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Benzo(*a*)pyrene diolepoxide adducts to albumin in workers exposed to polycyclic aromatic hydrocarbons: association with specific *CYP1A1*, *GSTM1*, *GSTP1* and *EHPX* genotypes

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We investigated whether the presence of (+)-*anti*-benzo(*a*)pyrene diolepoxide adducts to serum albumin (BPDE–SA) among workers exposed to benzo(*a*)pyrene (BaP) and unexposed reference controls was influenced by genetic polymorphisms of cytochrome P4501A1 (*CYP1A1*), microsomal epoxide hydrolase (*EHPX*), glutathione *S*-transferases M1 (*GSTM1*) and P1 (*GSTP1*), all involved in BaP metabolism. Exposed workers had significantly higher levels of adducts (0.124 ± 0.02 fmol BPT mg^{-1} SA, mean ± SE) and a higher proportion of detectable adducts (40.3%) than controls (0.051 ± 0.01 fmol BPT mg^{-1} SA; 16.1%) ($p = 0.014$ and $p = 0.012$). Smoking increased adduct levels only in occupationally exposed workers with the *GSTM1* deletion (*GSTM1* null) ($p = 0.034$). Smokers from the exposed group had higher adduct levels when they were *CYP1A1 *1/*1* wild-type rather than heterozygous and homozygous for the variant alleles (*CYP1A1 *1/*2* plus **2/*2*) ($p = 0.01$). The dependence of BPDE–SA adduct levels and frequency on the *CYP1A1 *1/*1* genotype was most pronounced in *GSTM1*-deficient smokers. Exposed workers with *GSTM1* null/*GSTP1* variant alleles had fewer detectable adducts than those with the *GSTM1* null/*GSTP1*A* wild-type allele, supporting for the first time the recent *in vitro* finding that *GSTP1* variants may be more effective in the detoxification of BPDE than the wild-type allele. Logistic regression analysis indicated that occupational exposure, wild-type *CYP1A1*1/*1* allele and the combination of *GSTM1* null genotype + *EHPX* genotypes associated with predicted low enzyme activity were significant predictors of BPDE–SA adducts. Though our findings should be viewed with caution because of the relatively limited size of the population analysed, the interaction between these polymorphic enzymes and BPDE–SA adducts seems to be specific for high exposure and might have an impact on the quantitative risk estimates for exposure to polycyclic aromatic hydrocarbons.

Keywords: benzo(*a*)pyrene diolepoxide adducts, occupational exposure, genetic polymorphism.

Introduction

Epidemiological evidence suggests that occupational exposure to mixtures of chemicals containing benzo(*a*)pyrene (BaP) and other polycyclic aromatic hydrocarbons (PAH) may be linked to an increase in the risk of mortality from

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neoplasms (WHO 1984, Boffetta *et al*. 1997). Consequently many different approaches have been proposed for assessing exposure to PAH in the workplace.

Dosimetry based solely on PAH ambient levels and PAH metabolites in body fluids has been recently complemented by measurements of covalent adducts between reactive PAH metabolites and DNA or blood proteins, with the aim of assessing the biologically effective dose and improving quantitative risk assessment at the individual level (Day *et al*. 1990, Skipper and Tannenbaum 1990, dell'Omo and Lauwerys 1993). The best known PAH is BaP, often used as a model compound for PAH exposure. BaP is metabolically activated to (+) *r*-7,*t*-8-dihydroxy-*t*-9,*t*-10-epoxy-7,8,9,10-tetrahydobenzo(*a*)pyrene (BPDE), the ultimate carcinogenic metabolite able to bind covalently to DNA and blood proteins (Gelboin 1980, Naylor *et al*. 1990, Skipper and Tannenbaum 1990). We recently showed that BPDE adducts to haemoglobin and albumin can be successfully monitored in populations with different levels of BaP exposure, but concentrations of adducts can vary considerably between persons with apparently similar exposure (Pastorelli *et al*. 1996, 1998, 2000). Although this variability may indicate imprecise estimates of PAH exposure, a significant proportion apparently comes from individual biological factors such as differences in metabolism (Wormhoudt *et al*. 1999).

The conversion of BaP into reactive BPDE is dependent on a cascade of biotransformations, including BaP oxidation by cytochrome P4501A1 (CYP1A1) into benzo(*a*)pyrene-7,8-oxide, hydration by microsomal epoxide hydrolase (mEH) and a final P450-dependent oxidation step giving rise to the highly carcinogenic BPDE. Intermediate metabolites and BPDE itself can be detoxified through different pathways like conjugation with glutathione, catalysed by the glutathione *S*-transferase superfamily (GST), which includes the isozymes GSTM1 and GSTP1.

The genes encoding the enzymes mentioned have been found to be polymorphic in humans, with relevance to the risk of cancer (WHO 1999). The *GSTM1* gene encodes for the detoxification enzyme GSTM1 and is deleted in 50% of the Caucasian population. The inherited absence of the *GSTM1* gene (the *GSTM1* null genotype) has been associated with an increased risk of several forms of cancer (Ketterer *et al*. 1992, WHO 1999) and its influence on various biomarkers of PAH exposure has been widely studied (Autrup *et al*. 1995, Binkova *et al*. 1998, Schoket *et al*. 1998, Alexandrie *et al*. 2000).

The *GSTP1* gene was recently found to be polymorphic in amino acid positions 105 and 114. Four alleles have been described: the wild-type *GSTP1*A* allele and three variant alleles, *GST1P1*B*, *GSTP1*C*, *GSTP1*D* (Ali-Osman *et al*. 1997, Watson *et al*. 1998). *GSTP1*B* differs from the wild-type by a single A → G transition at codon 105, resulting in an isoleucine:valine amino acid change (val105/ala114). In addition to possessing the A → G transition at codon 105, the *GSTP1*C* allele also contains a C → T transition at codon 114 which results in an alanine:valine amino acid change (val105/val114). *GSTP1*D* is a rare allele containing the wild-type codon 105 sequence and the polymorphic C → T transition at codon 114 (ile105/val114).

In vitro studies have recently shown that the variants of *GSTP1* differ in catalysing the detoxification of BPDE (Hu *et al*. 1997a,b, 1999), but no data are available on their *in vivo* effect. Thus their true importance is not yet fully understood, although an increased frequency of *GSTP1* variant alleles was

observed among patients suffering from lung cancer, bladder cancer and other smoking-related diseases (Harris *et al.* 1998, Jourenkova-Mironova *et al.* 1998, Matthias *et al.* 1998).

Another critical polymorphic gene in BaP metabolism, associated with an increased risk of smoking-related cancers (Benhamou *et al.* 1998, Jourenkova-Mironova *et al.* 2000), is the *EHPX* gene coding for the mEH. Its activity can be affected by two point mutations: one, at amino acid position 113 in exon 3, changes the tyrosine residue to histidine (Tyr113His) reducing the enzyme activity by at least 50% *in vitro*, the other, at amino acid position 139 in exon 4, changes histidine to arginine (His139Arg), increasing the enzyme activity by at least 25% (Hassett *et al.* 1994). There appear to be large individual differences in mEH activity in the human population, ranging from several times to 40-fold in various human tissues (Omiecinski *et al.* 1993). The mutant alleles have been related to an increased risk of developing aflatoxin B1–albumin adducts (McGlynn *et al.* 1995) and to an increased mutant frequency of the *HPRT* locus in exposed workers (Viezzer *et al.* 1999). We have found that *EHPX* variants may influence BPDE–SA adducts in lung cancer patients (Pastorelli *et al.* 1998), but few studies have addressed their impact on occupational PAH exposure biomarkers, even though mEH is an important control point for directing the pathway of BaP detoxification/activation.

However, the question whether metabolic polymorphisms act as modifiers of exposure biomarkers has been widely investigated for the *CYP1A1* gene which contributes to the bioactivation of BaP and other PAH. The *CYP1A1* Ile-Val (*m2*) mutation in the haem-binding region doubles the microsomal enzyme activity and it is in linkage disequilibrium in Caucasians with the *CYP1A1 Msp I* (*m1*) mutation which has also been associated experimentally with increased catalytic activity (Landi *et al.* 1994, Bartsch *et al.* 2000).

