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POLYCYCLIC AROMATIC HYDROCARBON BIOMARKERS OF INTERNAL EXPOSURE IN U.S. ARMY SOLDIERS SERVING IN KUWAIT IN 1991

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Biomarkers of exposure were applied to a cohort of U.S. Army soldiers who were deployed to Kuwait and Saudi Arabia in 1991 in the aftermath of the Persian Gulf War. The U.S. Army Environmental Hygiene Agency (currently the U.S Army Center for Health Promotion and Preventive Medicine) monitored air and soil for ambient PAHs. In addition, a group of 61 soldiers kept diaries of daily activities. These soldiers provided blood and urine samples in June in Germany before deployment to

Kuwait, in August after 8 weeks in Kuwait, and in October, one month after the return to Germany. DNA, prepared from white blood cells, was assayed for PAH-DNA adducts by immunoassay and bulky aromatic adducts by ^{32}P-postlabeling. Urinary 1-hydroxypyrene-glucuronide (1-OH-PG) was determined by synchronous fluorescence spectrometry. Contrary to expectations, environmental monitoring showed low ambient PAH levels in the areas where these soldiers were working in Kuwait. In addition, literature values for ambient PAH monitoring in Germany in 1990 suggest that the soldiers may have been exposed to higher levels of ambient PAHs in Germany than in Kuwait. Blood cell DNA adduct levels were lowest in Kuwait and increased significantly after the return to Germany. Also, urinary 1-OH-PG levels were lowest in Kuwait and highest in Germany. This study demonstrates modulations in PAH exposure biomarker levels that appear to correlate with ambient PAH exposure.

Keywords: DNA adducts; urinary metabolites; human biomonitoring; ambient monitoring.

INTRODUCTION

Large-scale pollution, occurring in the wake of the Persian Gulf War, caused the U.S. Army to investigate potential exposure of military personnel stationed in Kuwait to airborne polycyclic aromatic hydrocarbons (PAHs) and other toxicants. In May of 1991, the U.S. Army Environmental Hygiene Agency (AEHA) at Aberdeen Proving Ground, initiated a health risk assessment involving troops stationed in Fulda, Germany, who were deployed to Kuwait and later returned to Germany [1]. Environmental sampling and biological surveillance of military personnel included 2300 troops. A sub-group of the whole cohort (n = 61), with informed consent, kept daily diaries and filled out questionnaires to document their location and exposure during the study. These individuals provided blood and urine samples in Germany before going to Kuwait on June 10, after 8 wk of duty in Kuwait (August), and in Germany at the end of

October, about four weeks after departing Kuwait on September 20, 1991. While in Kuwait these soldiers were working about 9 miles south of 105 burning oil well fires, with prevailing winds from the north.

The blood and urine samples have been examined for biomarkers of PAH exposure. These include PAH-DNA adducts, measured by immunoassay, bulky aromatic DNA adducts, measured by ^{32}P-postlabeling and urinary 1-OH-pyrene-glucuronide (1-OH-PG), measured by synchronous fluorescence spectrometry (SFS). The PAH biomarker values were compared with air measurements of PAHs obtained from areas where the soldiers were working in Kuwait, and with literature values for ambient PAH concentrations taken Germany in 1990.

MATERIALS AND METHODS

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/day (EPA TO-13 method) were located near the soldiers' work sites between June and December of 1991. For particle-bound semi-volatile 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 photo-ionizing detectors.

Study population and sample collection.

Male army personnel (n = 61) of the 11th Armored Calvary Regiment gave informed consent and completed a questionnaire covering demographic and exposure information [1]. From 40 ml of blood, buffy coat samples were stored frozen until DNA was prepared. Spot urine samples were obtained at the time of blood draw, and stored frozen.

Determination of urinary 1-OH-PG.

