Biomonitoring of United States Army Soldiers Serving in Kuwait in 1991

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Abstract

Biomarkers of polycyclic aromatic hydrocarbon (PAH) exposure and genetic biomarkers of potential cancer susceptibility were determined in a group of United States Army soldiers who were deployed to Kuwait and Saudi Arabia in 1991 in the aftermath of the Persian Gulf War. Because hundreds of oil well fires were still burning, there was concern that ground troops stationed in Kuwait might be exposed to high levels of PAHs and other toxicants. The United States Army Environmental Hygiene Agency monitored air and soil for ambient PAHs. In addition, a group of 61 soldiers was involved in the biomonitoring study reported here. These soldiers kept diaries of daily activities and provided blood and urine samples in Germany (June) before deployment to Kuwait, after 8 weeks in Kuwait (August), and 1 month after the return to Germany (October). Here we present data for PAH-DNA adducts measured by immunoassay in blood cell DNA samples obtained at all three sampling times from 22 soldiers and bulky aromatic adducts measured by ^{32}P-postlabeling in blood cell DNA samples from 20 of the same soldiers. Urinary 1-hydroxypyrene-glucuronide levels were determined by synchronous fluorescence spectrometry in a matched set of samples from 33 soldiers. Contrary to expectations, environmental monitoring showed low ambient PAH levels in the areas where these soldiers were working in Kuwait. For both DNA adduct assays, levels were the lowest in Kuwait in August and increased significantly after the soldiers returned to Germany (October). Urinary 1-hydroxypyrene-glucuronide levels were also lowest in Kuwait and highest in Germany, but the differences were not statistically significant. The PAH-exposure biomarker levels were not significantly influenced by polymorphic variations of *CYP1A1 (Msp*I) and glutathione S-transferases M1 and T1. Overall, the data suggest that this group of soldiers was not exposed to elevated levels of PAHs while deployed in Kuwait.

Introduction

Large-scale pollution, occurring in the wake of the Persian Gulf War, caused the United States Army to investigate potential exposure of military personnel stationed in Kuwait and Saudi Arabia to airborne PAHs[2] and other toxicants. The United States Army Environmental Hygiene Agency at Aberdeen Proving Ground initiated a health risk assessment and biological surveillance involving troops stationed in Germany, who were deployed to Kuwait for approximately 4 months (1). This monitoring operation began May 1, 1991, and included environmental sampling as well as biological surveillance of military personnel. Ambient air sampling was performed with personal portable pumps as well as fixed high volume samplers placed near work locations. Measurements were taken daily through July and then every third day until December of 1991. The 2300 troops participating in the biological surveillance study were originally stationed in Fulda, Germany and deployed to Kuwait on June 10, 1991. A subgroup of 61 soldiers kept daily diaries and filled out questionnaires to document their location and exposure during the study. These individuals were asked to provide blood and urine samples in Germany before going to Kuwait (June), after 8 weeks of duty in Kuwait (August), and in Germany (October), 4 weeks after departing Kuwait on September 20, 1991.

This surveillance operation was begun with the expectation that the soldiers in Kuwait would be exposed to elevated levels of ambient PAHs, and the exposure biomarkers investigated were chosen because of their extensive application in the literature of human PAH biomonitoring (2). The blood samples were examined for PAH-DNA adducts, measured by immuno-

Received 11/4/97; revised 3/3/98; accepted 3/10/98.
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[2] The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; 1-OH-PG, 1-hydroxypyrene-glucuronide; SFS, synchronous fluorescence spectrometry; GST, glutathione *S*-transferase; DELFIA, dissociation-enhanced lantbanide fluoroimmunoassay; BPDE, (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; BP, benzo(*a*)pyrene; SCE, sister chromatid exchange.

assay, and bulky aromatic DNA adducts, measured by ^{32}P-postlabeling. DNA adduct formation in WBC DNA is considered to primarily reflect exposure received within days to a few weeks prior to sampling (3–5). Urinary 1-OH-PG was measured by SFS and is considered to reflect exposure within 24 h of the sampling time (6, 7). The biomarker results were then compared, stratified by polymorphisms in genes responsible for PAH metabolism (8, 9). These include *CYP1A1* (*Msp*I), which is presumed to confer enzyme inducibility and predispose to increased DNA adduct formation, and *GSTM1* and *GSTT1*, which have coding region deletions of 8 and 5 kb, respectively, that result in loss of detoxification function. The results of the biomarker analyses were compared with air and soil measurements of PAHs obtained from areas where the soldiers were working in Kuwait and with literature values for ambient PAH concentrations measured in Germany in 1992.