Positive associations between the presence of these variant alleles and increased PAH–DNA adducts have been reported but so have negative or weak associations (Mooney *et al.* 1997, Butkiewicz *et al.* 1998, Pastorelli *et al.* 1998, Rojas *et al.* 1998, Schoket *et al.* 1998). We found no published information about the relationship between combinations of these genotypes and PAH-related protein adducts. Although protein adducts are not considered to be directly involved in carcinogenesis, they indicate that exposure has occurred and a fraction of the carcinogen has reacted with endogenous macromolecules. Therefore the influence of inherited metabolic capabilities on the adduct profile may affect the quantitative risk estimates for PAH exposure.

The aim of the present study was to examine whether BPDE–SA adducts vary with *GSTM1*, *GSTP1*, *CYP1A1* and *EHPX* genotype, alone or in combination, as part of an ongoing comprehensive evaluation of biological markers among workers occupationally-exposed to PAH.

Materials and methods

Subjects and sample collection

The study was conducted in a graphite-electrode-producing plant in Central Italy, where 129 male workers were enrolled, all occupationally exposed to PAH. As a control group, 82 unexposed males were initially recruited from the technical and maintenance staff of the University of Perugia, Central Italy.

Each subject answered a structured questionnaire about personal data, occupation and smoking habits. As an indication of cumulative smoking exposure, pack-years were computed as the average number of packs smoked per day multiplied by years of smoking.

Table 1. Main characteristics of the reference group and graphite electrode plant workers

	Reference subjects	Exposed workers
Number	31	124
Age (mean years ± SD)	42.61 ± 6.10	43.91 ± 5.01
(range)	30.3–52.5	29.92–55.01
Smokers (%)	45.2	48.4
Pack-years (mean ± SD)	18.6 ± 14.3	24.2 ± 14.3
Cigarettes per day (mean ± SD)	15.43 ± 1	18.8 ± 1

Informed consent was obtained from all individuals enrolled in the study and a blood sample was collected at the end of a work shift, after at least four working days in the week. Samples for BPDE–SA adduct analysis were only available for 96% PAH-exposed workers and 38% reference subjects. Therefore the final study population consisted of 124 exposed workers and 31 controls, whose main characteristics are reported in table 1. Mean age and prevalence of smokers were similar in the control group and in the exposed workers. Duration of occupational exposure to PAH was 19.9 ± 5.2 years (mean ± SD).

Current individual exposure to PAH was assessed by measuring urinary 1-hydroxypyrene (1-HOP) in reference subjects and workers. Air concentrations of PAH, including BaP, in the workplace were determined. An extensive presentation of the work environment analyses will be given elsewhere (dell'Omo *et al.*, manuscript in preparation). Recruitment, questionnaire administration, and biological sampling were done by the Institute of Occupational Medicine and Toxicology, University of Perugia.

Immediately after blood withdrawal, plasma was separated by centrifugation and total DNA was extracted from peripheral blood using standard techniques. Plasma and DNA samples were stored at −80°C and then shipped on dry ice to the Department of Environmental Health Sciences, Istituto di Ricerche Farmacologiche Mario Negri (Milan), for BPDE–SA adducts and genotype analysis.

Determination of BPDE-SA adducts

BPDE adducts were analysed as benzo(*a*)pyrene tetrols (BPTs) released from serum albumin (SA) after acid hydrolysis and quantitated by high-resolution gas chromatography–negative ion chemical ionization–mass spectrometry with selected ion recording after Extrelut extraction and immunoaffinity purification, as described previously (Pastorelli *et al.* 1996, 2000). The detection limit was $\leq$0.05 fmol BPT mg^{-1} SA.

GSTM1,GSTP1, CYP1A1 and EHPX genotyping

Since DNA was not available for one exposed worker, genotyping was done on 123 exposed workers.

GSTM1 and *EHPX* gene were co-amplified by multiplex PCR reaction. The reaction mixture (50 µl total volume) consisted of 100–200 ng genomic DNA, 0.1 µM *GSTM1* primers (Bell *et al.* 1992), 1 µM *EHPX* exon 3 primers (Smith and Harrison 1997), 0.2 µM *EHPX* exon 4 primers (Gaedigk *et al.* 1994), 0.25 mM dNTPs, 10 mM Tris-HCl (pH9), 50 mM KCl, 2 mM MgCl2, 2 U Taq DNA polymerase. The initial denaturation step (94°C, 5 min) was followed by 35 cycles of melting (94°C, 30 s), annealing (61°C, 45 s), elongation (72°C, 45 s) and a final extension at 72°C for 5 min. The amplification of *EHPX* exons 3 and 4 was detected by the presence of bands at 162 and 381 bp respectively. The *GSTM1* polymorphism was detected by the presence or absence of a band at 215 bp.

The *EHPX* genotypes ascribed to exon 3 and exon 4 mutations were identified by restriction fragment length polymorphism (RFLP) analysis, carrying a single double-digestion reaction. PCR aliquots (15 µl) underwent simultaneous *EcoR* V and *Rsa* I restriction enzyme digestion in the appropriate buffer with bovine serum albumin (0.1 mg ml^{-1}) at 37°C for 5 h. Samples were then analysed by electrophoresis through a non-denaturating 8% polyacrylamide gel.

Individuals were screened for *GSTP1* codon 105 and codon 114 polymorphisms using PCR-restriction length polymorphism (PCR-RFLP) analysis as described by Harris *et al.* (1998). Data from individual PCR-RFLP analysis of both polymorphic sites were combined to determine the presence of the wild-type allele *GSTP1*A* (105ile/114ala,) and the three allelic variants *GSTP1*B* (105val/114ala), *GSTP1*C* (105val/114val) and *GSTP1*D* (105ile/114val) as describe in Park *et al.* (1999) for haplotype deduction. Individuals whose *GSTP1* genotypes were heterozygous or homozygous variants were termed *GSTP1* variant subjects, and individuals homozygous for the wild-type allele were *GSTP1* AA.

The T → C mutation (*m1*) in the 3′-flanking region of *CYP1A1* gene was detected by PCR–RFLP analysis using the enzyme *Msp* I. The allele carrying only this mutation was termed **2A*. The *CYP1A1* Ile/Val replacement (*m2*) was detected by the genotyping method, using *Bsr*DI–RFLP analysis (Cascorbi *et al*. 1996). The allele with only this mutation was termed **2C*. An allele with *m1* plus *m2* was termed **2B* (Ingelman-Sundberg *et al*. 2000).

Statistical analysis

To compute statistics for adduct values, subjects with unmeasurable levels were considered as having half the minimum detectable value.

Group differences in BPDE–SA adduct levels were tested by the Mann–Whitney two-tailed *U* test or Kruskal–Wallis test, as appropriate. Fisher's exact test was used to test the association between genotypes and adduct frequency dichotomized in undetectable and detectable.

The presence or absence of BPDE–SA adducts was modelled as a function of *GSTM1*, *GSTP1*, *CYP1A1*, *EHPX* status, smoking, exposure and age using logistic regression. Statistical analysis were done by SPSS 9.0 software (SPSS 9.0. SPSS Inc., Chicago, USA 1999).

Results

Analysis of BPDE–SA adducts

The average BPDE–SA adduct level in exposed workers was significantly higher (0.124 ± 0.02 fmol BPT mg^{-1} SA, mean ± SE) than in controls (0.051 ± 0.01 fmol BPT mg^{-1} SA, mean ± SE) ($p = 0.014$) as shown in figure 1. Adducts were detectable in 40.3% (50/124) exposed workers and in 16.1% (5/31) controls, the difference being significant ($p = 0.012$). In the exposed group, BPDE–SA adduct levels did not significantly correlate with the duration of occupational exposure.