Analysis of 1-OH-PG was performed as previously described [2,3]. Urine samples (10 ml) were purified on C18 Sep-Paks, (Waters Associates, Milford, MA). Eluates were dried and applied to immunoaffinity chromatography columns [4,5]. Materials captured by the antibodies were subjected to HPLC and fractions containing 1-OH-PG were pooled and the concentration determined by SFS ($\Delta\lambda$ 34) [4]. Urinary creatinine levels were determined spectro-photometrically (biochemical kit, Sigma Chemical Co., St. Louis, MO).

DNA preparation and assay of PAH-DNA adducts by dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA).

DNA was isolated by proteinase K digestion, phenol extraction and ethanol precipitation [6]. The concentration was determined by spectrophotometry (A_{260} / A_{280}) and adjusted to approximately 500 µg DNA/ml. The DELFIA, a variation of the standard competitive enzyme-linked immunosorbent assay performed with rabbit antiserum elicited against DNA modified with (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE) [7,8], has been extensively validated [9]. Calf thymus DNA modified with BPDE to 2.5 fmol/ug DNA (80 adducts/10^8 nucleotides) was used for the standard curve and the 50% inhibition was at 6.3 ± 2.4 fmol/well (mean ± SD, n=25). When 25 ug of human DNA was assayed the limit of sensitivity was 0.8 adducts/10^8 nucleotides. The antiserum recognizes DNA samples modified with diol-epoxides of several different PAHs [10].

DNA preparation and assay for aromatic adducts by ^{32}P-postlabeling.

The procedure was as described [11]. DNA (4ug), recovered from the DELFIA plates by phenol extraction was digested with 0.29 U of micrococcal nuclease, 1.2 mU of spleen phosphodiesterase and 0.38 U of Nuclease P1. Incubation with an excess of [γ - ^{32}P]ATP (2330-4760 Ci/mmole) and 3 U of T4 kinase was followed by 4-directional thin-layer chromatography. The diagonal radioactive zone was excised and adduct values were

obtained by calculation from the specific activity of the [γ - ^{32}P]ATP [12].

RESULTS

Air monitoring for particulate and volatile PAHs in Kuwait.
No PAHs were detected on filters from personal sampling pumps that were carried in Kuwait, so 31 different samples of much larger volumes of air were subsequently obtained near the soldiers' work sites. Filter extracts were analyzed for the presence of 23 PAHs and more than half of these compounds were undetectable. 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. Also shown in Table 1 are values for sampling performed in 1990 in two different areas of Germany [13] at an unknown time of year. All of the values obtained at both sites are higher than those measured by the U.S. Army in Kuwait.

Nucleated blood cell DNA adduct measurements by BPDE-DNA DELFIA and ^{32}P-Postlabeling.
Although 61 male army personnel were initially enrolled in the study in Germany, some did not provide biological samples at all time points and for some samples insufficient DNA was available for these assays. A total of 132 blood samples was assayed by the BPDE-DNA DELFIA (Figure 1). Negative samples comprised 44% of the 50 samples for June, 51% of the 37 samples for August and 13% of the 45 samples for October. The median PAH-DNA adduct values were 1.7, 0.6 and 3.5 adducts/10^8 nucleotides for June, August and October, respectively. The mean PAH-DNA adduct values were 2.6 ± 3.3, 2.5 ± 4.1 and 4.1 ± 3.2 adducts/10^8 nucleotides for June, August and October, respectively. For the difference between August and October the P value, by Wilcoxon sign rank test, was 0.0002. A complete sample set of DELFIA data (June, August and October) was available for 22 individuals (Table 2), and again, PAH-DNA adduct values were significantly lower in Kuwait in August than in Germany in October (P = 0.0009).

TABLE I PAH measurements of air samples taken in Kuwait (1991) and Germany (1990, from Jacob et al, [13])

PAH	Camp Thunderock Kuwait ng / m^3	Rural Germany: ng / m^3	Industrial Germany: Mecklenburg Saarland ng / m^3
Benzo[a]pyrene	≤ 0.23	3.0	1.5 - 7.0
Benz[a]anthracene	≤ 0.23	12.3	2.7 - 7.5
Chrysene and triphenylene		5.7	5.1
Chrysene	0.35		
Benzo[b and k]-fluoranthenes	0.27	11.0	4.2 - 17.0

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. The adduct levels in Kuwait (August) were significantly lower than those obtained in Germany in October (Table 2). Figure 2 shows autoradiograms of thin layer chromatographic profiles for 5 soldiers, for whom DNA samples were available at all 3 sampling times. The number and intensity of adduct spots decreased significantly in Kuwait, compared to Germany in June and October. Soldiers 2-5 had the most intense profiles in October.