Materials and Methods

Study Population and Sample Collection. Male Army personnel (n = 61) of the 11th Armored Calvary Regiment were selected from a group of 2300 individuals undergoing medical processing for deployment. They gave informed consent and completed a questionnaire covering demographic and exposure information. Blood specimens (40 ml) were obtained in heparinized tubes from soldiers in Germany before deployment to Kuwait on June 10, in August after 8 weeks in Kuwait, and in October 4 weeks after the return to Germany on September 20. Blood was centrifuged, and the buffy coat samples were transported on wet ice within 48 h to Microbiological Associates, Inc. (Rockville, MD), where WBCs were stored frozen until DNA was prepared. Spot urine samples were obtained at the time of blood draw and stored frozen.

Monitoring of Air in Kuwait. In Kuwait (1), soldiers carried personal sampling pumps that filtered ~10 m^3 of air/day. In addition, high-volume samplers that collected 2000 m^3 of air per day (EPA PM 10 method) and 275 m^3 of air per day (EPA TO-13 method) were located near the soldiers' work sites between June and December of 1991. For particle-bound, semivolatile material, filters from the high-volume samplers were analyzed (PM 10), and for particle-bound plus gaseous material, high-volume samplers containing filters and XAD resin were used (TO-13). Analyses were performed by gas chromatography/mass spectrometry using flame-ionizing and photoionizing detectors.

Preparation of Blood Cell DNA and Assay of PAH-DNA Adducts by DELFIA. DNA was isolated by proteinase K digestion, phenol extraction, and ethanol precipitation (10), and the concentration was determined by spectrophotometry (A_{260}).

DELFIA is a variation of the standard competitive ELISA performed with rabbit antiserum elicited against DNA modified with BPDE (11, 12) and has been validated extensively (13). The standard used for evaluation of biological samples was calf thymus DNA modified with BPDE to 2.5 fmol adducts/μg DNA (~80 adducts/10^8 nucleotides). The assay used a biotin-avidin amplification step and an end point based on excitation and fluorescence of a europium label. The 50% inhibition of the standard curve was at 6.3 ± 2.4 fmol adduct/well (mean ± SD, n = 25). In each well, 25 μg of human DNA were assayed, and the limit of sensitivity was 0.8 adducts/10^8 nucleotides. Because this antiserum recognizes DNA samples modified with diol-epoxides of several different PAHs, including chrysene, benz[a]anthracene, and benzo[k]fluoranthene (14), the values obtained from human samples are likely to reflect DNA adduct formation by multiple PAHs. The analysis was performed on a matched set of DNA samples from 22 individuals.

Assay of Aromatic Adducts by ^{32}P-Postlabeling. The procedure used was essentially that described previously by Schoket *et al.* (15). DNA (4 μg), recovered from the DELFIA plates by proteinase K digestion, phenol extraction, and ethanol precipitation was digested with 0.29 units of micrococcal nuclease, 1.2 milliunits of spleen phosphodiesterase in a 4.8 μl volume at 37°C overnight. A subsequent digestion was with 0.38 units of nuclease P1. Incubation with an excess of [γ-^{32}P]ATP (2330–4760 Ci/mmol) and 3 units of T4 polynucleotide kinase was followed by four-directional TLC on PEI cellulose sheets (Macherei-Nagel, Doren, Germany). For each sample, the diagonal radioactive zone of the chromatogram, containing well-resolved and partially resolved spots, was excised and counted. The calculation of the ^{32}P-labeled adducts was derived from the [γ-^{32}P]ATP-specific activity (16). Because nuclease P1 was used for enhancement of the sensitivity, it is unlikely that DNA adducts of aromatic amines are included in the values presented here. The analysis was performed on a matched set of DNA samples from 20 individuals, all of whom had samples analyzed by PAH-DNA DELFIA.