As shown in figure 2, smoking did not affect the levels of adducts in the study population. Smokers and non-smoker controls had similar levels of adducts (0.053 ± 0.01 fmol BPT mg^{-1} SA and 0.049 ± 0.02 fmol BPT mg^{-1} SA, mean ± SE,

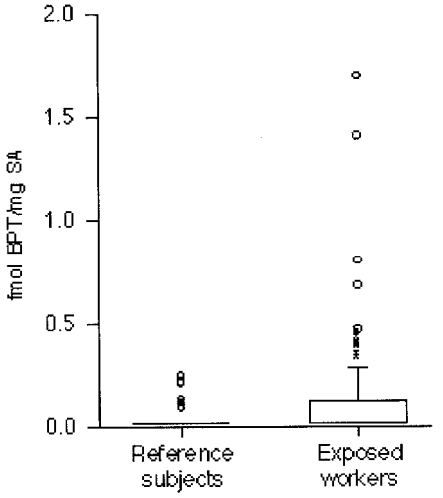

Figure 1. Box plots representing the levels of BPDE–SA adducts (fmol BPT mg^{-1} SA) in reference subjects ($n = 31$) and graphite electrode plant workers ($n = 124$). The box encompasses the 25th and 75th percentiles. Whiskers extend to the highest and lowest levels that are not outliers. *, outliers >1.5 box length from the 75th percentile. °, outliers >3 box length from the 75th percentile. BPDE–SA levels in reference subjects vs exposed workers: $p = 0.014$, Mann–Whitney two-tailed *U* test.

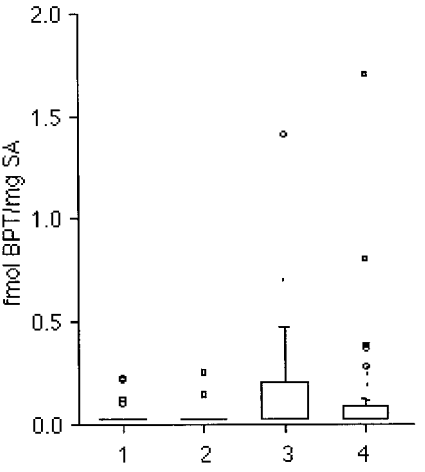

Figure 2. Box plots representing the levels of BPDE–SA adducts (fmol BPT mg^{-1} SA) among reference subjects and graphite electrode plant workers, according to their smoking habits. The box encompasses the 25th and 75th percentiles. Whiskers extend to the highest and lowest levels that are not outliers. *, outliers >1.5 box length from the 75th percentile. °, outliers >3 box length from the 75th percentile. 1: non-smokers reference subjects, 17 subjects, 2: smokers reference subjects, 14 subjects; 3: non-smoker exposed workers, 64 subjects; 4: smoker exposed workers, 60 subjects.

respectively). However, among exposed workers, non-smokers had a slightly but not significantly higher level of adducts (0.139 ± 0.02 fmol BPT mg^{-1} SA, mean ± SE) than smokers (0.107 ± 0.03 fmol BPT mg^{-1} SA, mean ± SE; $p = 0.085$), whose frequency of measurable adducts (33.3%) was slightly lower than non-smokers (46.9%) ($p = 0.124$).

Effect of GSTM1, EHPX and CYP1A1 genotype on BPDE–SA adducts

Table 2 shows the mean levels of BPDE–SA adducts and the frequency of detectable adducts according to *GSTM1* and *GSTP1* genotypes in controls and the exposed group.

In the reference group none of the genotypes analysed had any significance influence either on BPDE–SA adduct levels or frequency of detectable adducts. In the exposed workers, *GSTM1* null individuals had a slightly higher level of BPDE–SA adducts and a higher percentage of detectable adducts than those with the *GSTM1* positive genotype, though these differences did not reach statistical significance ($p = 0.0791$ and $p = 0.094$ respectively).

In reference subjects and exposed workers, individuals homozygous for the *GSTP1*A* wild-type allele (*GSTP1* AA) and those whose *GSTP1* genotypes were heterozygous or homozygous variants (*GSTP1* variants) had similar levels and frequency of detectable adducts. Considering each variant separately (*GSTP1*B*, *GSTP1*C*, *GSTP1*D*) there were no noteworthy effects on adduct levels or proportion of detectable adducts.

When *GSTM1* and *GSTP1* genotypes were analysed in combination, the frequency of detectable adducts was doubled in exposed workers with the *GSTM1* null/*GSTP1* AA genotype compared to the *GSTM1* positive/*GSTP1*

Table 2. BPDE–SA adduct levels and frequency of detectable adducts in relation to *GSTM1* and *GSTP1* genotypes in reference subjects and exposed workers

Genotype	Reference subjects		Exposed workers	
	BPT fmol ml^{-1} SA, mean ± SE	Frequency of detectable adducts	BPT fmol ml^{-1} SA, mean ± SE	Frequency of detectable adducts
GSTM1 null	0.044 ± 0.014 (15)	13.3%	0.156 ± 0.039 (50)	50.0%
GSTM1 positive	0.062 ± 0.018 (16)	18.8%	0.104 ± 0.023 (73)	33.0%
GSTP1 AA	0.042 ± 0.012 (12)	16.7%	0.103 ± 0.025 (63)	41.3%
GSTP1 Var[a]	0.061 ± 0.017 (19)	21.05%	0.148 ± 0.034 (60)	40.0%
GSTM1 null + *GSTP1* AA	0.037 ± 0.011 (8)	12.5%	0.084 ± 0.018 (20)	60.0%[b]
GSTM1 positive + *GSTP1* AA	0.053 ± 0.028 (4)	25.0%	0.112 ± 0.033 (43)	32.6%
GSTM1 null + *GSTP1* Var	0.053 ± 0.028 (7)	14.3%	0.203 ± 0.062 (30)	43.3%
GSTM1 positive + *GSTP1* Var	0.065 ± 0.023 (12)	25.0%	0.093 ± 0.023 (30)	36.7%

[a] *GSTP1* Var: individuals heterozygous for one polymorphic allele and individuals whose *GSTP1* genotypes are homozygous variants, with both alleles comprising the *GSTP1*B*, *GSTP1*C*, and/or *GSTP1*D* alleles. *GSTP1* AA are individuals homozygous for the wild-type allele *GSTP1*A*.

[b] Frequency of detectable adducts in exposed workers *GSTM1* null *GSTP1* AA vs *GSTM1* positive + *GSTP1* AA: $p = 0.05$, Fisher's exact two-tailed test.

None of the other differences were significant.

AA genotype ($p = 0.05$). In those with the *GSTP1* variants, the *GSTM1* genotype had no influence.

No influence of the *GSTM1* genotype was observed on formation of adducts in the reference group stratified by smoking habits (smokers with *GSTM1* null genotype: <0.05 fmol BPT mg^{-1} SA, with *GSTM1* positive genotype: 0.063 ± 0.027 fmol BPT mg^{-1} SA; non-smokers with *GSTM1* null genotype: 0.054 ± 0.021 fmol BPT mg^{-1} SA, with *GSTM1* positive genotype: 0.062 ± 0.027 fmol BPT mg^{-1} SA, mean ± SE). On the contrary, smoking-exposed workers with *GSTM1* null genotype had significantly higher adduct levels (0.210 ± 0.085 fmol BPT mg^{-1} SA, mean ± SE) than the *GSTM1* active genotype (0.052 ± 0.01 fmol BPT mg^{-1} SA, mean ± SE) ($p = 0.034$). Adducts were more frequent in smokers with the *GSTM1* null genotype (47.62%) than those with *GSTM1* positive (25.64%) but this tendency was not statistically significant ($p = 0.096$). None of the *GSTP1* genotypes had any significant influence either on BPDE–SA adduct levels or detectable adducts frequency (data not shown).

Table 3 shows the impact of *EHPX* genotypes on BPDE–SA adduct levels and frequency in the study population. These polymorphisms did not appear to influence the adducts, even when subjects were grouped by the three predicted EHPX enzymatic activity levels assigned according to current knowledge of the *in vitro* functional expression of variant alleles, as reported by Benhamou *et al.* (1998). When smoking habits were considered, the predicted levels of EHPX activity did not affect BPDE–SA adducts.

