons

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Objectives Airborne exposure to polycyclic aromatic hydrocarbons (PAH) in the potroom of an aluminum reduction plant was studied in relation to genotoxic or mutagenic effects, and the possibility of host genotypes of different metabolizing enzymes modifying associations between PAH exposure and genotoxic or mutagenic response was assessed.

Subjects and methods Ninety-eight male potroom workers and 55 male unexposed blue-collar workers constituted the study population. Micronuclei in CD4+ and CD8+ lymphocytes, DNA (deoxyribonucleic acid) single-strand breaks, hypoxanthine guanine phosphoribosyl transferase (HPRT) mutation frequency, and genotype for cytochrome P-4501A1, glutathione transferases M1, T1 and P1, and microsomal epoxide hydrolase were analyzed using peripheral mononuclear cells. Urine samples were collected for the analysis of 8-hydroxydeoxyguanosine.

Results Micronuclei in peripheral CD4+ and CD8+ lymphocytes, DNA single-strand breaks, HPRT mutation frequency, and 8-hydroxydeoxyguanosine in urine did not differ between the potroom workers and the unexposed referents. With the exception of an observed exposure-response relationship for potroom workers with Tyr/Tyr genotype for microsomal epoxide hydrolase, between airborne PAH and CD8+ micronuclei, no correlations were found between any of the genotoxicity biomarkers and any of the exposure measures (airborne particulate PAH, airborne gas phase PAH, length of employment in the potroom, 1-hydroxypyrene in urine, or PAH-DNA adducts in peripheral lymphocytes), also when genotypes for biotransformation enzymes were considered.

Conclusions The results indicate that the employed biomarkers of mutagenic or genotoxic effects are not appropriate for surveillance studies of potroom workers exposed to current airborne levels of PAH. The significance of the correlation between airborne PAH and CD8+ micronuclei in Tyr/Tyr genotype subjects should be evaluated.

Key terms aluminum reduction plant, biomarkers of genotoxic effect, cytochrome P-4501A1, glutathione transferases, microsomal epoxide hydrolase, peripheral lymphocytes.

Workers in potrooms of aluminum reduction plants are exposed to coal-tar pitch volatiles containing mutagenic and carcinogenic polycyclic aromatic hydrocarbons (PAH). There are convincing epidemiologic data showing increased risks for lung and bladder cancer in potroom workers (1). In a cohort of Canadian aluminum
smelter workers the increased risks for these tumors were associated with an estimated time-weighted average (TWA) airborne exposure of 10–20 µg/m³ to benzo[a]pyrene (BaP) (2, 3). The exposure levels have generally decreased since then. In our present study on potroom workers from a Swedish primary aluminum production plant, the median TWA BaP concentration in the breathing zone was only 1 µg/m³ (4). According to a risk assessment model derived from Canadian data (2), this exposure might, however, still result in a slightly less than doubled relative risk for bladder cancer. From a cancer preventive point of view there is therefore a need to survey workers exposed to such PAH levels.

The aim of the present study was to evaluate whether a median TWA airborne PAH exposure corresponding to 1 µg/m³ in the potroom of an aluminum reduction plant will result in genotoxic or mutagenic effects in the workers, when determined using a series of different effect biomarkers. Another aim was to assess whether the host genotype for different drug-metabolizing enzymes, such as cytochrome P-4501A1 (CYP1A1), glutathione transferase M1, T1 and P1 (GSTM1, GSTT1, GSTP1), and microsomal epoxide hydrolase (mEH), which are involved in the biotransformation of PAH (table 1), would modify the associations between exposure to PAH and genotoxic or mutagenic response.

### Subjects and methods

Ninety-eight male potroom workers from a Swedish primary aluminum Soderberg type of production plant constituted the exposed study group. The potroom workers were recruited from the 167 current workers. Of these 68 declined to participate or did not respond to the invitation. One subject had to be excluded due to a previous tumor diagnosis. The 55 male referents were blue-collar workers (mail carriers and city council employees) from the same town as the aluminum smelter, and they were not occupationally exposed to PAH during the preceding 5 years. All the subjects were interviewed by a physician (UC) with respect to medical history, smoking habits, alcohol consumption, earlier employment, and use of personal protection devices. Age, length of employment, and smoking habits are given for the study groups in table 2. Sixty-two percent of the potroom workers were using effective personal respiratory protection devices, and 38% used them only when they suspected that their work task might give high PAH exposures. Due to the policy of the plant, the use of respiratory protection devices had increased during the months preceding the examination. All the potroom workers used protective gloves and took a shower immediately after work. Personal measurements of airborne benzo[a]pyrene in the same plant a few years before the present study ranged from 1.9 to 36 (median 2.8) µg/m³ (5).