Analysis of Urine Samples for 1-OH-PG. Analysis of 1-OH-PG was performed as described previously (6, 7). Briefly, urine samples (10 ml) were acidified by the addition of HCl (37%; 80 μl) and heated for 3 h (90°C). The samples were initially purified using C18 Sep-Pack columns (Waters Associates, Milford, MA). The eluates (80% methanol fraction) were dried and applied to immunoaffinity chromatography columns containing antisera specific for PAH-DNA adducts (17, 18). Materials not bound by the antisera were washed through in Tris-HCl (100 mM, pH 7.4) and materials captured by the antibodies were eluted in methanol. Methanol fractions were dried, redissolved in water, subjected to HPLC on octadecasilane analytical columns (250 × 4.6 mm), and eluted with gradients of methanol (30–60%) in water at a flow rate of 1 ml/min. Fractions containing 1-OH-PG were pooled, and the concentration was determined by SFS ($\Delta\lambda$ 34; Ref. 17). Urine creatinine levels were determined spectrophotometrically after reaction with picrate (biochemical kit; Sigma Chemical Co., St. Louis, MO). The analysis was performed on a matched set of samples from 33 soldiers.

Genotyping of Human Metabolic Polymorphisms by PCR. For coamplification of *CYP1A1* and *GSTM1* sequences from normal genomic WBC DNA, amplified *CYP1A1* acts as a positive control for analysis of *GSTM1*. Amplification consisted of 35 cycles (melting, 94°C, 1 min; annealing, 52°C, 1 min; synthesis, 72°C, 1 min; final extension, 72°C, 7 min). The reaction mixture (100 μl) contained 100 ng of genomic DNA; primers (forward 5′-TAGGAGTCTTGTCTCATGCCT-3′ and reverse 5′-CAGTGAAGAGGTGTAGCCGCT-3′ for *CYP1A1*; primers forward 5′-CTGCCCTACTTGATTGATGGG-3′ and reverse 5′-CTGGATTGTAGCAGATCATGC-3′ for *GSTM1*; 20 pmol of Tris-HCl (10 mM; pH 8.3); 50 mM KCl; 3 mM $MgCl_2$; 200 nmol of deoxynucleotide triphosphates; and 2.5 units of polymerase (Perkin-Elmer, Norwalk, CT). Following amplification, a portion of the reaction mixture (40 μl) was subjected to agarose gel (2%) electrophoresis containing ethidium bromide for determination of *GSTM1* genotype. Bands were observed at 339 and 694 bp for *CYP1A1* and *GSTM1*. A second portion of the reaction mixture (40 μl) was subjected to *Msp*I restriction enzyme digestion (1 unit in Tris-HCl [pH 7.5; 10 mM], 10 mM $MgCl_2$/KCl, 1 mM β-mercaptoethanol, and 0.1 mg/ml BSA 37°C for 2 h) and agarose gel (1%)

electrophoresis with ethidium bromide staining for *CYP1A1* PCR products. Cleavage products of 134 and 205 bp were observed for the *Msp*I site present.

For determination of the *GSTT1* genotype, PCR amplification consisted of 35 cycles (melting, 94°C, 1 min; annealing, 60°C, 1 min; synthesis, 72°C, 1 min; final extension, 72°C, 10 min). The reaction mixture (100 μl) contained 100 ng of genomic DNA, *GSTT1* primers (forward 5′-TTCCTTACTG-GTCCTCACATCTC-3′ and reverse 5′-TCACCGGATCATG-GCCAGCA-3′; 150 pmol), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μmol deoxynucleotide triphosphates, and 1 unit of polymerase. After amplification, a portion of the reaction mixture (40 μl) was subjected to agarose gel (2%) electrophoresis with ethidium bromide. Individuals functional for *GSTT1* have a band at 500 bp.

Statistics. Summary data are presented as means ± SD and median and are portrayed graphically by box-and-whisker plots. Samples with values lower than the limit of detection were assigned a value half-way between zero and the limit of detection. The statistical significance of differences in levels of each PAH exposure marker between the first samples collected in Germany (in June) and those collected in Kuwait (in August) and between the samples collected in Kuwait and the second set of samples from Germany (in October) were tested by the Wilcoxon sign rank test for the subset of subjects who provided samples at all three time points. Assessment for potential confounding of the relationship between time interval (*i.e.*, June-August and August-October) and biomarker level was tested by multiple linear regression, where month tested and current number of cigarettes smoked per day during that month were the independent variables and the natural log of each biomarker outcome was the dependent variable. In addition, the influence of change in the number of cigarettes smoked per day on change in each biomarker over the June-August and August-October time intervals was tested by the Spearman correlation test. The influence of each genotype on PAH biological markers of exposure (both separately, from each of the three collection times, and pooled) was tested by the Wilcoxon rank sum test. Linear regression was used to adjust the influence of each genotype and smoking on each PAH biomarker; values were log transformed to improve normality. Spearman correlation was used to test the correlation between PAH exposure biomarkers. Two-tailed *P*s less than 0.05 were considered significant.