Interestingly, *Msp I* analysis of the *CYP1A1* gene indicated that in exposed workers the homozygous wild type genotype was associated with approximately double levels of adducts and the proportion of positive adducts was higher than in

Table 3. BPDE–SA adduct levels and frequency of detectable adducts in relation to *EHPX* genotypes in reference subjects and exposed workers

Genotype	Reference subjects		Exposed workers	
	BPT fmol ml^{-1} SA, mean ± SE	Frequency of detectable adducts	BPT fmol ml^{-1} SA, mean ± SE	Frequency of detectable adducts
EHPX				
Tyr113Tyr	n.d.[a] (9)	0%	0.125 ± 0.043 (33)	33.3%
Tyr113His	0.072 ± 0.018 (19)	26.0%	0.124 ± 0.029 (71)	42.2%
His113His	n.d.[a] (3)	0%	0.127 ± 0.035 (19)	47.4%
His129His	0.048 ± 0.031 (20)	15.0%	0.133 ± 0.025 (82)	41.5%
His139Arg			0.120 ± 0.042 (36)	41.5%
Arg139Arg	0.064 ± 0.02 (11)[b]	27.3%[b]	0.037 ± 0.012 (5)	20.0%
EHPX activity[c]				
High	n.d.[a] (4)	0%	0.141 ± 0.072 (19)	37.0%
Intermediate	0.064 ± 0.02 (11)	27.3%	0.093 ± 0.022 (36)	39.0%
Low	0.053 ± 0.016 (16)	18.7%	0.138 ± 0.03 (68)	43.0%

Number of individuals analysed are in parentheses.

[a] Not detectable; this group was considered as having 0.025 fmol BPT mg^{-1} SA (half the minimum detectable value).

[b] *His139Arg* + *Arg139Arg*, since only one control was *Arg139Arg*.

[c] High activity: individuals with *Tyr113Tyr* and *His139Arg* genotypes, or *Tyr113Tyr* and *Arg139Arg* genotypes or *Tyr113His* and *Arg139Arg*. Intermediate activity: individuals with *Tyr113Tyr* and *His139His* genotypes, or *Tyr113His* and *His139Arg* genotypes, or *His113His* and *Arg139Arg* genotypes. Low activity: individuals with *His113His* and *His139His* genotypes, *His113His* and *His139Arg* genotypes or *Tyr113His* and *His139His* genotypes (25).

None of the differences were significant.

those with at least one copy of the variant allele ($p = 0.0461$ and $p = 0.037$ respectively) (table 4). The Ile/Val polymorphism of the *CYP1A1* gene did not show any influence on BPDE–SA adduct levels and frequency. When both these polymorphisms were analysed together, controls did not show any differences in levels and proportion of BPDE–SA adduct, whereas exposed workers with no mutated alleles (*CYP1A1*1/*1*) had significantly higher BPDE adduct level than those with at least one mutated allele (*CYP1A1*1/*2* and *CYP1A1*2/*2*) ($p = 0.033$). When the adduct levels were classified as non-detectable and detectable, the percentage of measurable adducts was significantly higher in wild-type *CYP1A1*1/*1* subjects than in those with heterozygous and homozygous variants *CYP1A*1/*2* and *CYP1A1*2/*2* ($p = 0.023$).

Evidence of a *CYP1A1* genotype dosage effect among smokers was provided by the 3.4 times higher BPDE–SA adduct levels in carriers of the *CYP1A1*1/*1* genotype (43 subjects) (0.134 ± 0.043 fmol BPT mg^{-1} SA, mean ± SE) compared with those having at least one copy of the variants alleles (17 subjects) (0.040 ± 0.020 fmol BPT mg^{-1} SA, mean ± SE) ($p = 0.01$). This effect was not observed among non-smokers, where *CYP1A1*1/*1* individuals (46 subjects) had levels of adducts (0.151 ± 0.035 fmol BPT mg^{-1} SA, mean ± SE) similar to individuals with *CYP1A1* variants (17 subjects) (0.112 ± 0.035 fmol BPT mg^{-1} SA, mean ± SE).

When *GSTM1* and *CYP1A1* were analysed in combination, no effect on BPDE–SA adduct levels and frequency was observed in reference subjects (table 5). However the level of adducts and proportion of detectable adducts was

Table 4. BPDE–SA adduct levels and frequency of detectable adducts in reference subjects and exposed workers grouped according to *CYP1A1* genotypes and alleles combination

Genotype	Reference subjects		Exposed workers	
	BPT fmol ml^{-1} SA, mean ± SE	Frequency of detectable adducts	BPT fmol ml^{-1} SA, mean ± SE	Frequency of detectable adducts
CYP1A1				
Msp/RFLP				
w1/m1 + m1/m1	0.048 ± 0.02 (5)	20.0%	0.078 ± 0.02[c] (33)	24.2%[d]
w1/w1	0.055 ± 0.013 (26)	15.4%	0.143 ± 0.027 (90)	46.7%
IIe/Val RFLP				
w2/m2 + m2/m2	n.d.[a] (3)	0%	0.112 ± 0.042 (13)	38.5%
w2/w2	0.057 ± 0.013 (28)	17.9%	0.127 ± 0.023 (110)	41.0%
**1/*1*	0.056 ± 0.014 (25)	20.0%	0.144 ± 0.028[e] (89)	47.0%[f]
**1/*2*plus **2/*2*[b]	0.044 ± 0.019 (6)	16.7%	0.076 ± 0.02 (34)	23.5%

Number of individuals analysed are in parentheses.

[a] Not detectable; this group was considered as having 0.025 fmol BPT mg^{-1} SA (half the minimum detectable value).

[b] *1/*2 includes *1/*2A, *1/*2B, *1/*2C; *2/*2 includes *2A/2A, *2B/*2B, *2A/*2B.

[c] BPDE–SA adduct levels in exposed workers *CYP1A1 w1/m1 + m1/m1* vs *w1/w1*: $p = 0.046$, Mann–Whitney two-tailed *U* test.

[d] Frequency of detectable adducts in exposed workers *CYP1A1 w1/m1 + m1/m1* vs *w1/w1:* $p = 0.037$, Fisher's exact test, two-tailed.

[e] BPDE–SA adduct levels in exposed workers *CYP1A1*1/*1* vs *CYP1A1*1/*2* plus**2/*2:* $p = 0.033$, Mann–Whitney two-tailed *U* test.

[f] Frequency of detectable adducts in exposed workers *CYP1A1*1/*1* vs *CYP1A1*1/*2* plus**2/*2:* $p = 0.023$, Fisher's exact test, two-tailed.

None of the other differences were significant.

Table 5. Influence of combined *GSTM1* genotype and *CYP1A1* alleles on BPDE–SA adduct levels and frequency of detectable adducts in reference subjects and exposed workers

GSTM1/CYP1A1 genotype combinations	Reference subjects		Exposed workers	
	fmol BPT mg^{-1} SA, mean ± SE	Frequency of detectable adducts	fmol BPT mg^{-1} SA, mean ± SE	Frequency of detectable adducts
1. *GSTM1* null + *CYP1A1 *1/*1*	0.049 ± 0.017 (12)	16.7%	0.175 ± 0.049[b] (38)	55.26%[c]
2. All others	0.056 ± 0.016 (19)	21.05%	0.103 ± 0.020 (85)	34.12%
Smokers				
3. *GSTM1* null + *CYP1A1 *1/*1*	n.d.[a] (4)	0%	0.266 ± 0.116[d] (15)	60.00%[e]
4. All others	0.059 ± 0.024 (10)	20%	0.054 ± 0.010 (45)	24.44%
Non-smokers				
5. *GSTM1* null + *CYP1A1 *1/*1*	0.061 ± 0.026 (8)	25%	0.155 ± 0.026 (23)	52.17%
6. All others	0.054 ± 0.021 (9)	22%	0.157 ± 0.041 (40)	45.00%

Number of individuals analysed are in parentheses.

[a] Not detectable; this group was considered as having 0.025 fmol BPT mg^{-1} SA (half the minimum detectable value).

[b,c] 1 vs 2, $p = 0.03$, Mann–Whitney two-tailed *U* test and $p = 0.03$, Fisher's exact test, respectively.

[d,e] 3 vs 4, $p = 0.004$, Mann–Whitney two-tailed *U* test and $p = 0.024$, Fisher's exact test, respectively.