### Venous blood sampling

Venous blood samples were obtained in the morning, during an 8-week period. In most cases, the samples were drawn from both the potroom workers and the referents during the same mornings. The shift workers were sampled on the second or third workday morning after a preceding 6-day work leave. Blood samples for lymphocyte preparation were collected in heparinized Vacutainer®

### Table 1. Genotypes for biotransformation enzymes in the 98 potroom workers and in the 55 referents.

|                  | Potroom workers (N) | Referents (N) |
|------------------|---------------------|--------------|
| CYP1A1           |                     |              |
| Ile/Ile          | 91                  | 48           |
| Ile/Val          | 7                   | 7            |
| Val/Val          | 0                   | 0            |
| CYP1A1 (104)     |                     |              |
| m1/m1            | 80                  | 46           |
| m1/m2            | 15                  | 8            |
| m2/m2            | 3                   | 1            |
| GSTM1            |                     |              |
| -                | 52                  | 32           |
| +                | 46                  | 23           |
| GSTT1            |                     |              |
| -                | 17                  | 5            |
| +                | 81                  | 50           |
| GSTP1 (113)      |                     |              |
| Ala/Ala          | 85                  | 51           |
| Ala/Val          | 13                  | 4            |
| Val/Val          | 0                   | 0            |
| mEH (exon 3)     |                     |              |
| Tyr/Tyr          | 51                  | 24           |
| Tyr/His          | 37                  | 20           |
| His/His          | 10                  | 11           |
| mEH (exon 4)     |                     |              |
| His/His          | 61                  | 38           |
| His/Arg          | 35                  | 16           |
| Arg/Arg          | 2                   | 1            |

*These numbers differ somewhat with respect to each outcome variable, which is specified in the text or in the tables.

### Table 2. Age, employment time and smoking habits of the 98 potroom workers and the 55 referents.

|                  | Age (years) | Employment time in potroom (years) | Number of current smokers | Cigarettes smoked per day (smokers only) |
|------------------|-------------|-----------------------------------|---------------------------|-----------------------------------------|
| Potroom workers  | Median Range| Median Range                       | Median Range              | Median Range                            |
| Referents        | 35          | 22–60                             | 7.0                       | 1.3–32                                  |

50 | 30 | 10 | 40

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CPT™ tubes. Within 1.5 hours after the sampling, the tubes were centrifuged at 1500 g for 20 minutes at room temperature. The centrifugation separated the mononuclear cells. The blood samples were transported by air freight in cold boxes, and they reached the laboratories within 6 hours of the sampling.

**Urine sampling**

For the shift workers urine samples were collected in the morning before the shift and in the late evening after the shift on the 6th workday. For the daytime potroom workers, the urine samples were collected in the morning before work and in the afternoon after work, on the 4th or 5th workday of the week. For the referents urine samples were collected on the same morning as the blood samples were drawn.

**Isolation of deoxyribonucleic acid**

Deoxyribonucleic acid (DNA) from lymphocytes was prepared using a commercial kit based on solid phase, anion-exchange chromatography (QIAGEN Genomic-tip 100G).

**Cytochrome P-4501A1 genotype**

The polymerase chain reaction (PCR) analyses of the closely linked MspI and Ile/Val polymorphisms in cytochrome P-4501A1 (CYP1A1) were performed essentially as described by Hayashi et al (6). Detailed information of the PCR assay is given elsewhere (7). The MspI polymorphism in the 3'end of CYP1A1 is due to a base substitution (C→T) 264 base pairs downstream to the poly(A) signal. The absence or presence of a MspI restriction site identifies the ml and the m2 allele. PCR products including the MspI site were digested and subjected to agarose gel electrophoresis to identify the genotypes ml/ml, ml/m2, and m2/m2. The Ile/Val polymorphism in the 7th exon arises from a A→G base change, which results in a replacement of isoleucine by valine at residue 462 in the heme binding region of the enzyme. Two primers, differing by only 1 base in the 3'end according to the polymorphic site, were separately used together with a common upstream primer in an allele-specific PCR. The 2 resulting PCR products were subjected to agarose gel electrophoresis in parallel lanes to identify the Ile/Ile, Ile/Val, or Val/Val genotypes.