Results

Study Subjects. Although 61 male Army personnel were initially enrolled in the study in Germany, a substantial number did not provide biological samples at the later times (19). A further constraint was the amount of DNA available from the collected samples. Therefore, in this report, for each biomarker we present only the data for subjects who contributed samples on all three occasions. For the largest sample set, the 33 individuals for whom urinary 1-OH-PG was assayed, the mean ± SD age was 24.4 ± 6.2 years. Twenty of the soldiers (61%) were Caucasian, 7 (21%) were African-American, 4 (12%) were Hispanic, and 1 was Native American (data missing for one subject). For the 27 subjects with smoking data available for both June and August, 13 (48.1%) were current smokers in June and 8 (29.6%) were current smokers in August. For the 33 subjects with smoking data available for both August and October, 10 (30.3%) were current smokers in August and 7 (21.2%) were current smokers in October. Age and race distribution were similar for the 22 individuals with available PAH-DNA DELFIA data and the 20 individuals with available ^{32}P-postlabeling data. Among this group, the proportion of current smokers was minimally changed between June and August and declined between August and October.

Air Monitoring for Particulate and Volatile PAHs in Kuwait. No PAHs were detected on media from personal sampling pumps that the soldiers carried in Kuwait; therefore, 31 different samples of much larger volumes of air were subsequently obtained near the soldiers' work sites. Material extracted from the high-volume samples was analyzed by gas chromatography/mass spectrometry. The filter extracts were analyzed for the presence of 23 PAHs, and more than half of these compounds were undetectable. Ambient concentrations of BP and other carcinogenic PAHs likely to be adducted to human DNA and detected by the BPDE-DNA DELFIA are shown in Table 1.

DNA Adduct Measurements. For the PAH-DNA DELFIA, data for 66 samples from 22 soldiers are presented in Fig. 1. Forty-five % of the samples for June, 45% of the samples for August, and 9% of the samples for October were below the limit of detection. The mean ± SD PAH-DNA adduct values were 3.1 ± 4.1, 1.6 ± 1.8, and 3.9 ± 3.5 adducts/10^8 nucleotides for June, August, and October, respectively. The difference between August and October values was statistically significant by Wilcoxon sign rank test ($P = 0.0009$). Although adduct values were lower in August than in June, the difference was not significant ($P = 0.25$). The significance of the August-October time interval on adduct levels determined by linear regression ($P = 0.0003$) was unchanged after adjustment for cigarette use. In addition, the change in number of cigarettes smoked per day did not significantly correlate with the change in adduct levels between June and August ($r = 0.35$, $P = 0.16$) or August and October ($r = 0.02$, $P = 0.94$).

Sixty matched DNA samples from 20 of the same 22 soldiers with complete DELFIA data were assayed for bulky aromatic DNA adducts by ^{32}P-postlabeling (Fig. 2), and all samples contained detectable levels of adducts. The mean ± SD adduct levels were 2.8 ± 1.6, 1.7 ± 0.9, and 3.0 ± 2.1 adducts/10^8 nucleotides for June, August, and October, respectively. Again, the adduct levels in Kuwait were the lowest, and the August values were significantly lower than those obtained in Germany in October ($P = 0.0003$ by Wilcoxon sign rank test). The June-August difference was also significant ($P = 0.004$). The significance of the June-August time interval on adduct levels determined by linear regression ($P = 0.004$) was minimally changed after adjustment for current cigarette use ($P = 0.007$), and the significance of the August-October time interval ($P = 0.004$) was unchanged after adjustment. In addition, the change in number of cigarettes smoked per day did not correlate with change in adduct levels between June and August ($r = 0.26$, $P = 0.36$) or between August and October ($r = -0.14$, $P = 0.54$).

A comparison of DELFIA and ^{32}P-postlabeling values for each sample showed no correlation between the two assays (Spearman correlation, $r = -0.02$, $P = 0.87$), suggesting that immunoassays using the BPDE-DNA antiserum and ^{32}P-postlabeling do not detect the same adducts in human DNA samples.