None of the other differences were significant.

Table 6. Influence of combined *GSTM1* genotype and predicted *EHPX* enzyme activity on BPDE-adduct levels and frequency of positive adducts in reference subjects and exposed workers

	Reference subjects		Exposed workers	
	fmol BPT mg^{-1} SA, mean ± SE	Frequency of detectable adducts	fmol BPT mg^{-1} SA, mean ± SE	Frequency of detectable adducts
1. *GSTM1* null + EHPX low	0.049 ± 0.02 (8)	12.5%	0.219 ± 0.063[b] (29)	62.0%[c]
2. All others[a]	0.055 ± 0.01 (23)	21.7%	0.096 ± 0.018 (94)	34.0%

Number of individuals analysed are in parentheses.
[a] Including *GSTM1* null + EHPX high/intermediate activity, *GSTM1* positive + EHPX low activity, *GSTM1* positive + EHPX high/intermediate activity.
[b] 1 vs 2, $p = 0.003$, Mann–Whitney two-tailed U test.
[c] 1 vs 2 $p = 0.01$, Fisher's exact test.

significantly higher in exposed workers with the *GSTM1* null/*CYP1A1*1/*1* genotype. Furthermore, *GSTM1* null/*CYP1A1*1/*1* workers who smoked had approximately a six times higher average adduct level and 2.5 times higher frequency of detectable adducts than those with other *GSTM1*/ *CYP1A1* combinations. In non-smoking workers BPDE–SA adducts did not show any *GSTM1*/*CYP1A1* genotype dependence.

Among exposed workers those with the *GSTM1* null genotype combined with *EHPX* genotypes associated with low enzyme activity had significantly higher adduct levels and a higher frequency of measurable adducts than those with all other possible genotypes combinations, as shown in table 6. No gene-related influence was observed in controls.

Furthermore, exposed workers with the GST*M1* null genotype + EHPX predicted low activity and no variants in *CYP1A1* alleles (22 subjects) had double the concentrations of adducts (0.250 ± 0.080 fmol BPT mg^{-1} SA, mean ± SE) of individuals with all other possible combinations of the three genes (101 subjects) (0.098 ± 0.018 fmol BPT mg^{-1} SA, mean ± SE) ($p = 0.002$) (figure 3). Again, BPDE–SA adducts were more frequent in workers with this three-genes combinations genotype (68.18%) than in those with all other combinations (34.65%) ($p = 0.007$).

Multivariate analysis of BPDE–SA adduct levels

Logistic regression analysis for the presence or absence of detectable adducts with the variable PAH exposure, smoking, age, *GSTM1*, *GSTP1*, *CYP1A1*, *EHPX* status, indicated that exposure and *CYP1A1* dichotomized as wild-type *CYP1A1 *1/*1* versus *CYP1A1 *1/*2* plus **2/*2* (heterozygous plus homozygous variants) had a significant effect ($p = 0.02$ and $p = 0.025$, respectively). No other variables influenced the presence or absence of measurable adducts in the overall population.

Combined genotypes were then included in the regression model to explore gene–gene interactions. This analysis indicated that the combination of the *GSTM1* null genotype + EHPX predicted low activity genotypes ($p = 0.032$) was a significant predictor of the presence of adducts.

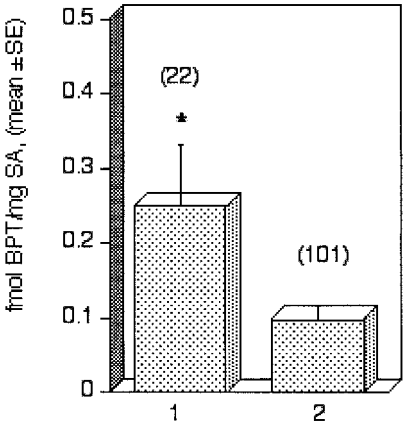

Figure 3. BPDE–SA adduct levels (fmol BPT mg^{-1} SA) in exposed workers, in relation to combined *CYP1A1*, *GSTM1* and predicted EHPX enzyme activity genotypes. 1: *CYP1A1*1*/**1* + *GSTM1* null + *EHPX* low activity genotypes, 2: all other possible genotype combinations. Number of individuals analysed are in parentheses. $*p = 0.002$, Mann–Whitney two-tailed *U* test.

Discussion

Influence of occupational exposure on BPDE–SA adducts

In the present study we found that the percentage of detectable BPDE–SA adducts and their levels were significantly higher (about 2.5 times) in workers than in controls, in accordance with previous reports of higher concentrations of BaP-derived albumin adducts in occupationally PAH exposed subjects (Sherson *et al.* 1990, Tas *et al.* 1994). However, the BPDE–SA adduct levels we found were lower than those reported by these authors. This could be mainly due to the fact that the BaP concentrations in air samples from our graphite-electrode-producing plant were generally lower (range 0.005–3.11 μg B(*a*)P m^{-3}) (dell'Omo *et al.*, manuscript in preparation) than those reported for instance by Tas *et al.* (1994), who gave a range of 0.002–72.91 μg BaP m^{-3} in steel foundries and graphite electrode plant.

Methodological aspects might also explain the low levels of adducts we reported. Using gas chromatography–mass spectrometry, we were able to measure BPT as the exact chemical species responsible for SA adduction, whereas methods like immunoassays or HPLC with fluorescence detection, used by other authors, lack chemical specificity, measuring several types of PAH adducts.

The lack of association between BPDE–SA adducts and smoking comes as no surprise, since we already reported that in non-occupationally exposed subjects who were moderate smokers (average of 15 cigarettes per day) BPDE–SA adduct levels (0.09 ± 0.02 fmol BPT mg^{-1} SA) were not really different from those in non-smokers (0.06 ± 0.01 fmol BPT mg^{-1} SA) (Pastorelli *et al.* 2000). These figures are similar to those observed in the control subjects in this study. The lack of association between cigarette smoking status and BPDE–SA adducts has also been observed when SA adducts were formed by a different BPDE enantiomer (Ozbal *et al.* 2000).

The present and other studies found that BPDE protein adduct levels in workers exposed to PAH are not significantly affected by smoking (Sherson *et al.* 1990, Omland *et al.* 1994). It is reasonable to assume that the estimated BaP

inhaled by a moderate smoker (15 cigarettes per day = 0.3–0.6 μg BaP day^{-1}) might be masked by the amount of BaP inhaled by workers (2.9 μg BaP day^{-1}, due to an average airborne exposure of 0.145 μg BaP m^{-3}). However, in exposed workers, we found slightly higher levels and proportion of measureable adducts in non-smokers than smokers, though this difference was not statistically significant. Whether this reflects some interference from different components of smoke with enzyme activities, or different metabolic pathways related to the route of BaP exposure, remains to be seen.

Influence of GSTM1,GSTP1, CYP1A1 and EHPX genotypes on BPDE–SA adducts

The main point of interest in the present study was to investigate the effects of four metabolic susceptibility genes involved in BaP metabolism on BPDE–SA adduct formation, in order to understand whether the wide variability of this biomarker could be ascribed to specific genetic makeup. However, when measuring formation of adducts, it is necessary to stress that the metabolic activities of enzymes, closely involved in adducts' formation, may not always be the reflection of genetic variants (Wormhoudt *et al*. 1999).

The distribution of *GSTM1*, *GSTP1*, *CYP1A1* and *EHPX* genotypes was similar in reference subjects and exposed workers and agreed with the frequencies described in the literature for the Caucasian population (Ketterer *et al*. 1992, Benhamou *et al*. 1998, Harris *et al*. 1998).

When subjects were stratified on the basis of exposure, gene-related effects on adduct formation emerged only in occupationally-exposed individuals. The homozygous deletion of *GSTM1* increased the level of adducts in workers who smoked, but not in non-smokers.

