Biomarker Studies in Northern Bohemia

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Studies were conducted in northern Bohemia to simultaneously evaluate personal exposures to air pollution in the form of respirable particles containing polycyclic aromatic hydrocarbons (PAHs) and biomarkers of exposure, biological effective dose, genetic effects, and metabolic susceptibility. The series of biomarkers included PAH metabolites in urine, urine mutagenicity, PAH-DNA adducts in white blood cells determined by 32P-postlabeling, PAH-albumin adducts determined by enzyme-linked immunosorbent assay (ELISA), DNA damage in lymphocytes detected by comet assay, chromosomal aberrations, sister chromatid exchanges, and glutathione S-transferase M1 (GSTM1) genotypes. For these studies, a group of women who work outdoors about 30% of their daily time was selected. In a pilot study, a group of women from a polluted area of the Teplice district (northern Bohemia) was compared with a group of women from a control district of southern Bohemia (Prachatice). In a follow-up repeated studies, a group of nonsmoking women from Teplice was sampled repeatedly during the winter season of 1993 to 1994. Personal exposure monitoring for respirable particles (<2.5 μm) was conducted for the 24-hr period before collection of blood and urine. Particle extracts were analyzed for carcinogenic PAHs. In the pilot study and in the follow-up study, a highly significant correlation between individual personal exposures to PAHs and DNA adducts was found (r = 0.54, p = 0.016; r = 0.710, p < 0.001, respectively). The comet parameter (percentage DNA in tail; %T) correlated with exposures to respirable particles (n = 0.304, p = 0.015). The GSTM1 genotype had a significant effect on urinary PAH metabolites, urine mutagenicity, and comet parameters (%T and tail moment) when the GSTM1 genotype was considered as a single factor affecting these biomarkers. Multifactor analysis of variance considering exposure and adjusting the data for GSTM1, age, and diet showed that the effect of personal exposures to PAHs on the variability of biomarkers (DNA adducts, comet parameters, urine mutagenicity) might be higher than the effect of the GSTM1 genotype. These results show the importance of considering all potential factors that may affect the biomarkers being analyzed. — Environ Health Perspect 104(Suppl 3):591–597 (1996)

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Introduction

Northern Bohemia, a highly industrialized brown coal basin, was one of the most polluted areas in all of Europe (1). High-sulfur brown coal is used not only by power plants (this area produced 35% of the electricity used in the former Czechoslovakia), glasswork, and chemical and petrochemical industries but also for heating homes, large apartment complexes, and business facilities. In addition to industry, heavy automobile traffic en route to Germany contributes to the air pollution in this area. The health consequences of environmental pollution are a major concern of the Czech Government and the public. Early studies provided evidence of a high incidence of cancer and reproductive and behavioral effects (2,3). Teplice, one of the mining districts in northern Bohemia, was designated in 1991 as a model district for improved environmental monitoring and research on the health effects of air pollution as part of a large interdisciplinary project called the Teplice Program. The studies reported here are part of the project Biomarkers of Exposure to Mutagens and Carcinogens. The objectives of this project are to simultaneously evaluate personal exposures and internal measures of exposure, biological effective dose, early biologic effects, and metabolic susceptibility using a series of biomarkers.

The first studies in which biomarkers detected seasonal differences in exposure and genetic damage from environmental pollution were conducted in a population from a highly polluted area in Silesia (Poland) (4–7). In these studies, the investigators were not able to determine the relationship between individual exposure to air pollution and biomarkers of exposure and genetic damage because personal exposure data were not collected.
In our studies, polycyclic aromatic hydrocarbons (PAHs) were selected as the air pollution marker for monitoring of personal exposures. PAHs and their nitro-derivatives associated with respirable particle fraction (RSP) have been identified as a major source of carcinogenic risk in urban areas \((8,9)\). An air quality monitoring and receptor modeling study \((10)\) conducted in the Teplice district during 1992 to 1994 identified emissions from residential heating and vehicles as the major sources of organic carbon, including carcinogenic PAHs. These compounds are primarily activated through an oxidative metabolic pathway to electrophilic intermediates capable of covalent binding to protein and DNA. Furthermore, they are detoxified by the glutathione transferase enzymes from the glutathione \(S\)-transferase (GST) genes \((e.g.,\ glutathione \(S\)-transferase M1 \([\text{GSTM1}]\))