Determination of Urinary 1-OH-PG. Urine samples were taken at the time of blood draw, and PAH metabolites were concentrated by immunoaffinity chromatography, eluted, and further purified by HPLC and analyzed for pyrene fluorophores by SFS. A matched set of samples from 33 individuals was available, and the values presented are corrected for concentration of urinary creatinine (Fig. 3). The mean ± SD values in

Table 1 Mean PAH measurements at Camp Thunderock, Kuwait, in air samples obtained between June and December of 1991

PAH[a]	Hi-Vol. Rf. PM 10		EPA method TO-13	
	No. positive/ Total	ng/m^3	No. positive/ Total	ng/m^3
Benzo[*a*]pyrene	0/20	≤0.23[b]	0/11	≤0.8[b]
Benz[*a*]anthracene	0/19	≤0.23[b]	0/11	≤0.8[b]
Chrysene	5/20	0.35	1/11	0.9
Benzo[*k*]fluoranthene	1/20	0.23[b]	0/11	≤0.8[b]
Benzo[*b*]fluoranthene	2/20	0.28	0/11	≤0.8[b]

[a] DNA samples modified with the diol-epoxides of these compounds cross-react with the anti-BPDE-DNA antiserum (10).
[b] Below the limit of detection.

fmol 1-OH-PG/μmol creatinine (mean ± SD) were 15.0 ± 15.1, 10.0 ± 8.5, and 11.2 ± 8.8 for June, August, and October, respectively. Whereas there were no statistically significant differences among any of these groups, the lowest values were found in the samples obtained in Kuwait. The change in number of cigarettes smoked per day did not correlate with change in 1-OH-PG levels observed between June and August ($r = -0.11$, $P = 0.58$) or between August and October ($r = 0.10$, $P = 0.59$).

By Spearman correlation, comparing three assays from 20 subjects for both DNA adduct analyses and three assays from 16 subjects for the 1-OH-PG, there were no statistically significant correlations among the three markers.

Genetic Polymorphisms of *CYP1A1* (*Msp*I), *GSTM1*, and *GSTT1*. DNA was subjected to PCR, and the amplified products were analyzed by gel electrophoresis. For *CYP1A1*, subjects homozygous for the absence of the *Msp*I restriction were designated M−/M−. Heterozygotes were M+/M−, where M+ is the putative at-risk variant. No rare M+/M + homozygotes were found in this group. For *GSTM1*, functional homozygotes (1/1) and functional heterozygotes (1/0) were grouped together and compared to null individuals (0/0), with an 800-bp coding region deletion. Similar groupings were made for the *GSTT1* polymorphisms.

For the matched set of individuals for whom PAH-DNA adducts were measured by DELFIA, values at all three sampling times were included in an analysis comparing genotypic variants, and there were no significant differences in PAH-DNA adduct levels stratified by polymorphic variant (Table 2). A similar result was observed for aromatic DNA adducts measured by ^{32}P-postlabeling and urinary 1-OH-PG (Table 2). Also, no significant differences were detected when bioassay data for a single sampling time were stratified by genotype. In a multiple regression model that adjusted each genotype for all others and current cigarette smoking, none of the genotypes had a significant impact on any bioassay outcome. Furthermore, the trends in PAH biomarker levels across the three sampling periods (Figs. 1–3) were generally similar when stratified by each genotype.

Discussion

In June of 1991, 515 of the original 605 Kuwait oil well fires were active in Kuwait. Of these, 105 were to the north, with the closest northern fires burning ~9 miles from the soldiers' location. Northern fires were most likely responsible for any exposure because the prevailing winds were southerly. Contrary to expectations, the environmental monitoring data showed that ambient levels of PAHs were very low in the areas