A similar gene–dosage effect of smoking was observed by Butkiewicz *et al*. (1998) who found high PAH–DNA adduct levels in granulocytes from healthy smokers with the *GSTM1* null genotype. Viezzer *et al*. (1999) observed a similar *GSTM1* genotype effect on PAH–DNA adducts exclusively in coke-oven workers who smoked. Unfortunately there are no studies investigating the impact of metabolic gene polymorphisms on biologically effective dose markers such as blood protein adducts. The only data relate to heavy smokers lung cancer patients who had no differences in BPDE–SA adduct levels in relation to *GSTM1* genotype, possibly because of the small sample size (Pastorelli *et al*. 1998). The sources and routes of exposure might also have influenced the results. Smoking lung cancer patients presumably received most of their PAH by inhalation (tobacco-related exposure), whereas workers who smoke are exposed to PAH not only through inhalation but also by skin penetration and possible ingestion of particulate-bound PAH.

We found that in workers with the *GSTM1* deletion, the frequency of detectable adducts was double in those homozygous for the *GSTP1*A* wild-type allele, even though the statistical significance was modest. In carriers of *GSTP1* variants, the *GSTM1* genotype did not have any influence. This is particularly interesting because it suggests for the first time that the *GSTP1*A* wild-type allele might become a host factor that increases the presence of BPDE–SA adducts only when *GSTM1* is not active. Such a genotype combination is in apparent contrast with the findings of Butkiewitz *et al*. (2000) and Ryberg *et al*.

(1997) who reported a significant increase of PAH-related DNA adducts in *GSTM1* null subjects with the *GSTP1* variants.

Although different kinds of biomarkers, like DNA and blood proteins, may be differently modulated by polymorphic genes (Pastorelli *et al*. 1998), a possible explanation of the discrepancy could also come from the recent *in vitro* finding that the allelic variants of *GSTP1* differ in their activity toward the non-planar PAH diol-epoxide and the planar PAH diol-epoxide such as (+) *anti*-BPDE (Hu *et al*. 1997a, 1999). The presence of the polymorphic sites on the *GSTP1* gene is more effective than the wild-type allele in catalysing the GSH conjugation of (+) *anti*-BPDE. This protective role of *GSTP1* variants may therefore emerge only when the (+) *anti*-BPDE adduct is specifically detected as with our method, and not when other less specific methods are employed.

Another interesting aspect of the current study is the negative effect on adduct levels driven by *CYP1A1* variants (*CYP1A1 *1/*2* plus **2/*2*). This was mostly evident in exposed workers, where those having at least one copy of the variant alleles had significantly lower adduct levels and a smaller proportion of detectable adducts than carriers of the *CYP1A1 *1/*1* genotype. The presence of the wild-type *CYP1A1 *1/*1* was another factor that only increased BPDE–SA adduct levels in exposed workers who smoked.

Our findings on *CYP1A1*'s effect on adduct levels was unexpected, since we recently reported that in lung cancer patients the proportion of detectable BPDE–SA adducts was higher among those with the *CYP1A1* variants genotype (*CYP1A1 *1/*2* plus **2/*2*). Similarly, Rojas *et al*. (2000) showed that coke oven workers with the *CYP1A1* variants genotype had higher BPDE–DNA adduct levels, although the difference was significant only for detectable adducts. However, the relationship between *CYP1A1* variants and the formation of PAH adducts is still controversial. Several studies have reported a weak or no effect of *CYP1A1* polymorphism on adduct levels (Shields *et al*. 1993, Hemminki *et al*. 1997, Schoket *et al*. 1998), whereas Ichiba *et al*. (1994) and Rothman *et al*. (1995) found significantly higher bulky-DNA adducts in chimney sweepers and fire-fighters with the CYP1A1 **1/*1* genotype than in workers carrying the *Msp I* mutation, in accordance with the results of the present study.

CYP1A1 polymorphism is often explained as enhanced inducibility, leading to higher enzymatic activity to activate precarcinogens. Consequently the activation of BaP to the ultimate carcinogenic *anti*-BPDE might be enhanced by the presence of *CYP1A1* variants. But *CYP1A1* is considered to be primarily an extrahepatic enzyme, so its apparently enhanced activity in extrahepatic districts might be responsible for the smaller amount of BPDE in hepatocytes and lower concentrations of BPDE–SA adducts. It should be emphasized that the pathway mediated by *CYP1A1* is one of several in the metabolism of BaP. Therefore a high concentration of PAH in the environment, such as those encountered by these workers, might induce different metabolic pathways leading to less BPDE being available for SA adduction.

We found that the dependence of BPDE–SA adduct levels and frequency on the *CYP1A1 *1/*1* genotype was strongest in *GSTM1*-deficient smokers who were occupationally-exposed to PAH, supporting the evidence that the *GSTM1* null genotype might become a strong susceptibility factor in the presence of certain gene–gene and environment combinations (Ketterer *et al*. 1992).

When occupational and tobacco-related PAH exposures were considered together, BPDE–SA adduct formation was mainly influenced by an unfavourable detoxifying genotype. The mechanistic background of this effect is still not clear, but smoking habits might confer a unique susceptibility on individuals with an inefficient detoxifying mechanism, who are occupationally-exposed to PAH, probably by interfering with enzymes involved in PAH activation or detoxification pathways.

A further important observation is the dependence of the presence of BPDE–SA adducts on the *GSTM1* deletion genotype combined with *EHPX* genotypes associated with low enzyme activity. Although it is plausible to relate increased adduct formation with decreased *GSTM1* activity , it is far from clear why the genotypes associated with putative EHPX low activity, potentially responsible for decreased BPDE formation, should lead more BPDE–SA adducts. One possible explanation is the weak correlation between *EHPX* polymorphism and enzyme activity. Recent studies were unable to assign hepatic EHPX protein/enzymatic activity levels solely on the basis of the two polymorphic loci (Hassett *et al.* 1997, Laurenzana *et al.* 1998), suggesting that additional factors regulate *EHPX* expression and phenotype. Thus, *GSTM1* deletion may become a predictor of increased BPDE–SA adduct levels in the presence of *EHPX* genotypes combinations that could be linked to some still unknown determinants of EHPX activity. No such interaction of genes was observed in our previous study, where lung cancer patients had similar levels and frequency of BPDE–SA adducts, regardless of the *GSTM1*–EHPX predicted enzymatic activity status (Pastorelli *et al.* 1998). This might be due to the lower frequency of *EHPX* genotypes associated with low enzymatic activity found in our lung cancer patients (38%) compared with the workers in the current study (55.3%), as expected from the different *EHPX* genotypes distribution in cancer patients and healthy subjects (Benhamou *et al.* 1998). On the other hand, the different effects on the same biomarker by the same genotype combination may depend on the PAH exposure route and the PAH mixture, which are presumably different in occupationally exposed workers and lung cancer patients, who were mainly exposed to tobacco-related PAH.

In conclusion, this study indicated that the most important determinants for the presence of BPDE–SA adducts were not only occupational PAH exposure, but also the *CYP1A1* wild-type (*CYP1A1*1/*1*) allele and the combined *GSTM1* deletion-*EHPX* putative low activity genotypes. Such factors may, at least partly, explain the wide interindividual differences in adduct levels in the population studied.

One limitation of this study is the relatively small size of the analysed population. Because of the multiple comparison performed, our findings should be viewed with caution and as hypothesis-generating. Nevertheless, the data suggest that integration for BPDE–SA adducts with inherited metabolic traits might be relevant to the validation of this biomarker in risk assessment of PAH exposure.

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References

Alexandrie, A. K., Warholm, M., Carstensen, U., Axmon, A., Hagmar, L., Levin, J. O., Ostman, C. and Rannug, A. 2000, CYP1A1 and GSTM1 polymorphisms affect urinary 1-hydroxypyrene levels after PAH exposure. *Carcinogenesis*, **21**, 669–676.

Ali-Osman, F., Akande, O., Antoun, G., Mao, J. X. and Buolamwini, J. 1997, Molecular cloning, characterization, and expression in *Escherichia coli* of full-length cDNAs of three human glutathione *S*-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. *Journal of Biological Chemistry*, **272**, 10004–10012.

Autrup, H., Vestergaard, A. B. and Okkels, H. 1995, Transplacental transfer of environmental genotoxins: polycyclic aromatic hydrocarbon-albumin in non-smoking women, and the effect of maternal GSTM1 genotype. *Carcinogenesis*, **16**, 1305–1309.