On the basis of this knowledge, the following exposure and dosimetry biomarkers were selected for our studies. The PAH metabolites in urine and urine mutagenicity were used as markers of internal exposure reflecting uptake, metabolism, and excretion. PAH–DNA adducts detected by \(^{32}\)P-postlabeling and PAH–albumin adducts detected by enzyme-linked immunosorbent assay (ELISA) were used as markers of biological effective dose. Selected biomarkers of genetic damage include chromosomal aberrations (CA) and sister chromatid exchanges (SCE) evaluated by cytogenetic analysis. \(\text{GSTM1}\) genotypes were determined as one of the markers of metabolic susceptibility.

A population of women who worked outdoors about 30% of their daily time was selected for our studies. In a pilot study, a group of women working as postal workers or gardeners from a highly polluted area of the Teplice district was compared with the group of women working as postal workers and nursery school teachers in the town of Prachatice, a control district in southern Bohemia \((11)\). There were 21 nonsmokers and 9 light smokers in the Teplice group and 30 nonsmokers in the Prachatice group. We did not find any significant differences in DNA and protein adducts, SCE, and the urinary mutagenicity results when nonsmokers from these two districts were compared; CA was an exception. The higher values of CA in the control group from Prachatice resulted from elevations only in the nursery school teachers. The measurement for exposure to carcinogenic PAHs indicated significantly lower exposure in the control district, as reflected in the urinary PAHs and metabolites; however, the range of exposures overlapped between these two districts. The higher mutagenic response was observed in the urine of smokers in all mutation assays as compared to nonsmokers from the both districts, but these data were not significant due to high interindividual variability within this relatively small group \((12)\).

Within the nonsmokers from Teplice, significant correlation of personal exposure to carcinogenic PAHs with DNA adducts was found \((r = 0.54, p = 0.016)\) \((13)\).

Based on the results obtained in the pilot study, we started a follow-up study with a group of nonsmoking women from the town of Teplice. This report is an update of the ongoing investigation providing information about the relationship between individual environmental exposures to carcinogenic PAHs, the \(\text{GSTM1}\) genotype, and biomarkers of exposure and genetic damage.

**Methods**

**Subjects and Sampling**

Informed consent was obtained from each subject prior to beginning this study. A questionnaire was administered to each subject to determine her individual lifestyle \((e.g.,\ diet, smoking habits, passive smoking exposure, alcohol consumption, residence in district)\). Any persons with medical treatment, radiography, or vaccination within the previous 3 months were not included in the study.

In the pilot study, a group of 30 women from Teplice city from 18 to 51 years of age \((\text{median} = 36\) years\) and 30 women from Prachatice city from 28 to 54 years of age \((\text{median} = 42\) years\) were selected, as described in detail elsewhere \((11)\). Most of the subjects were lifetime residents of these districts. In the Teplice group, 9 women were smokers \((\text{smoking} 3–15\) cigarettes/day\), whereas women in the Prachatice group were all nonsmokers. The pilot study was conducted during the last week of November 1992 in Teplice and the first week of December 1992 in Prachatice and lasted 2 days for each group.

In this study we selected 19 nonsmoking women from 18 to 52 years of age \((\text{median} = 31.5\) years\) with residence in Teplice city \(13\) postal workers and \(6\) gardeners). Ten of these women were also part of the pilot study. The nonsmoking status was verified by determination of cotinine in urine samples using radioimmunoassay \((14)\). The study was performed during the winter season and included 4 sampling days \((\text{whole group sampled in 1 day})\) from the end of October 1993 until February 1994, when this study was interrupted due to a long period of unusual meteorological conditions \((\text{high wind and lack of inversion conditions})\).

Personal exposure monitoring \((\text{PEM})\), starting at the beginning of the working shift, was conducted for 24 hr. Blood and urine samples were collected at the end of PEM.