**Glutathione transferase M1 genotype**

Glutathione transferase M1 (GSTM1) genotyping was performed essentially according to methods described by Brockmoller and his collaborators (8). A detailed description has been given elsewhere (7). In addition, β-actin primers (Stratagene) were added to give a positive yield (868 bp) for the DNA samples. Deficiency in GSTM1 activity has been ascribed to a homozygous deletion of the gene. Consequently, the PCR assay detects the presence or absence of the GSTM1 gene but does not differentiate between the heterozygous and homozygous carriers. PCR products were analyzed by agarose gel electrophoresis.

**Glutathione transferase P1 genotype**

The Ile/Val polymorphism in exon 5 (amino acid 104) (9) was determined by PCR amplification with primers P1 (5'-AGTCTCTCATCCTCCACGC) and P2 (5'-CTCTATTCCATTTGCCTGACC), yielding a 950 bp fragment, and subsequent cleavage with restriction enzyme Alw26I. PCR reactions were carried out in a 20 μl volume containing 10 mM tris(hydroxymethyl)aminoethanehydrochloride acid (Tris/HCl), pH 8.4, 50 mM potassium chloride (KCl), 1.4 mM magnesium chloride (MgCl₂), 0.2 mM deoxynucleoside triphosphate (dNTP), 0.5 μM of each primer, 0.75 units of Taq DNA polymerase and 0.24 μg DNA. The PCR conditions were 94°C for 2 minutes, followed by 30 cycles of 94°C for 24 seconds, 60°C for 46 seconds, and 74°C for 1 minute. All the PCR runs had negative (no DNA) and positive controls. A 10-μl amount of each PCR product was digested with 1.5 units of Alw26I (MBI Fermentas) under standard conditions. The DNA fragments were separated on 1.8% agarose and visualized by ethidium bromide staining. Band lengths of 476 bp and 318 bp indicate homozygous Ile, while 318 bp, 254 bp and 222 bp indicate homozygous Val genotypes. Samples from persons with the heterozygous Ile/Val genotype give rise to fragments of 476 bp, 318 bp, 254 bp, and 222 bp.

The Ala/Val polymorphism in exon 6 (amino acid 113) was determined by allele-specific PCR with primers P1 (5'-AGCTCTCATCCTCCACGC) and P3(V) (5'-CATAGTCATCCTTGCCCA) or P4(A) (5'-CATAGTCATCCTTGCCCG), yielding a 1133 bp fragment. PCR reactions were carried out in a 20-μl volume containing 10 mM Tris/HCl, pH 8.4, 50 mM KCl, 1.6 mM MgCl₂, 0.2 mM dNTPs, 0.5 μM of each primer, 0.75 units of Taq DNA polymerase and 0.24 μg DNA. The PCR conditions were 94°C for 2 minutes, followed by 25 cycles of 94°C for 24 seconds, 66°C for 46 seconds, and 74°C for 1 minute. All the PCR runs had negative (no DNA) and positive controls.

**Glutathione transferase T1 genotype**

The persons who were glutathione transferase T1 (GSTT1) null were identified by PCR with β-actin as a positive control, as described previously (10, 11).

**Microsomal epoxide hydrolase genotype**

The Tyr/His polymorphism in exon 3 (amino acid 113) of microsomal epoxide hydrolase (mEH) was analyzed
by allele-specific PCR with primers EH1 (5'-GGTTTGCTGTTGTTTCTG) and EH2 (5'-AGCTTGAAGTTGGAGGTC), yielding a 259 bp fragment. PCR reactions were carried out in a 25 μl volume containing 10 mM Tris/HCl, pH 8.4, 50 mM KCl, 1.0 mM MgCl₂, 0.2 mM dNTPs, 0.5 μM of each primer, 0.75 units of Taq DNA polymerase and 0.3 μg DNA. The PCR conditions were 94°C for 2 minutes, followed by 27 cycles of 94°C for 24 seconds, 53°C for 46 seconds, and 72°C for 1 minute. All the PCR runs had negative (no DNA) and positive controls.