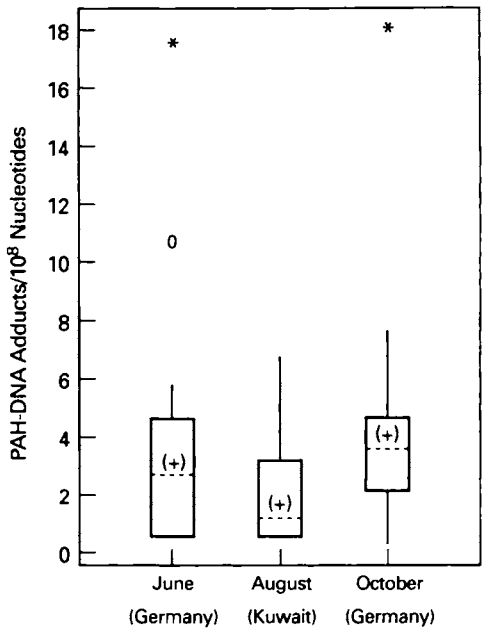

Fig. 1. PAH-DNA adducts for a matched set (June, August, and October) of samples from 22 soldiers measured by anti-BPDE-DNA DELFIA. Box-and-whisker plots indicate the following: —, median; +, mean; upper and lower edges of the box represent the 75th and 25th percentiles, respectively; *0*, outliers >1.5 × the range within the boxes; *, extreme outliers >3 × the range within the boxes. Nondetectable values were given a value of 0.4 adducts/10^8 nucleotides, half-way between zero and the lower limit of assay detection. Mean (± SD) values are 3.1 ± 4.1, 1.6 ± 1.8, and 3.9 ± 3.5 adducts/10^8 nucleotides for June, August, and October, respectively. Median values are 2.2, 0.8, and 3.2 for the same times. *P* for the June-August difference is 0.25, and for the August-October difference, $P = 0.0009$ (Wilcoxon sign rank test).

where these soldiers were working in Kuwait. This observation may be consistent with evidence that PAH emissions are lower when fuels burn in an uncontrolled manner with unrestricted access to oxygen, as compared to oxygen-starved or controlled burning conditions that lead to higher PAH emissions. Consistent with the ambient monitoring data, the lowest levels of PAH-DNA adducts and aromatic-DNA adducts observed in this study were in blood cell DNA samples obtained while these soldiers were deployed in Kuwait. The increase in DNA adducts upon the return to Germany, as compared with the adduct levels in Kuwait, was statistically significant. In addition, urinary 1-OH-PG levels were the lowest in the samples obtained in Kuwait, although differences for this biomarker were not statistically different among sampling times. Overall, the low levels of environmental PAHs found in the immediate area of the soldiers' duty stations in Kuwait support the observation of low levels of exposure biomarkers found in blood and urine samples taken in Kuwait in August. It is important to underscore that it is uncertain to what extent these observations apply to other soldiers stationed in the area at this time. Ambient PAH concentrations are likely to have been high in the immediate vicinity (<1 mile) of the oil well fires (20, 21), but no samples were taken, and therefore, projection of these biomarker findings to the majority of military personnel stationed in Kuwait at that time would not be appropriate.

There were no environmental PAH measurements obtained by the Army in Fulda, Germany. The town, which is ~55 miles east of Frankfurt am Main, is relatively small and without large-scale industrialization. It is located near what was, in

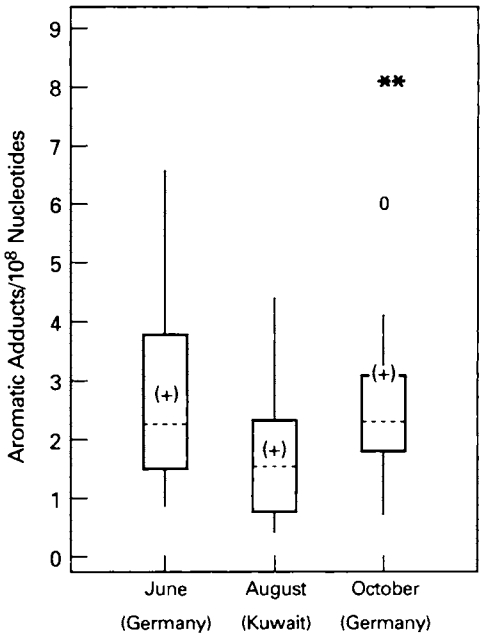

Fig. 2. Bulky DNA adducts for a matched set (June, August, and October) of samples from 20 soldiers measured by ^{32}P-postlabeling. Box-and-whisker plot details are given in the legend to Fig. 1. Mean (± SD) values are 2.8 ± 1.6, 1.7 ± 0.9, and 3.0 ± 2.1 adducts/10^8 nucleotides for June, August, and October, respectively. Median values are 2.2, 1.5, and 2.2 for the same times. *P* for the June-August difference is 0.004, and for the August-October difference, *P* = 0.0003 (Wilcoxon sign rank test).