**Personal Exposure Monitoring**

Personal monitoring was provided using a personal sampler from the U.S. Environmental Protection Agency \((\text{U.S. EPA})\) \((15,16)\). Respirable particles smaller than 2.5 \(\mu\)m were collected on Teflon-impregnated glass fiber filters. The sampler was connected to a pump powered by batteries and operated continuously for a 24-hr period. The sampler inlet was attached to the individual near the breathing zone and was located by her bed during the night. Particles from the filter were extracted by sonication with dichloromethane for analysis of the carcinogenic PAHs by high-performance liquid chromatography \((\text{HPLC})\) with fluorimetric detection \((17)\). The carcinogenic PAHs reported here include chrysene, benzo[\(a\)]anthracene, benzo[\(b\)]fluoranthene, benzo[\(k\)]fluoranthene, benzo[\(a\)]pyrene, dibenzo[\(ah\)]anthracene, indeno[1,2,3-\(cd\)]pyrene and benzo[\(ghi\)]perylene. Detailed sampling and analytical methods are reported elsewhere \((11,15–17)\).

**Urine Mutagenicity and PAH Metabolite Analysis**

Urine samples were tested for mutagenicity in the plate incorporation assay using tester strains \(\text{T}A98\) and \(\text{Y}G1041\) and in the Kado-microsuspension assay using the \(\text{Y}G1041\) strain because these strains are the most sensitive to PAHs and nitro-PAHs present in air samples from north Bohemia \((16)\). Both assays were performed with and without metabolic activation. The detailed procedures are described by Cernd \(\text{et al.}\) \((12)\).

For detection and quantitation of the parent PAHs and their metabolites \((\text{PAH/ metabolite})\) in urine, two independent methods \((\text{HPLC})\) and gas chromatography–mass spectrometry \((\text{GC/MS})\) were used as described in detail by Lewtas \(\text{et al.}\) \((11)\).

**DNA Adduct Analysis by \(^{32}\)P-postlabeling**

White blood cells \((\text{WBC})\) were isolated from fresh heparinized blood by centrifugation at \(900 \times \text{g}\) for 20 min followed by lysing of
the contaminating red cells. DNA was isolated, as previously described (18), using RNases A and T1 and proteinase K treatment followed by phenol/chloroform/isoamylalcohol extraction.

DNA adducts were analyzed by $^{32}$P-postlabeling according to the method of Gupta et al. (19) with minor modifications as described in detail elsewhere (13). Briefly, 3.0 μg DNA was enzymatically hydrolyzed to 3'-nucleoside monophosphates. The adducted nucleotides were enriched by butanol extraction (18). Adducts were labeled with [γ-$^{32}$P]ATP in the presence of T-4 polynucleotide kinase. The radiolabeled samples were analyzed by multidimensional PEI-cellulose thin-layer chromatography (TLC). To calculate adduct levels, the aliquots of DNA enzymatic digestion product from each individual sample were labeled with the same radiolabeled mix. Intensifying screen-enhanced autoradiography was used to detect the presence of radiolabeled adducts on TLC plates. The total DNA adduct levels reported here are for the diagonal radioactive zone (DRZ), inclusive of all distinct spots, and were evaluated from at least two independent experiments from the pilot or follow-up studies.

**PAH–Protein Adducts by Competitive ELISA**

Competitive ELISA with the monoclonal antibody 8E11 (20) was used to analyze for benzo[a]pyrene (B[a]P) tetrads released from plasma albumin, basically as described by Lee and Santella (21) with modifications described in detail elsewhere (11). The values for each subject are the mean of two replicate wells and are expressed as femtomes of B[a]P tetrads per microgram protein. Samples with less than 20% inhibition in the ELISA were considered to be below detectable limits, and the concentrations corresponding to 10% inhibition on the standard curve were assigned to these samples for statistical analysis.

**Cytogenetic Analysis**

Lymphocyte cultures were prepared from the whole blood as previously described by Sorsa et al. (22). The microscopic slides were stained with Giemsa (for detection of CA) or with fluorescence plus Giemsa (for detection of SCE). Chromosomal aberrations were evaluated in 100 metaphase cells per sample by two independent cytogeneticists using coded slides. Chromatid and chromosome breaks and chromatid and chromosome exchanges were scored according to Buckton and Evans (23) and used to calculate the number of cells carrying aberrations per 100 cells (% CA). SCE analysis was based on screening of 50 metaphase cells for each determination. SCE were evaluated according to the method of Perry and Wolff (24).