The His/Arg polymorphism in exon 4 (amino acid 139) was determined by PCR and restriction fragment length polymorphism (RFLP), as described by Hassett et al (12).

**Micronuclei in peripheral CD4+ and CD8+ lymphocytes**

Isolated mononuclear leukocytes were incubated in RPMI 1640 medium (RPMI = Roswell Park Memorial Institute) with 15% fetal calf serum for 72 h. Phytohemagglutinin (Gibco; 1 ml/100 ml medium) was used as a mitogen. After the incubation, the lymphocytes were separated by magnetic attraction into CD4+ and CD8+ fractions using Dynabeads, as previously described (13). The cells were then smeared on slides and stained with May-Grünewald Giemsa. Micronuclei were analyzed in the cytoplasm as described by Hogstedt (14). The aim was to analyze 3000 activated cells for each cell type. For CD4+ cells this goal was reached for 115 specimens, while for another 28 specimens the separation procedure could not be used. For CD8+ cells this goal was, however, reached only for 50 specimens, due to the separation procedure employed. For another 42 specimens, 1000 to 2999 cells were analyzed. Specimens for which less than 1000 cells could be analyzed were excluded from further analyses. Only 1, very experienced, scorer participated, and coded slides were used.

**Single-cell gel electrophoresis assay for strand breaks of deoxyribonucleic acid**

The mononuclear cells were embedded in 3 layers of agarose gel on glasses, which had been prepared with a layer of plastic film (GelBondFilm) in order to increase the adhesion of cells. The mononuclear cells were lysed in the gel by detergents with high salt concentrations. The unwinding of DNA was achieved in an alkaline solution during the following electrophoresis at 25 V and 300 mA. The cells were stained by ethidium bromide, which binds to DNA. Cell nuclei with DNA damage showed an increased level of single-stranded DNA outside the nucleus similar to a tail of a comet. Ninety-nine mononuclear cells were analyzed for each person. The cells were identified in a fluorescence microscope connected to a computer with an image analyzing program (AutoCell, Dynamic Data Links Ltd, Cambridge, United Kingdom), which automatically calculates the total area of each tail, its absolute average intensity, and its distance to the center position of the head. From these data the program calculates several parameters of DNA damage, of which we have used tail length, tail area, and tail inertia (ie, the moment of inertia of the tail). The used modification of the original method (15) has earlier been described by Hellman et al (16).

**Hypoxanthine guanine phosphoribosyl transferase mutant frequency**

The culture media used and the T-cell cloning procedure have been described in detail previously (17). Briefly, all the lymphocytes were washed in phosphate buffer saline (PBS) and stimulated for 44 hours with 0.3% phytohemagglutinin (Difco, USA) in RPMI 1640-based medium containing 5% fetal calf serum and 5% human serum. Two 96-well nonselection plates were then prepared by inoculating 1 or 2 target cells and 2x10⁴ lethally X-irradiated lymphoblastoid (RJK853) feeder cells in each well with a growth medium containing 20% T-cell growth-factor-enriched conditioned medium. The selection plates received 2x10⁴ target cells and 1x10⁴ feeder cells per well, and 6-thioguanine (Sigma) (2 μg/ml) was added to the growth medium. After 2 weeks, cell growth in the wells was scored visually using an inverted microscope. The cloning efficiency was calculated from the proportion of negative wells on the assumption of a Poisson distribution (18). Mutant frequency was obtained by

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### Table 3. Airborne particulate and gaseous phase total polycyclic aromatic hydrocarbons (PAH) in the respiratory zone during a full workday, 1-hydroxypyrene in urine sampled immediately after work, and PAH-DNA in peripheral mononuclear blood cells of the potroom workers and referents. a (DNA = deoribonucleic acid)

|                      | Potroom workers | Referent subjects |
|----------------------|-----------------|-------------------|
|                      | N    | Median | Range   | N    | Median | Range   |
| Airborne particulate PAH (μg/m³) | 97   | 13.2   | 0.01—270 | 3    | 0.11   | 0.01—0.37 |
| Airborne gaseous PAH (μg/m³) | 96   | 16.3   | 0.01—131 | 5    | 0.20   | 0.008—0.41 |
| 1-Hydroxypyrene in urine after work (μmol/mol creatinine) | 96   | 4.31   | 0.09—17.7 | 5    | 0.13   | 0.06—0.75 |
| PAH-DNA-adducts in mononuclear blood cells (per 10⁴ nucleotides) | 88   | 2.62   | 1.44—6.87 | 53   | 2.47   | 1.28—8.88 |

a Data originally published by Carstensen et al (4).
dividing the cloning efficiency in the presence of 6-thioguanine with that in the absence of 6-thioguanine (mean value).