Determination of urinary 1-OH-PG.

For urine samples taken at the time of blood draw, a matched set of 33 samples was available and the values were corrected for concentration of urinary creatinine (Table 2). Although the levels were lower in Kuwait, none of the differences was statistically significant.

FIGURE 1 Determination of PAH-DNA adducts by BPDE-DNA DELFIA in nucleated blood cell DNA from U.S. Army soldiers stationed in Germany and Kuwait in 1991.

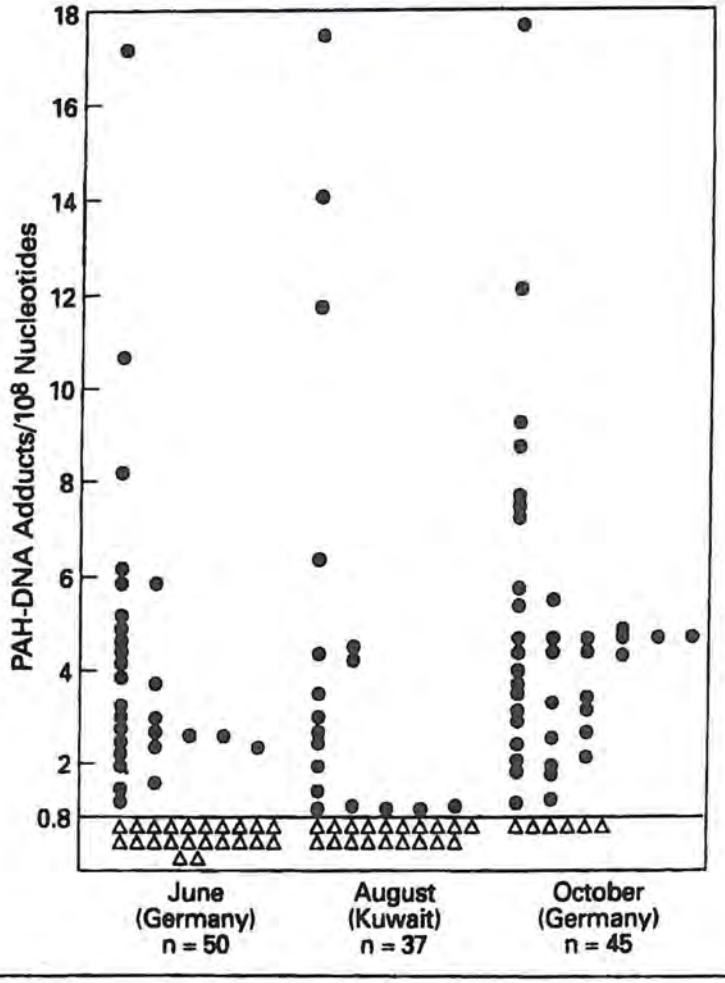

FIGURE 2 Results of ^{32}P-postlabeling TLC and autoradiography of blood cell DNA samples from 5 U.S. Army soldiers. Chromatography conditions were designed to detect bulky aromatic DNA adducts. For each soldier there are three autoradiograms (a, b and c for soldier 1) representing samples taken in June, August and October, respectively.