Bartsch, H., Nair, U., Risch, A., Rojas, M., Wikman, H. and Alexandrov, K. 2000, Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. *Cancer Epidemiology Biomarkers & Prevention*, **9**, 3–28.

Bell, D. A., Thompson, C. L., Taylor, J., Miller, C. R., Perera, F., Hsieh, L. L. and Lucier, G. W. 1992, Genetic monitoring of human polymorphic cancer susceptibility genes by polymerase chain reaction: application to glutathione transferase mu. *Environmental Health Perspectives*, **98**, 113–117.

Benhamou, S., Reinikainen, M., Bouchardy, C., Dayer, P. and Hirvonen, A. 1998, Association between lung cancer and microsomal epoxide hydrolase genotypes. *Cancer Research*, **58**, 5291–5293.

Binkova, B., Topinka, J., Mrackova, G., Gajdosova, D., Vidova, P., Stavkova, Z., Peterka, V., Pilcik, T., Rimar, V., Dobias, L., Farmer, P. B. and Sram, R. J. 1998, Coke oven workers study: the effect of exposure and GSTM1 and NAT2 genotypes on DNA adduct levels in white blood cells and lymphocytes as determined by 32P-postlabelling. *Mutation Research*, **416**, 67–84.

Boffetta, P., Jourenkova, N. and Gustavsson, P. 1997, Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. *Cancer Causes and Control*, **8**, 444–472.

Butkiewicz, D., Grzybowska, E., Hemminki, K., Ovrebo, S., Haugen, A., Motykiewicz, G. and Chorazy, M. 1998, Modulation of DNA adduct levels in human mononuclear white blood cells and granulocytes by CYP1A1 CYP2D6 and GSTM1 genetic polymorphisms. *Mutation Research*, **415**, 97–108.

Butkiewicz, D., Grzybowska, E., Phillips, D. H., Hemminki, K. and Chorazy, M. 2000, Polymorphisms of the GSTP1 and GSTM1 genes and PAH–DNA adducts in human mononuclear white blood cells. *Environmental Molecular Mutagenesis*, **35**, 99–105.

Cascorbi, I., Brockmoller, J. and Roots, I. 1996, A C4887A polymorphism in exon 7 of human CYP1A1: population frequency, mutation linkages, and impact on lung cancer susceptibility. *Cancer Research*, **56**, 4965–4969.

Day, B. W., Naylor, S., Gan, L. S., Sahali, Y., Nguyen, T. T., Skipper, P. L., Wishnok, J. S. and Tannenbaum, S. R. 1990, Molecular dosimetry of polycyclic aromatic hydrocarbon epoxides and diol epoxides via hemoglobin adducts. *Cancer Research*, **50**, 4611–4618.

Dellomo, M. and Lauwerys, R. R. 1993, Adducts to macromolecules in the biological monitoring of workers exposed to polycyclic aromatic hydrocarbons. *Critical Reviews in Toxicology*, **23**, 111–126.

Gaedigk, A., Spielberg, S. P. and Grant, D. M. 1994, Characterization of the microsomal epoxide hydrolase gene in patients with anticonvulsant adverse drug reactions. *Pharmacogenetics*, **4**, 142–153.

Gelboin, H. V. 1980, Benzo[*alpha*]pyrene metabolism, activation and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. *Physiological Reveiws*, **60**, 1107–1166.

Harris, M. J., Coggan, M., Langton, L., Wilson, S. R. and Board, P. G. 1998, Polymorphism of the Pi class glutathione S-transferase in normal populations and cancer patients. *Pharmacogenetics*, **8**, 27–31.

Hassett, C., Aicher, L., Sidhu, J. S. and Omiecinski, C. J. 1994, Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants [published erratum appears in *Hum Mol Genet* 1994 Jul;**3**(7):1214]. *Human Molecular Genetics*, **3**, 421–428.

Hassett, C., Lin, J., Carty, C. L., Laurenzana, E. M. and Omiecinski, C. J. 1997, Human hepatic microsomal epoxide hydrolase: comparative analysis of polymorphic expression. *Archives of Biochemistry and Biophyisics*, **337**, 275–283.

Hemminki, K., Dickey, C., Karlsson, S., Bell, D., Hsu, Y., Tsai, W. Y., Mooney, L. A., Savela, K. and Perera, F. P. 1997, Aromatic DNA adducts in foundry workers in relation to exposure, life style and CYP1A1 and glutathione transferase M1 genotype. *Carcinogenesis*, **18**, 345–350.

Hu, X., Ji, X., Srivastava, S. K., Xia, H., Awasthi, S., Nanduri, B., Awasthi, Y. C., Zimniak, P. and Singh, S. V. 1997a, Mechanism of differential catalytic efficiency of two polymorphic forms of human glutathione *S*-transferase P1-1 in the glutathione conjugation of carcinogenic diol epoxide of chrysene. *Archives of Biochemistry and Biophyisics*, **345**, 32–38.

Hu, X., Xia, H., Srivastava, S. K., Herzog, C., Awasthi, Y. C., Ji, X., Zimniak, P. and Singh, S. V. 1997b, Activity of four allelic forms of glutathione *S*-transferase hGSTP1-1 for diol epoxides of polycyclic aromatic hydrocarbons. *Biochemical and Biophysical Research Communications*, **238**, 397–402.

Hu, X., Herzog, C., Zimniak, P. and Singh, S. V. 1999, Differential protection against benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide-induced DNA damage in HepG2 cells stably transfected with allelic variants of pi class human glutathione *S*-transferase. *Cancer Research*, **59**, 2358–2362.

Ichiba, M., Hagmar, L., Rannug, A., Hogstedt, B., Alexandrie, A. K., Carstensen, U. and Hemminki, K. 1994, Aromatic DNA adducts, micronuclei and genetic polymorphism for CYP1A1 and GST1 in chimney sweeps. *Carcinogenesis*, **15**, 1347–1352.

Ingelman-Sundberg, M., Daly, A. K., Oscarson, M. and Nebert, D. W. 2000, Human cytochrome P450 (CYP) genes: recommendations for the nomenclature of alleles [letter]. *Pharmacogenetics*, **10**, 91–93.

Jourenkova-Mironova, N., Wikman, H., Bouchardy, C., Voho, A., Dayer, P., Benhamou, S. and Hirvonen, A. 1998, Role of glutathione *S*-transferase GSTM1, GSTM3, GSTP1 and GSTT1 genotypes in modulating susceptibility to smoking-related lung cancer. *Pharmacogenetics*, **8**, 495–502.

Jourenkova-Mironova, N., Mitrunen, K., Bouchardy, C., Dayer, P., Benhamou, S. and Hirvonen, A. 2000, High-activity microsomal epoxide hydrolase genotypes and the risk of oral, pharynx, and larynx cancers. *Cancer Research*, **60**, 534–536.

Ketterer, B., Harris, J. M., Talaska, G., Meyer, D. J., Pemble, S. E., Taylor, J. B., Lang, N. P. and Kadlubar, F. F. 1992, The human glutathione *S*-transferase supergene family, its polymorphism, and its effects on susceptibility to lung cancer. *Environmental Health Perspectives*, **98**, 87–94.

Landi, M. T., Bertazzi, P. A., Shields, P. G., Clark, G., Lucier, G. W., Garte, S. J., Cosma, G. and Caporaso, N. E. 1994, Association between CYP1A1 genotype, mRNA expression and enzymatic activity in humans. *Pharmacogenetics*, **4**, 242–246.

Laurenzana, E. M., Hassett, C. and Omiecinski, C. J. 1998, Post-transcriptional regulation of human microsomal epoxide hydrolase. *Pharmacogenetics*, **8**, 157–167.

Matthias, C., Bockmuhl, U., Jahnke, V., Harries, L. W., Wolf, C. R., Jones, P. W., Alldersea, J., Worrall, S. F., Hand, P., Fryer, A. A. and Strange, R. C. 1998, The glutathione *S*-transferase GSTP1 polymorphism: effects on susceptibility to oral/pharyngeal and laryngeal carcinomas. *Pharmacogenetics*, **8**, 1–6.