8-Hydroxydeoxyguanosine in urine
8-hydroxydeoxyguanosine (8OHdG) in urine was determined by automated coupled-column high-performance liquid chromatography and electrochemical detection as described elsewhere (19, 20). The values were corrected to a urine density of 1.024.

Statistical methods
Mann-Whitney’s U-test and Joncheere-Terpstra’s test were used for group-wise comparisons, and the correlations were assessed by Spearman’s rank test. Multiple linear regression models for log-transformed outcome variables were used to adjust for confounders and to identify interactions. The P-values reported are 2-sided.

Results
As presented in more detail elsewhere (4), the respiratory zone samples from the potroom workers contained, as a median, 13.2 μg/m^{3} of 22 airborne particulate PAH congeners and 16.3 μg/m^{3} of 7 gas phase PAH congeners, which was 2 orders of magnitude higher than for samples from the referents (table 3). Moreover, the median urinary concentration of 1-hydroxypyrene in the urine sampled immediately after work was 4.31 μmol/mol creatinine, as compared with 0.13 μmol/mol creatinine among the referents. In contrast, the PAH-DNA adducts did not differ between the potroom workers and referents.

Age was weakly correlated with the micronucleus frequency of CD4\(^+\) in both the potroom workers (r\(_a\)=0.33, P=0.002) and the referents (r\(_r\)=0.30, P=0.03). The corresponding figures for CD8\(^+\) cell micronuclei were r\(_a\)=0.45 (P<0.001) and r\(_r\)=0.38 (P=0.06), respectively. The smokers had significantly higher frequencies of micronuclei in their CD4\(^+\) lymphocytes (P=0.03), but only among the potroom workers (table 4). Smoking did not affect the frequencies of micronuclei in the CD8\(^+\) lymphocytes.

The frequencies of micronuclei in peripheral CD4\(^+\) and CD8\(^+\) lymphocytes did not differ between the potroom workers and the referents (table 4). However, among the potroom workers, the micronuclei frequencies of the CD8\(^+\) lymphocytes, but not of the

| Table 4. Micronuclei in peripheral CD4\(^+\) and CD8\(^+\) lymphocytes (per 1000 cells) in the potroom workers and referents, with respect to smoking habits. |
|---|---|---|---|
| Potroom workers | N | Median | Range | N | Median | Range | P-value\(^b\) |
| CD4\(^+\) | Smokers | 30 | 1.0 | 0.0—2.7 | 11 | 0.7 | 0.0—1.7 | 0.10 |
| | Nonsmokers | 62 | 0.7 | 0.0—2.7 | 40 | 0.7 | 0.0—2.5 | 0.4 |
| | All | 92 | 0.7 | 0.0—2.7 | 51 | 0.7 | 0.0—2.5 | 0.08 |
| | P-value\(^a\) | | | | | | | 0.03 |
| | | | | | | | >0.5 |
| CD8\(^+\) | Smokers | 24 | 0.7 | 0.0—4.7 | 5 | 0.8 | 0.0—1.3 | >0.5 |
| | Nonsmokers | 42 | 0.6 | 0.0—2.3 | 20 | 0.8 | 0.0—3.0 | 0.07 |
| | All | 66 | 0.7 | 0.0—4.7 | 26 | 0.8 | 0.0—3.0 | 0.11 |
| | P-value\(^a\) | | | | | | | 0.37 |
| | | | | | | | >0.5 |
| a | Comparison between the potroom workers and the referents. |
| b | Comparison between the smokers and nonsmokers. |

Table 5. Associations between airborne particulate polycyclic aromatic hydrocarbons (PAH) and micronuclei in CD8\(^+\) lymphocytes in 66 potroom workers, adjusted for age in a multiple linear regression model, stratified for microsomal epoxide hydrolase (mEH) genotype. The 95% confidence intervals (95% CI) for the regression coefficients and the proportions of variance (r\(^2\)) explained by the variables are given.