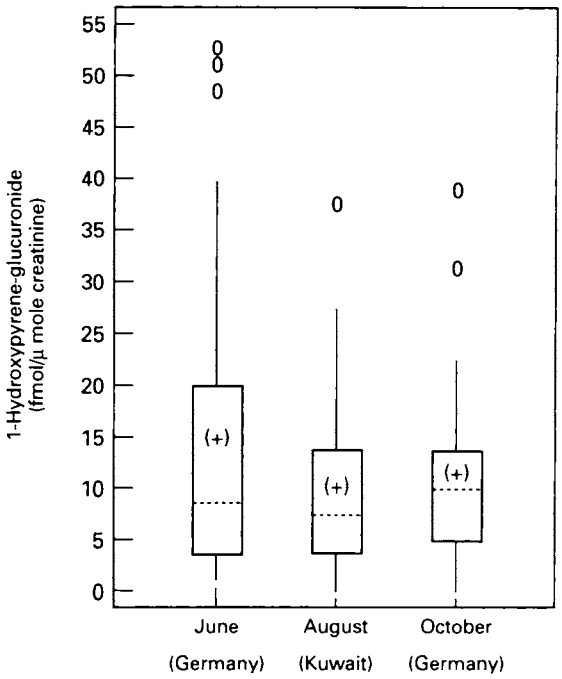

Fig. 3. Urinary 1-OH-PG for a matched set (June, August, and October) of samples from 33 soldiers, including 16 analyzed for DNA adducts. Box-and-whisker plot details are given in the legend to Fig. 1. Mean (± SD) values are 15.1 ± 15.1, 10.2 ± 8.7, and 11.2 ± 8.7 fmol 1-OH-PG/μmol creatinine for June, August, and October, respectively. Median values are 9.1, 7.8, and 9.6 for the same times. *P*s for all comparisons were not significant (Wilcoxon sign rank test).

1991, the East German border. Literature values for some carcinogenic PAHs are available for two different areas in Germany (22). Sampling was performed in 1990, at an unknown time of year, in an industrialized region near Saarbrucken, and a rural northern area of what was then East Germany, in Mecklenburg. The Saarland site is ~165 miles west of Fulda, where the soldiers were stationed. For the Saarland location, ambient values for BP, benz[*a*]anthracene, chrysene, triphenylene, benzo[*b*]fluoranthene, and benzo[*k*]fluoranthene ranged from 7.0 to 16.8 ng/m^3. For the Mecklenburg site, values for the same compounds ranged from 3.0 to 15.7 ng/m^3. All of these values are considerably higher than those measured by the United States Army in Kuwait (Table 1) and may therefore have contributed to the formation of enhanced levels of blood cell DNA adducts in Germany.

Coal combustion exhaust apparently plays an important role in the ambient PAH levels in Germany (22), suggesting that higher pollution levels should be demonstrable in winter. A year-long air monitoring exercise carried out in Julich, Germany, a semi-rural site (23), demonstrated low levels of ambient BP (0.4 ng/m^3) in the summer of 1992, with levels as high as 3.0 ng/m^3 measured in winter. Data from Poland (24) have demonstrated 5-fold higher human lymphocyte aromatic DNA adduct levels in winter as compared with summer, whereas the wintertime air-borne levels of 35 ng of BP/m^3 were also much higher than the 5.5 ng BP/m^3 observed in summer. These studies make plausible the hypothesis that the soldiers' elevated DNA adduct levels observed in Germany at the end of October may be partially due to the increased use of combustion sources for heating and meteorological conditions that consistently result in elevated PAH levels in urban air sheds in winter.

Diet has been shown to contribute to blood cell PAH-DNA adducts and to correlate with 1-OH-PG urinary metabolites. Studies in California firefighters and volunteers in a controlled dietary study have demonstrated a correlation between ingestion of heavily charcoal-broiled food cooked over an open, flaming grill and levels of blood cell PAH-DNA adducts (3, 4, 7). In addition, urinary 1-OH-PG was correlated with ingestion of charcoal-grilled hamburger in a controlled dietary study reported by Kang *et al.* (7). In the Kuwait study, the contribution of diet could not be addressed directly. The questionnaire data gathered in Kuwait were not used in this analysis because some of the subjects reported eating charcoal or flame-broiled food while in Kuwait, which contradicted direct observations that the military mess halls and local eating establishments did not make use of open-flame cooking. The fact that blood cell PAH-DNA and bulky aromatic DNA adducts were lowest in Kuwait suggests that dietary intake of PAHs may not have been substantial at this time. In contrast, when in Fulda the soldiers would have had access to broiled and flame-cooked meat in fast-food eating establishments on the base.