**Comet Assay Analysis**

Lymphocytes for the comet assay were isolated from 5 ml of fresh heparinized blood using Histopaque 1077. Cells were washed in RPMI 1640 medium twice and mixed with freezing medium containing 90% inactivated fetal calf serum and 10% dimethyl sulfoxide (DMSO). The frozen cells were stored in a polystyrene box at −80°C; after 24 hr cells were stored in liquid nitrogen until they were thawed for the assay. The comet assay was performed according to Singh et al. (25) with a slight modification (R Tice, personal communication). Briefly, fully frosted slides were covered with the first layer of 1% agarose (120 μl). The lymphocytes were diluted to a density of 10,000 cells/slide and mixed with 0.75% low melting-point agarose (total volume 85 μl per slide). The second layer of agarose with cells was poured over the first layer. Both layers of agarose were solidified at 4°C. Lysis was carried out for 60 min (the lysing solution contained 2.5 M NaCl, 0.1 M Na$_2$EDTA, 10 mM Tris, 1% Triton X-100, 10% DMSO, pH 10), unwinding for 60 min, and electrophoresis for 40 min at 20 V (0.67 V/cm, 300 mA). The same buffer was used for both unwinding and electrophoresis (300 mM NaOH, 1 mM Na$_2$EDTA, pH, 13.0), and these procedures were performed at 4°C under dim light. After electrophoresis neutralizing buffer was applied (0.4 M Tris, pH 7.5) and the nuclei were stained with ethidium bromide (20 μg/ml), the samples were analyzed using an image analyzer system (Comet, version 2.3; Kinetic Imaging, Ltd, United Kingdom). The following comet parameters were evaluated for 100 images per sample: percentage of DNA in tail (%T), tail length (TL), and tail moment (TM = %T × TL). Medians of these parameters for 100 images were used for the characterization of each individual subject.

**Determination of GSTM1 Genotype**

The GSTM1 polymorphism was determined by polymerase chain reaction (PCR) with a slight modification (using DNA isolated from WBC) described by Zhong et al. (26). Two of the three primers used could also anneal to another class M gene (GSTM4), while the third was specific for the GSTM1 gene. The GSTM1 null genotype was identified on the basis of the absence of the GSTM1-specific fragment. The consistent presence of the other fragment was used as an internal standard to detect failure of the amplification reaction.

**Statistical Analysis**

Statistical analysis was performed using the STATGRAPHICS Plus 7.0 package (Magnaustics, Inc., Rockville, MD). Non-parametric methods were chosen for a group-wide evaluation of the individual data that did not follow a normal distribution. The Mann-Whitney rank sum U-test was used for comparison of two samples and the Kruskal-Wallis one-way analysis of variance by ranks was used for evaluation of the data in overall studies. Correlations were performed by the Spearman rank correlation test. Using log-transformed data, the multifactor analysis of variances procedure was used to analyze the association between biomarkers and independent variables while controlling for the potential influence of the other variables.

**Results**

We report here the relationship between biomarkers, analyzed in a pilot study, and GSTM1 genotype (Table 1). The GSTM1 gene was present in 48% of Teplice nonsmokers and only in 37% of Prachaticke nonsmokers. In the group of smokers from Teplice (n = 9), there were only two persons lacking the GSTM1 gene; therefore, it was not reasonable to evaluate smokers according to the presence of this gene. We did not observe any significant differences in DNA and protein adducts, CA, and SCE between GSTM1-positive and -negative groups in both districts. A significantly increased level of urinary PAHs and their metabolites were observed in the GSTM1-negative group compared with GSTM1-positive group in nonsmokers from Teplice. The elevated exposure to carcinogenic PAHs by the GSTM1-negative group, as determined by personal air sampling, may have contributed to the significant difference observed in urine. In contrast, the elevated PAH exposure by the GSTM1-positive group from Prachaticke was probably responsible for the lack of a difference in PAH metabolite levels in the urine samples between these two groups. Nevertheless, the PAH levels as determined by air monitoring and in urine are higher for the Teplice group as compared with the Prachaticke group.
Table 1. Effect of GSTM1 genotype on the biomarkers of dose and effect for nonsmokers from Teplice (polluted region) and Prachatice (control region) analyzed in the pilot study.