| mEH Tyr/Tyr genotype | Regression coefficient | 95%CI | P-value |
|---|---|---|---|
| Particulate PAH | 0.02 | 0.01—0.04 | 0.001 |
| Age | 0.01 | -0.004—0.02 | 0.16 |

| mEH Tyr/His or His/His genotype | Regression coefficient | 95%CI | P-value |
|---|---|---|---|
| Particulate PAH | 0.003 | -0.01—0.004 | 0.4 |
| Age | 0.05 | 0.01—0.09 | 0.01 |
| r\(^2\) | | | 0.23 |
CD4+ lymphocytes, were significantly correlated with both airborne particulate PAH (r=0.32, P=0.01), airborne gas phase PAH (r=0.32, P=0.01), and the length of employment in the potroom (r=0.26, P=0.04), but not with 1-hydroxypyrene in urine or PAH-DNA adducts. After adjustment for age in a multiple regression analysis, these associations disappeared for the 30 potworkers with the mEH (exon 3) Tyr/His or His/His genotypes but, with respect to airborne PAH, remained for the 35 workers with the Tyr/Tyr genotype (particulate PAH, P=0.001; gas phase PAH P<0.001) (table 5). None of the other genotypes for the biotransformation enzymes interacted with the micronuclei frequency.

The frequency of DNA strand breaks in peripheral mononuclear blood cells, in the single cell gel electrophoresis (SCGE) assay, can be expressed in several ways. The median values for the tail area, tail length, or tail inertia did not differ significantly between the potroom workers and the referents, irrespective of whether the analysis was stratified for smoking or not (table 6). Moreover, among the potroom workers, no correlations were found between any of the exposure measures and any of the measures of DNA strand breaks. In the total group, the 26 subjects with a m1/m2 or m2/m2 genotype for CYP1A1 showed increased median tail inertias in the SCGE assay (P=0.05), as compared with the 122 with a m1/m1 genotype. Neither age, smoking habits, nor any of the other genotypes significantly correlated with the occurrence of DNA strand breaks. With respect to the median tail inertias, there were no statistically significant interactions between any of the exposure measures and the genotype for CYP1A1.

The HPRT (hypoxanthine guanine phosphoribosyl transferase) mutant frequency in the peripheral lymphocytes was only analyzed for a consecutive series of the 58 potroom workers and 33 referents first sampled. No significant difference in the HPRT mutant frequency could be observed between the exposed workers and the referents.
The median TWA BaP concentration in the breathing zone was 1 µg/m³ among the examined potroom workers (4). This level represents a hundredfold increase if the potroom workers are compared with the referents. The exposure contrast was similar for total PAH also.

In spite of the contrast in PAH exposure, no significant genotoxic or mutagenic effects were observed in the potroom workers as compared with the referents. However, among the potroom workers an exposure-response relationship was observed between airborne PAH and CD8+ micronuclei, but only for subjects with the Tyr/Tyr genotype for mEH. The lack of more substantial genotoxic effects was not due to a effective usage of personal protection devices among the potroom workers, as we also observed 30-fold higher urinary concentrations of 1-hydroxypyrene for the potroom workers when compared with the referents. This result cannot be explained by the choice of reference group either. The age distribution and smoking prevalence were similar for the groups. Moreover, the referents were blue-collar workers recruited from the same town as the potroom workers, but without any occupational PAH exposure. We also do not believe that the lack of substantial genotoxic effects in our study was due to a lack of statistical power. The number of subjects studied was large enough to permit detection of even moderate increases in the rates of the biological end points.

Previous cyto genetic studies on potroom workers have not shown any increased rates of chromosome aberrations (21) or sister chromatid exchanges (22) in peripheral lymphocytes. The micronucleus assay has, however, never before been employed on potroom workers. The results from micronucleus studies on other PAH-exposed groups provide a contrast. In coke-oven workers (22–24) and graphite electrode plant workers (22), there were no increased micronuclei frequencies in peripheral lymphocytes. In 1 study of chimney sweeps (25), but not in another (7), an increased frequency of micronuclei was observed. In the latter study the micronuclei frequency was, however, correlated with the levels of aromatic DNA adducts (26). In previous studies the effect of smoking on the micronucleus frequency was the most pronounced in CD8+ lymphocytes (27, 28), which raised the hypothesis that some subclasses of lymphocytes are more susceptible to micronucleus formation after exposure to xenobiotics. In contrast, we observed a more pronounced effect of smoking on the CD4+ micronuclei, however only in exposed subjects, in our study. Among the exposed subjects an exposure-response relationship was found between airborne PAH and CD8+ micronuclei, but only in potroom workers with the Tyr/Tyr genotype for mEH. The combined influence of the lymphocyte subclass analysis and mEH genotype on micronuclei frequency has not been evaluated previously. Whether the present result is of any biological significance or a random finding has to be assessed in future studies.