In contrast to our observations showing decreased levels of exposure biomarkers in Kuwait, McDiarmid *et al.* (19), who evaluated SCEs in the same blood samples from this group of soldiers, found elevated levels of SCEs in Kuwait. These investigators showed that the predeployment baseline samples taken in June had levels of SCEs that were significantly lower than those taken in Kuwait in August ($P < 0.0001$) and in Germany in October ($P < 0.0001$). Postulated causes of these increased SCEs were stress, immunoglobulin injections, silica (in sand), and pesticides (19). Whereas the exact cause will be difficult to determine, the results of our study indicate that environmental pollution and events leading to PAH-DNA and

Table 2 PAH biomarker levels stratified by genetic polymorphic variant[a]

Gene	Genotype	Biomarker					
		PAH-DNA adducts		Bulky DNA adducts		1-OH-PG	
		n^b	Median	n^b	Median	n^b	Median
CYP1A1 (*Msp*I)	Site absent (M−/M−)	14	2.0	13	2.0	19	8.6
	Heterozygote (M−/M+)	7	2.6	6	2.4	10	8.7
GSTM1	Functional (1/1, 1/0)	15	2.2	14	1.9	17	8.0
	Null (0/0)	7	2.4	6	2.4	12	11.1
GSTT1	Functional (1/1, 1/0)	15	2.4	13	2.1	22	8.6
	Null (0/0)	6	0.8	6	2.4	7	9.1

[a] *P*s (not shown) for comparison of variants with each biomarker were determined by Wilcoxon rank sum test, and none were significant. Analyses include only individuals for which a matched set of samples was available for each biomarker.
[b] Includes measurements for a matched set of samples taken at all three times; therefore, the number of biomarker measurements is three times higher than the number of individuals (*n*).

bulky-DNA adduct formation are not likely to have induced the SCE increases in Kuwait.

In one study, bulky DNA adduct formation, determined by ^{32}P-postlabeling, was measured in civilian firefighters working in Kuwait in 1991. Darcey *et al.* (25) monitored lymphocytes from nine volunteer firefighters before going to Kuwait and within 3 weeks of their return to the United States, after spending a 6-week tour of firefighting duty in Kuwait. These investigators did not observe a difference in adduct levels in pre- and postexposure samples.

Polymorphisms in genes involved with PAH metabolism had no influence on PAH biomarker levels. This may be due, in part, to the small study sample, which resulted in low power to detect significant differences. Multiple studies have evaluated a correlation between genotypes that alter carcinogen metabolism and exposure biomarker levels, with varying results. Adduct levels in human lung correlated well with activity of lung microsomal aryl hydrocarbon hydroxylase (8, 26). A similar relationship for (7*R*)-N^2-{10-[7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo(α)pyrene]-yl}-deoxyguanosine (BPdG) and aryl hydrocarbon hydroxylase was observed in human placenta (27). However, no association has been found between elevated BP-specific adduct level in human lung samples from autopsy donors (28, 29) or in WBC DNA of California firefighters (30) and the *CYP1A1* exon 7 polymorphism. A group of chimney sweeps was shown to have significant differences in WBC bulky aromatic DNA adducts determined by ^{32}P-postlabeling when *CYP1A1 Msp*I site-absent individuals were compared with those with the site present (31). For the *GSTM1* polymorphism, a significant proportion of lung samples with measurable BPdG adducts were from *GSTM1* null individuals (28, 29), but blood cell DNA adduct levels, measured by BPDE-DNA immunoassay, were not different between functional and null individuals (30, 32). The complications of the interrelationships involved are discussed by Vineis (9).

In conclusion, there was no evidence of an increase in levels of the three PAH exposure biomarkers in soldiers stationed in Kuwait. There was, in fact, some evidence of a decrease in levels of these biomarkers measured in samples obtained in Kuwait as compared with those obtained in Germany. The lower biomarker levels were consistent with the low levels of ambient PAH exposure documented in the areas of the soldiers' duty stations in Kuwait.

Acknowledgments

Appreciation is extended to Margaret Taylor for editorial assistance.

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Cancer Epidemiol Biomarkers Prev 1998;7:545-551.