TABLE II DNA Adduct measurements of blood cell DNA samples, and urinary 1-OH-PG, in U.S. Army soldiers

Month	DNA Adducts/10^8 nucleotides					
	PAH-DNA DELFIA (n = 22)[a]		^{32}P-postlabeling (n = 20)[a]		Urinary 1-OH-PG (n = 33)[c] fmol /μ mole creatinine	
	Mean ± SD[b]	Median	Mean ± SD	Median	Mean ± SD	Median
June	3.1 ± 4.1	2.2	2.8 ± 1.6	2.2	15.1 ± 15.1	9.1
August	1.6 ± 1.8	0.8	1.7 ± 0.9	1.5	10.2 ± 8.7	7.8
October	4.0 ± 3.5	3.2	3.0 ± 2.1	2.2	11.2 ± 8.7	9.6

[a] P values were determined by Wilcoxon sign rank test for matched group comparisons. P values for the June-August comparison were 0.25 by DELFIA and 0.004 by ^{32}P-postlabeling. P values for the August-October comparison were 0.0009 by DELFIA and 0.0003 by. ^{32}P-postlabeling.
[b] Non-detectables (only DELFIA), 45% for June and August and 9% for October, were given a value of 0.4 adducts/10^8 nucleotides, that is half way between zero and the lower limit of assay detection.
[c] P values for all comparisons were not significant by Wilcoxon sign rank test.

DISCUSSION

The environmental monitoring data showed that ambient levels of PAHs were frequently undetectable in the areas where soldiers were working in Kuwait. Similarly, levels of PAH-DNA adducts and aromatic-DNA adducts were the lowest in the samples obtained in Kuwait. In addition, urinary 1-OH-PG levels were lowest in August, although no differences were statistically significant. 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. These observations apply only to this cohort of soldiers, since PAH concentrations are likely to have been high in the immediate vicinity of the burning oil well fires [14,15]. Therefore, projection of these biomarker findings to the majority of military personnel stationed in Kuwait at that time would not be appropriate.

Coal combustion exhaust apparently plays an important role in the ambient PAH levels in Germany [13]; a year-long air monitoring exercise carried out in Julich, Germany (a semi-rural site) [16] 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 [17] have demonstrated five-fold higher human lymphocyte aromatic DNA adduct levels in winter as compared to summer, while the wintertime airborne levels of 35 ng 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 in October may be partially due to indoor heating.

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 laboratory volunteers 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 [3,18,19]. In the Kuwait study the contribution of diet could not be addressed directly, although the military mess halls and the local eating

establishments did not make use of open-flame cooking or grilling.

In contrast to our observations showing decreased levels of exposure biomarkers in Kuwait, McDiarmid et al. [20], who evaluated sister chromatid exchanges (SCEs) in the same cohort of soldiers and the same blood samples, found high levels of SCEs in Kuwait. These investigators showed that the pre-deployment baseline samples taken in June had levels of SCEs that were significantly lower than those taken in Kuwait in August ($P < 0.0001$) and Germany in October ($P < 0.0001$). Outcome was unaffected by known personal SCE modifiers including smoking, age and diet. Because the SCE is not an exposure-specific biomarker, the exact cause of the increase will be difficult to determine. The results of our study indicate that environmental pollution and events leading to PAH-DNA and bulky-DNA adduct formation are not likely to have induced the SCE increases in Kuwait.

The most important observation of this study is the concordant modulation of DNA adduct levels with ambient PAH concentrations. It is possible that indoor heating and the accessibility of charcoal-broiled food in the diet in Germany may have contributed to the increased DNA adduct levels observed in Germany in October. It seems likely that the low ambient PAH concentrations observed in Kuwait in August contributed to the low levels of blood cell DNA adducts found in samples obtained at that time.

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References

[1.] Kuwait Oil Fire Health Risk Assessment for 5 May - 3 December, 1991 (No. 39-26-L192-91. Department of the Army, U. S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD, 1994).

[2.] A. Weston, E. D. Bowman, P. Carr, N. Rothman, and P. T. Strickland, Carcinogenesis, 14, 1053 (1993).

[3.] D. H. Kang, N. Rothman, M. C. Poirier, A. Greenberg, C. H. Hsu, B. S. Schwartz, M. E. Baser, J. D. Groopman, A. Weston, and P. T. Strickland, Carcinogenesis, **16**, 1079 (1995).

[4.] A. Weston and E. D. Bowman, Carcinogenesis, **12**, 1445 (1991).