The SCGE assay has thus far only been employed in a few studies assessing genotoxic effects in exposed workers. Styrene-exposed workers had significantly higher levels of DNA strand breaks than factory controls (29), but no significant difference was found between workers exposed to 1,3-butadiene and unexposed referents.

**Discussion**

The median TWA BaP concentration in the breathing zone was 1 µg/m³ among the examined potroom workers (4). This level represents a hundredfold increase if the potroom workers are compared with the referents. The exposure contrast was similar for total PAH also.

In spite of the contrast in PAH exposure, no significant genotoxic or mutagenic effects were observed in the referents (medians 5.4 versus 5.6×10⁶) (table 7). Age was correlated with the HPRT mutant frequency (t=0.28, P=0.007). Moreover, the smokers had a higher HPRT mutant frequency than the nonsmokers (P=0.02). The 13 subjects with the His/His genotype for mEH (exon 3) had a median HPRT mutant frequency of 8.7×10⁶, as compared with 5.5×10⁴ for the 37 with Tyr/His, and 5.1×10⁶ for the 41 persons with the Tyr/Tyr genotype (P=0.02). None of the other genotypes correlated with the HPRT mutant frequency. A weak but significant univariate correlation was seen between 1 of the exposure measures, length of employment in the potroom, and the HPRT mutant frequency (t=0.26, P=0.05), but when adjusted for age, smoking habits, and mEH (exon 3) in a multiple linear regression model, no significant effect of the exposure variable remained.

The concentration of 80HdG in the preshift urine did not differ significantly between the 90 potroom workers [median 22.3 (range 8—56) nmol/l] and the 50 referents [median 19.5 (range 10—55) nmol/l, P=0.04]. This effect was restricted to the nonsmokers (medians 22.8 and 18.7 nmol/l, P=0.02) and was not seen at all among the smokers (P>0.5). It was also observed that the 73 subjects with the GSTM1 null genotype had a higher concentration of 80HdG in urine. However, the 79 subjects with a GSTM1 null genotype had a higher concentration of 80HdG in urine than the 61 subjects with at least 1 copy of the GSTM1 gene (medians 22.9 and 19.6 nmol/l, P=0.04). This effect was restricted to the nonsmokers (medians 22.8 and 18.7 nmol/l, P=0.02) and was not seen at all among the smokers (P>0.5). It was also observed that the 73 subjects with the GSTP1 (amino acid 104) Ile/Val or Val/Val genotypes had higher 80HdG concentrations than the 67 subjects with the Ile/Ile genotype (medians 23.1 and 20.0 nmol/l, P=0.05). This effect was restricted to the nonsmokers (medians 23.2 and 18.9 nmol/l, P=0.01) and was not seen at all among the smokers (P>0.5). None of the other genotypes for drug-metabolizing enzymes correlated with 80HdG in urine. There were no statistically significant interactions between any of the exposure measures and the genotype for GSTM1 or GSTP1 with respect to 80HdG in urine.
We did not find higher HPRT mutation frequencies among the potroom workers than among the unexposed referents. Neither was there any correlation between aromatic DNA adducts and HPRT mutation frequency. This result is in contrast with the findings among considerably less exposed foundry workers (<0.005 to 0.06 μg BaP/m³), for whom a dose-response association for HPRT mutation frequency was found (32). In the foundry study a significant positive correlation was also seen between PAH-DNA adducts, measured by an immunoassay, and HPRT mutation frequency. Moreover, when the examination was repeated a year later, the airborne BaP levels had decreased in parallel with a decrease in the HPRT mutation frequency (33). Bus maintenance workers exposed to diesel exhausts containing PAH had significantly higher aromatic DNA adduct levels than unexposed referents, but these groups did not differ with respect to HPRT frequency. Overall, a significant increase in HPRT mutant frequency with adduct level was, however, observed in the bus maintenance workers (34). In agreement with earlier reports (review in reference 35), our study showed weak positive correlations between both age and smoking, and the HPRT mutation frequency.