| Biomarkers                        | Teplice     | Prachatice |
|----------------------------------|-------------|------------|
|                                  | GSTM1+ (n=10) | GSTM1− (n=11) | GSTM1+ (n=11) | GSTM1− (n=19) |
| DNA adducts per 10⁶ nucleotides   | 4.00 (0.93–12.17) | 4.22 (0.81–7.49) | 4.77 (1.05–9.58) | 3.86 (0.61–16.27) |
| Protein adducts, fm/mg protein   | 11.1 (9.0–29.5) | 11.3 (6.0–18.4) | 12.0 (6.0–15.7) | 9.5 (6.0–18.9) |
| Chromosomal aberrations, %       | 1.0 (0–4) | 1.0 (0–4) | 3.0 (0–6) | 2.0 (0–4) |
| Sister chromatid exchanges       | 4.48 (3.4–6.6) | 4.84 (4.0–6.4) | 4.2 (3.5–5.1) | 4.9 (3.5–6.2) |
| Urinary PAH/metabolitesb         | 180.7 (96–304) | 272.9* (112–357) | 137.1 (62–186) | 131.3 (60–215) |
| Carcinogenic PAH exposure (ng/m³) | 7.7 (2.9–22.6) | 12.5 (8.3–26.0) | 8.3 (3.2–13.3) | 5.0 (2.7–18.8) |

*Median (range). *Urinary parent PAHs and their metabolites are expressed as ng/mg creatinine. *Carcinogenic PAHs evaluated from personal monitors include chrysene, benzo[a]anthracene, benzo[a]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, indeno[1,2,3-c,d]pyrene and benzo[g,h,i]perylene. *p=0.037 comparing GSTM1-positive and -negative groups from Teplice (Mann-Whitney rank sum U-test).

The results of urine mutagenicity analyzed with respect to the presence of the GSTM1 gene for nonsmokers from Teplice are in Table 2. The higher mutagenic response observed in the null GSTM1 genotype group was significant (p = 0.033) only in strain YG 1041, which contained elevated levels of nitroreductase and O-acetyltransferase activities (27) using a microsuspension assay.

A further analysis of the effect of external personal exposures was conducted by applying a multifactor analysis of variance procedure to log-transformed data. Considering personal exposure and adjusting data for age, GSTM1 genotype, and diet (evaluated from the questionnaire as the consumption of fried or smoked meat during 24 hr before collection of blood), the effect of the GSTM1 genotype on mutagenicity became less pronounced, but its effect on the urinary PAH/metabolites (Table 1) remained significant (p = 0.030).

The follow-up repeated-measures study was conducted with a group of 19 non-smoking women from Teplice that were sampled repeatedly for 4 additional sampling days (all on the same day) during the winter season of 1993 to 1994. Elevated ambient concentrations of pollutants exceeded the daily maximal acceptable levels only on 1 day (30 November 1993) of these samplings. The comet assay used for evaluation of DNA damage in lymphocytes was added as a new biomarker for the follow-up study. A summary of the median values for DNA adducts, CA, and comet assay parameters (T% and TM) together with personal exposures is shown in Figure 1. Personal exposures to carcinogenic PAHs (p < 0.01) were significantly higher in the second sampling (30 November 1993) as compared with the first, third, and fourth ones (median = 36.2, compared with 11.3, 13.7, and 12.6 ng/m³). There was a significant effect (Kruskal-Wallis test: p < 0.01) of sampling days on DNA adduct levels and comet parameters T% and TM. DNA adducts levels were elevated (p < 0.05) in the second sampling period (median = 6.54, compared to 4.07, 4.05, and 3.79 adducts/10⁶ nucleotides). Comet parameters T% and TM exhibited significantly increased values (p < 0.01) only in comparison with the fourth sampling period (T% median = 7.12, compared to 5.35; TM median = 0.42, compared to 0.34). There were no significant differences in CA between sampling periods, which is consistent with cumulative dose for a longer period time, but there is a trend toward lower CA following the third and fourth samplings (see Figure 1).

The average ambient concentrations of carcinogenic PAHs for weeks and months before sampling days in the follow-up study are shown in Figure 2. The data before the first sampling on 25 October were not available. The highest differences in the ambient PAH concentrations between the second and the two additional samplings were on the day of sampling (123.2 vs 30.1 and 32.3 ng/m³, respectively); the differences in the 1-week-before samplings were less (46.20 versus 28.98 and 6.44 ng/m³, respectively). The average monthly concentrations of ambient carcinogenic PAHs before these sampling periods were very similar (before the second: 27.9; third: 30.4; and fourth: 22.8 ng/m³).