McGlynn, K. A., Rosvold, E. A., Lustbader, E. D., Hu, Y., Clapper, M. L., Zhou, T., Wild, C. P., Xia, X. L., Baffoe-Bonnie, A., Ofori-Adjei, D., Chen, G., London, W. T., Shen, F. and Buetow, K. H. 1995, Susceptibility to hepatocellular carcinoma is associated with genetic variation in the enzymatic detoxification of aflatoxin B1. *Proceedings of the National Academy of Sciences of the USA*, **92**, 2384–2387.

Mooney, L. A., Bell, D. A., Santella, R. M., Van Bennekum, A. M., Ottman, R., Paik, M., Blaner, W. S., Lucier, G. W., Covey, L., Young, T. L., Cooper, T. B., Glassman, A. H. and Perera, F. P. 1997, Contribution of genetic and nutritional factors to DNA damage in heavy smokers. *Carcinogenesis*, **18**, 503–509.

Naylor, S., Gan, L. S., Day, B. W., Pastorelli, R., Skipper, P. L. and Tannenbaum, S. R. 1990, Benzo[*a*]pyrene diol epoxide adduct formation in mouse and human hemoglobin: physicochemical basis for dosimetry. *Chemical Research in Toxicology*, **3**, 111–117.

Omiecinski, C. J., Aicher, L., Holubkov, R. and Checkoway, H. 1993, Human peripheral lymphocytes as indicators of microsomal epoxide hydrolase activity in liver and lung. *Pharmacogenetics*, **3**, 150–158.

Omland, O., Sherson, D., Hansen, A. M., Sigsgaard, T., Autrup, H. and Overgaard, E. 1994, Exposure of iron foundry workers to polycyclic aromatic hydrocarbons: benzo(*a*)pyrene-albumin adducts and 1-hydroxypyrene as biomarkers for exposure. *Occupational and Environmental Medicine*, **51**, 513–518.

Ozbal, C. C., Skipper, P. L., Yu, M. C., London, S. J., Dasari, R. R. and Tannenbaum, S. R. 2000, Quantification of (7*S*,8*R*)-dihydroxy-(9*R*,10*S*)-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene adducts in human serum albumin by laser-induced fluorescence: implications for the *in vivo* metabolism of benzo[*a*]pyrene [In Process Citation]. *Cancer Epidemiology Biomarkers & Prevention*, **9**, 733–739.

Park, J. Y., Schantz, S. P., Stern, J. C., Kaur, T. and Lazarus, P. 1999, Association between glutathione *S*-transferase pi genetic polymorphisms and oral cancer risk [published erratum appears in *Pharmacogenetics* 2000 Jun;**10**(4):371]. *Pharmacogenetics*, **9**, 497–504.

Pastorelli, R., Restano, J., Guanci, M., Maramonte, M., Magagnotti, C., Allevi, R., Lauri, D., Fanelli, R. and Airoldi, L. 1996, Hemoglobin adducts of benzo[*a*]pyrene diolepoxide in newspaper vendors: association with traffic exhaust. *Carcinogenesis*, **17**, 2389–2394.

Pastorelli, R., Guanci, M., Cerri, A., Negri, E., La Vecchia, C., Fumagalli, F., Mezzetti, M., Cappelli, R., Panigalli, T., Fanelli, R. and Airoldi, L. 1998, Impact of inherited polymorphisms in glutathione *S*-transferase M1, microsomal epoxide hydrolase, cytochrome P450 enzymes on DNA, and blood protein adducts of benzo(*a*)pyrene-diolepoxide. *Cancer Epidemiology Biomarkers & Prevention*, **7**, 703–709.

Pastorelli, R., Guanci, M., Cerri, A., Minoia, C., Carrer, P., Negri, E., Fanelli, R. and Airoldi, L. 2000, Benzo(*a*)pyrene diolepoxide–hemoglobin and albumin adducts at low levels of benzo(*a*)pyrene exposure. *Biomarkers*, **5**, 245–251.

Rojas, M., Alexandrov, K., Cascorbi, I., Brockmoller, J., Likhachev, A., Pozharisski, K., Bouvier, G., Auburtin, G., Mayer, L., Kopp-Schneider, A., Roots, I. and Bartsch, H. 1998, High benzo[*a*]pyrene diol-epoxide DNA adduct levels in lung and blood cells from individuals with combined CYP1A1 MspI/Msp-GSTM1*0/*0 genotypes. *Pharmacogenetics*, **8**, 109–118.

Rojas, M., Cascorbi, I., Alexandrov, K., Kriek, E., Auburtin, G., Mayer, L., Kopp-Schneider, A., Roots, I. and Bartsch, H. 2000, Modulation of benzo[*a*]pyrene diolepoxide–DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. *Carcinogenesis*, **21**, 35–41.

Rothman, N., Shields, P. G., Poirier, M. C., Harrington, A. M., Ford, D. P. and Strickland, P. T. 1995, The impact of glutathione *S*-transferase M1 and cytochrome P450 1A1 genotypes on white-blood-cell polycyclic aromatic hydrocarbon–DNA adduct levels in humans. *Molecular Carcinogenesis*, **14**, 63–68.

Ryberg, D., Skaug, V., Hewer, A., Phillips, D. H., Harries, L. W., Wolf, C. R., Ogreid, D., Ulvik, A., Vu, P. and Haugen, A. 1997, Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk. *Carcinogenesis*, **18**, 1285–1289.

Schoket, B., Phillips, D. H., Kostic, S. and Vincze, I. 1998, Smoking-associated bulky DNA adducts in bronchial tissue related to CYP1A1 MspI and GSTM1 genotypes in lung patients. *Carcinogenesis*, **19**, 841–846.

Sherson, D., Sabro, P., Sigsgaard, T., Johansen, F. and Autrup, H. 1990, Biological monitoring of foundry workers exposed to polycyclic aromatic hydrocarbons. *British Journal of Industrial Medicine*, **47**, 448–453.

Shields, P. G., Bowman, E. D., Harrington, A. M., Doan, V. T. and Weston, A. 1993, Polycyclic aromatic hydrocarbon–DNA adducts in human lung and cancer susceptibility genes. *Cancer Research*, **53**, 3486–3492.

Skipper, P. L. and Tannenbaum, S. R. 1990, Protein adducts in the molecular dosimetry of chemical carcinogens. *Carcinogenesis*, **11**, 507–518.

Smith, C. A. and Harrison, D. J. 1997, Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema [see comments]. *Lancet*, **350**, 630–633.

Tas, S., Buchet, J. P. and Lauwerys, R. 1994, Determinants of benzo[*a*]pyrene diol epoxide adducts to albumin in workers exposed to polycyclic aromatic hydrocarbons. *International Archives of Occupational & Environmental Health*, **66**, 343–348.

Viezzer, C., Norppa, H., Clonfero, E., Gabbani, G., Mastrangelo, G., Hirvonen, A. and Celotti, L. 1999, Influence of GSTM1, GSTT1, GSTP1, and EPHX gene polymorphisms on DNA adduct level and HPRT mutant frequency in coke-oven workers [see comments]. *Mutation Research*, **431**, 259–269.

Watson, M. A., Stewart, R. K., Smith, G. B., Massey, T. E. and Bell, D. A. 1998, Human glutathione *S*-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis*, **19**, 275–280.

Wormhoudt, L. W., Commandeur, J. N. M. and Vermeulen, N. P. E. 1999, Gentic polymorphisms of human *N*-acetyltransferase, cytochrome P450, glutathione-*S*-transferase, and epoxide hydrolase enzymes: relevance to xenobiotic metabolism and toxicity. *Critical Reviews in Toxicology*, **29**, 59–124.

World Health Organization 1984, Polynuclear aromatic compounds. Part 3: Industrial exposure in aluminum production, coal gasification, coke production and iron and steel founding., *IARC Monographs: Evaluation of Carcinogenic Risk of Chemicals to Humans*, Vol. 34 (Lyon: IARC).

World Health Organization 1999, Metabolic polymorphisms and susceptibility to cancer. *IARC Scientific Publication* No. 148 (Lyon: IARC).