8OHdG is a chemical modification of DNA caused by reactive oxygen species in the cell, and its presence can be used as a biomarker for oxidative stress. Increased 8OHdG levels in urine have previously been found among workers in the asbestos, rubber, and azo dye industries (36). A correlation has also been found between urinary 8OHdG and exposure to benzene (37, 38). The 8OHdG assay has not previously been used as a biomarker for occupational PAH exposure. The presently observed lack of association between any indicator of PAH exposure and the excretion of 8OHdG in urine indicates that this assay may not be useful for the surveillance of PAH-exposed workers, at least not potroom workers. Loft et al (39) found that smoking was an important determinant for 8OHdG, but, on the other hand, several other studies showed little influence of smoking on urinary 8OHdG (20, 36–38). In our investigation, we could not detect any association between smoking and the excretion of 8OHdG.

The question of whether environmental carcinogenesis is modulated by host polymorphism is currently under extensive investigation. The enzymes of interest in the context of exposure to PAH include CYP1A1, mEH, GSTM1, and GSTP1, which are involved in the activation of PAH to reactive epoxides and their inactivation through hydrolysis or conjugation with glutathione. We also included another polymorphic glutathione transferase, GSTT1, in this investigation, an enzyme which catalyzes the glutathione conjugation of biologically active epoxides like butadiene-derived epoxides and ethylene oxide (40, 41). In previous studies on lymphocytes from PAH-exposed chimney sweeps and bus garage workers, as already mentioned, correlations were observed between micronuclei and aromatic DNA adducts and between HPRT mutations and aromatic DNA adducts (26, 34). In the study on chimney sweeps, the correlation between aromatic DNA adduct levels and micronuclei was the most marked for people lacking the GSTM1 gene (26). The GSTM1 null genotype seemed thus to confer higher susceptibility to PAH. The findings were interpreted to indicate an underlying variation in susceptibility to PAH between people and to explain some of the difficulties in establishing exposure-dependent increases in biological effects. Levels of cytogenetic damage in peripheral lymphocytes from smokers have, in some studies, been found to be higher in donors with the GSTM1 null genotype when compared with GSTM1-positive donors (42, 43).

In a recent study by Rojas et al, the influence of biotransformation polymorphisms on the formation of anti-benz[a]pyrene diol-epoxide adducts in DNA from smokers was shown (44). Their work presents very convincing evidence for the involvement of GSTM1 in the protection against DNA damage caused by PAH exposure. In a total of 40 individual DNA samples — from lung tissue from smoking lung cancer patients and white blood cells from PAH-exposed coke oven workers — no adducts were observed in any of the 23 persons carrying active GSTM1 genes, and all persons with the GSTM1 null genotype had measurable levels of DNA adducts. In contrast to the work by Rojas and his colleagues, no increased adduct levels were observed among the PAH-exposed workers in our study (4), and we also did not observe correlations between PAH adducts and any of the effect markers. Biotransformation genotypes did, however, influence the urinary excretion of 1-hydroxypyrene in our study (Alexandrie et al, unpublished observations), but there were no significant interactions of these genotypes on the associations between the exposure measures and any of the biological effect markers.

As detailed in the Results section, some additional findings were made with regard to biotransformation genotypes and the occurrence of micronuclei, DNA strand breaks, HPRT mutations, and 8OHdG in both the exposed and unexposed subjects. The statistically
significant associations observed may be explained as chance findings, depending on the large number of statistical comparisons made, but they may also indicate some inherited differences in susceptibility to DNA damage. Biomarker studies with a large number of persons, like this study, will be needed to establish such potential differences in susceptibility.

In conclusion, none of the tested biomarkers for mutagenic or genotoxic effects was increased at current exposure levels for PAH in Swedish potroom workers. This finding indicates that these biomarkers are not appropriate for further surveillance studies of such a workplace. The present mainly negative results are, however, not very surprising, as positive findings have been rare in previous studies on potroom workers. This is a puzzle because the PAH exposure for this work group has generally been higher than for other exposed groups in which genotoxic effects have been observed. There is no obvious explanation for this discrepancy. Whether the observed exposure-response relationship in potroom workers with Tyr/Tyr genotype for mEH, between airborne PAH and CD8+ micronuclei, is of any biological significance has to be assessed in other studies.

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