In the overall follow-up study, a significant correlation between individual

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*Median (range). *Plate incorporation assay. *KAD0 microsuspension assay. *p=0.033 comparing GSTM1 positive and negative groups (Mann-Whitney rank sum U-test).
Table 3. Effect of the GSTM1 genotype on DNA adducts and comet assay parameters.

| Parameters                        | GSTM1 +          | GSTM1 -          | Significance |
|-----------------------------------|------------------|------------------|--------------|
| Overall study                     |                  |                  |              |
| Sample size                       | 23               | 21               |              |
| DNA adducts/10⁶ nucleotides        | 4.32 (2.70–7.78) | 5.58 (3.01–11.1) | *p = 0.101   |
| Carcinogenic PAH exposure (ng/m³) | 14.2 (5.3–76.0)  | 16.8 (5.3–53.9)  | *p = 0.512   |
| Follow-up study                   |                  |                  |              |
| Sample size                       | 32               | 33               |              |
| DNA percentage in comet T (%)     | 6.07 (4.31–8.59) | 7.03 (3.39–10.09)| *p = 0.002   |
| TM                                | 0.35 (0.16–0.80) | 0.44 (0.16–1.11) | *p = 0.009   |
| Carcinogenic PAH exposure (ng/m³) | 13.9 (5.0–76.0)  | 16.8 (4.3–304)  | *p = 0.189   |

*Mann-Whitney rank sum U-test. *Overall study of 10 nonsmoking women participating in a pilot study and 4 additional sampling days of follow-up study; the whole set of DNA samples was run in the same experiments (see Methods) and the frequency of GSTM1 null genotype was 50%. *Median (range). *Carcinogenic PAHs include chrysene, benzo(a)pyrene, benzo(a)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene, indeno[1,2,3-c,d]pyrene and benzo[ghi]perylene. *Follow-up study of 19 nonsmoking women (4 sampling days); frequency of GSTM1 null genotype was 53%. *TM = T(%) × T (μm).

Biological personal exposures to carcinogenic PAHs and DNA adducts was found (r = 0.710; p < 0.001). The comet parameter T(%) correlated significantly (r = 0.304; p = 0.015) with personal exposures to respirable particles (mass concentrations). The personal 24-hr exposures were unrelated to the results of cytogenetic analysis.

The frequency of the GSTM1 null genotype in the follow-up study group was 53%. Group-wide comparison resulted in nonsignificant elevated DNA adduct levels by the null GSTM1 genotype group and significant increased values of both the comet parameters (Table 3). In a multifactor analysis of variance, after accounting for the PAH exposure, sampling, age, and diet, the effect of the GSTM1 genotype on comet parameters became less pronounced (Table 4).

Discussion

Biomarkers of human exposure, dose, and genetic effects in the general population have recently been studied with respect to exposures from environmental pollution (5–7,11,13–28–32). Perera et al. (5) found that the exposure to environmental pollution in the Silesian region of Poland was associated with a significant increase of DNA adducts, SCE, and CA, as well as with a frequency of ras oncogene overexpression. In this study, DNA adducts were significantly correlated with chromosomal aberrations, providing a possible link between environmental exposure and genetic alterations relevant to diseases. Autrup et al. (31) reported that nonsmoking pregnant women living in a suburban area had higher PAH–albumin adduct levels than comparable women living in a rural area. When the suburban and rural groups were combined, the daily transportation time of these women appeared to be a major factor contributing to the protein adducts, suggesting the importance of exposure to incomplete combustion products from either vehicle exhaust or local heating. In this study, the GSTM1 genotype did not alter these PAH–protein adduct levels. None of these studies employed personal exposure monitoring to quantitatively relate individual exposure to specific air pollutants to the levels of biomarkers analyzed.

The studies reported here have measured both ambient environmental levels of airborne carcinogenic PAHs and personal exposure to carcinogenic PAHs, which in both cases are adsorbed to respirable particles. The purpose of this study was to determine the relationship between personal external exposure to PAHs and biomarkers of internal exposure, dose, genetic damage, and metabolic susceptibility. The GSTM1 genotype, reported here, is the first metabolic susceptibility marker examined in this population.