[5.] R. M. Santella, C. D. Lin, W. L. Cleveland, and I. B. Weinstein, *Carcinogenesis*, **5**, 373 (1984).

[6.] Purification of nucleic acids, in Molecular Cloning, A Laboratory Manual, edited by J. Sambrook, E. F. Fritsch, and T. Maniatis (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1988), Volume 3, Appendix E, p. E.3.

[7.] M. C. Poirier, R. Santella, I. B. Weinstein, D. Grunberger, and S. H. Yuspa, Cancer Res., **40**, 412 (1980).

[8.] R. M. Santella, A. Weston, F. P. Perera, G. T. Trivers, C. C. Harris, T. L. Young, D. Nguyen, B. M. Lee, and M. C. Poirier, Carcinogenesis, **9**, 1265 (1988).

[9.] B. Schoket, W. A. Doty, I. Vincze, P. T. Strickland, G. Assennato, and M. C. Poirier, Cancer Epidemiol. Biol. and Prev., **2**, 349 (1993).

[10.] A. Weston, D. K. Manchester, M. C. Poirier, J. S. Choi, G. E. Trivers, D. L. Mann, and C. C. Harris, Chem. Res. Toxicol., **2**, 104 (1989).

[11.] B. Schoket, M. C. Poirier, and I. Vincze, Sci. Total Environ., **163**, 153 (1995).

[12.] M. V. Reddy and K. Randerath, Carcinogenesis, **7**, 1543 (1986).

[13.] J. Jacob, G. Grimmer, and A. Hildebrandt, in Polycyclic Aromatic Compounds, Proceedings of the 13th International Symposium on Polynuclear Aromatic Hydrocarbons, edited by P. Garrigues and M. Lamotte (Gordon and Breach Science Publs, Malaysia, 1993), p. 43.

[14.] S. Bakan, A. Chlond, U. Cubasch, J. Feichter, H. Graf, H. Grassl, K. Hasselmann, I. Kirchner, M. Latif, E. Roeckner, R. Sausen, U. Schlese, D. Schriever, I. Schult, U. Schumann, F. Sielmann, and W. Welke, Nature, **351**, 367 (1991).

[15.] K. A. Browning, R. J. Allam, S. P. Ballard, R. T. H. Barnes, D. A. Bennetts, R. H. Maryon, P. J. Mason, D. McKenna, J. F. B. Mitchell, C. A. Senior, A. Slingo, and F. B. Smith, Nature, **351**, 363 (1991).

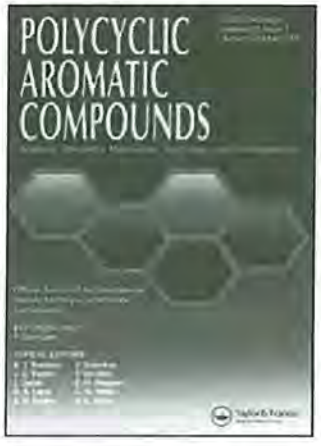
[16.] G. Kloster, R. Niehaus, O. Ollech, and H. Stania, in Polycyclic Aromatic Compounds, (Gordon and Breach Scientific Pbls, Malaysia, 1994), Volume 5, p.149.

[17.] E. Grzybowska, K. Hemminki, and M. Chorazy, Environ. Health Perspect., **99**, 77 (1993).

[18.] N. Rothman, M. C. Poirier, M. E. Baser, J. A. Hansen, C. Gentile, E. D. Bowman, and P. T. Strickland, Carcinogenesis, **11**, 1241 (1990).

[19.] N. Rothman, A. Correa-Villasenor, D. P. Ford, M. C. Poirier, R. Haas, J. A. Hansen, T. OToole, and P. T. Strickland, Cancer Epidemiol. Biomarkers and Prev., **2**, 341 (1993).

[20.] M. A. McDiarmid, D. Jacobson-Kram, K. Koloder, D. P. Deeter, R. M. Lachiver, B. G. Scott, B. Petrucelli, D. Gustavison, and D. Putman, Mutagenesis, **10**, 263 (1995).



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