Only one of the sampling periods (the second) in the follow-up study had substantially elevated ambient air pollution where the 24-hr personal exposures to PAHs (e.g., benzo[a]pyrene at 7.5 ± 3.6 ng/m³) reached values comparable to those of foundry workers during an 8-hr shift (6 ± 3 ng/m³) [33]. During most of the winter period of this study (1993–1994), unusually windy conditions prevented the winter inversion episodes typical of the Teplice district and surrounding districts in northern Bohemia. For this reason, this study will continue for several more years. During three of the four repeated sampling periods the personal exposures were relatively low and comparable to those found in other urban areas of North America and Europe. In spite of this, within the population studied, there was a sufficiently high interindividual variability in the personal exposures to PAHs to permit correlation analysis between individual personal exposure and biomarker measures. In previous studies comparing personal exposure and ambient air concentrations of air pollutants, it has been reported that such individual variation in personal exposure should be expected due to different microenvironmental concentrations indoors and outdoors and different times spent in each microenvironment [9,15,16]. For example, the postal workers in Teplice have different postal routes within the city that could account for variability in their personal exposures during working hours. During nonworking hours, they return to homes in different sections of the city.

The Teplice nonsmokers in our pilot study that lacked the GSTM1 gene (null genotype) exhibited significantly increased levels of urinary PAH metabolites and increased urine mutagenicity in strain YG1041 without metabolic activation (direct acting mutagens) when group-wide comparison was used. This finding is consistent with the report that the urine of smokers lacking this gene was several times more mutagenic than that of smokers with the gene [34]. Since we had only two smokers lacking the GSTM1 gene, we were unable to confirm this finding in smokers in our study (n = 9). Since GSTs are important in the detoxification of PAHs and other carcinogens by catalyzing conjugation of their reactive electrophilic forms to glutathione, it is expected that individuals with the null genotype may be at greater risk. The human polymorphism of the GSTM1 gene has been shown to be associated with an increased risk of cancer [35,36], as well as with susceptibility to mutagen-induced cytogenetic damage [37]. When we analyzed the effect of the GSTM1 genotype on the other biomarkers
in our pilot study (e.g., DNA adducts, protein adducts, and cytogenetic markers) by analysis of variance, we did not find a relationship between the presence of the GSTM1 gene and the other biomarkers. This may be due to the relatively small sample size \( (n = 21) \). It has been assumed that lack of the GSTM1 gene would result in an increased level of binding of genotoxic compounds with cellular macromolecules (e.g., as measured by DNA and protein adducts). In a study of Swedish chimney sweeps, those with the GSTM1 null genotype had only slightly, but not significantly, elevated DNA adduct levels \( (38) \). No effect of the GSTM1 genotype on PAH–albumin adduct levels was reported in the study of Danish pregnant women exposed to urban air pollution \( (31) \).

In our study, we have examined the relationship between the GSTM1 genotype and PAH exposure, DNA adducts, and the DNA damage parameters as measured by the comet assay. DNA adducts were slightly, but not significantly, elevated in the GSTM1 null genotype. The comet assay parameters (T% and TM) were both significantly elevated \( (p < 0.05) \) in the null GSTM1 genotype, consistent with a report that GSTM1 null individuals exposed to epoxides have a higher induction of cytogenetic damage \( (37) \).

In both studies the questionnaire data were used to explore the other factors that may affect interindividual variations seen in the biomarker data such as age, consumption of fried or smoked foods, and exposure to tobacco smoke due to passive smoking during the 24-hr period of sampling. These parameters generally were not significant.

In the above discussion of the influence of the GSTM1 genotype, the influence of genotype is considered to be a single factor. However, if we perform a multifactor analysis of variance, considering exposure and adjusting the data for GSTM1, age, and diet, these significances disappear. If you do not consider personal exposure, then the group-wide comparison (Mann-Whitney rank sum U-test) or one-way analysis of variance (Kruskal-Wallis test) will show an erroneously high significance for the effect of the GSTM1 null genotype, such as on both comet parameters in our study. Other biomarker studies in progress will be analyzed by this more rigorous multifactor analysis method. These results show the importance of considering all potential factors that may affect the biomarkers being analyzed. In the ideal case, simultaneous biological and statistical significance of differences obtained should be considered.

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