Scand J Work Environ Health 1990;16(2):135-143

<https://doi.org/10.5271/sjweh.1806>

Issue date: 01 Apr 1990

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This article in PubMed: www.ncbi.nlm.nih.gov/pubmed/2353197

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Detection of adducts of deoxyribonucleic acid in white blood cells of roofers by ³²P-postlabeling

Relationship of adduct levels to measures of exposure to polycyclic aromatic hydrocarbons

by Robin Herbert, MD,¹ Michele Marcus, PhD,¹ Mary S Wolff, PhD,¹ Frederica P Perera, DrPH,² Leslie Andrews, DrPH,² James H Godbold, PhD,¹ Marilyn Rivera, BA,¹ Marina Stefanidis, MS,² Xiao Quing Lu, MS,² Philip J Landrigan, MD,¹ Regina M Santella, PhD²

HERBERT R, MARCUS M, WOLFF MS, PERERA FP, ANDREWS L, GODBOLD JH, RIVERA M, STEFANIDIS M, LU XQ, LANDRIGAN PJ, SANTELLA RM. Detection of adducts of deoxyribonucleic acid in white blood cells of roofers by ³²P-postlabeling: relationship of adduct levels to measures of exposure to polycyclic aromatic hydrocarbons. *Scand J Work Environ Health* 1990;16:135-43. To assess the utility of adducts of deoxyribonucleic acid (DNA) as biomarkers of exposure to carcinogens in an industrial population, a pilot study of roofers occupationally exposed to polycyclic aromatic hydrocarbons was conducted. DNA isolated from white blood cells of roofers and nonoccupationally exposed comparison subjects matched for age, sex, and smoking status was analyzed for DNA adducts with the use of ³²P-postlabeling methods. Occupational exposures to polycyclic aromatic hydrocarbons were assessed by personal air sampling and skin wipes. Ten of the 12 roofers, but only 2 of the 12 comparison subjects, had detectable levels of aromatic DNA adducts in the ³²P-postlabeling assay. Among the roofers, the post-shift levels of polycyclic aromatic hydrocarbons in the skin wipes were correlated with the DNA adduct levels. These results suggest that ³²P-postlabeling assay may be useful for monitoring internal exposures to complex mixtures of aromatic hydrocarbons in industrial populations.

Key terms: benzo[a]pyrene, coal-tar pitch, dermal exposure, exposure assessment.

Occupational exposure to coal-tar pitch has been associated with an increased risk of various malignancies, including lung and skin cancers. The International Agency for Research on Cancer, in reviewing the evidence regarding the carcinogenicity of exposure to coal tars and related products, concluded that there is a causal association between exposure to coal-tar pitch and cancer in humans (1). Occupational groups with potential exposure to coal-tar pitch volatiles include roofers, coke oven workers, foundry workers, and aluminum reduction workers. Carcinogenesis among workers exposed to coal-tar products is believed to be due to exposure to polycyclic aromatic hydrocarbons (PAH).

Roofing workers sustain both respiratory and dermal exposure to PAH when applying hot coal-tar pitch, when removing old pitch roofs, and, to a less extent, when applying hot asphalt roofs. Epidemiologic studies of roofers include a large cohort study

conducted by Hammond et al (2), which found significantly elevated mortality rates for cancer of the lung, oropharynx and esophagus, stomach, and bladder, and for nonmelanoma skin cancer.

More recently, studies of PAH-exposed populations, including roofing workers, have been conducted utilizing a variety of biological markers of exposure to mutagenic and carcinogenic substances (3-5). These markers, which include assays for carcinogenic DNA adducts, hold promise for incorporation into human epidemiologic studies, for use in exposure and risk assessment, and for the biological monitoring of groups exposed to chemical carcinogens. Measurement of carcinogenic DNA adduct formation is particularly promising. Because the formation of chemical addition products with DNA appears to be a critical event in carcinogenesis, measurement of carcinogenic DNA adducts should provide biologically relevant information which reflects an integration of human exposure, absorption, metabolism, and DNA-adduct formation and repair rates (6, 7). Once validated, these assays may allow a more timely detection of human carcinogens in the environment and workplace than is currently possible.

³²P-postlabeling is a sensitive method for the detection of hydrophobic carcinogenic DNA adducts (8-10). It has been used to study adduct formation and repair in a large number of animal studies and to monitor human exposure to environmental carcinogens (11,

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12). This assay has the advantage of not requiring advance knowledge of the adduct of interest and can thus be applied to a wide range of exposure situations.

This pilot study was performed to compare the levels of carcinogenic DNA adducts among industrial workers with well-characterized exposures to PAH to levels in an appropriately matched nonoccupationally exposed group.

Subjects and methods

Population

The study group consisted of 12 roofing workers and 12 comparison subjects who were matched for sex, age (within 11 years), and smoking status and were without occupational exposure to PAH. The potential subjects were excluded if they had cancer, were known to be carriers of human immunodeficiency virus (HIV), or they reported exposure to any of the following potentially mutagenic agents (because sister chromatid exchange frequencies and urinary mutagens were being evaluated in related studies): ethylene oxide, styrene, benzene, chemotherapeutic agents, or vinyl chloride. A roofing site at which there was exposure to PAH was identified with the assistance of Local 8 of the United Union of Roofers, Waterproofers, and Allied Workers. A field survey was conducted at this site in June 1987. Of 14 roofers at the site at the beginning of the survey, one was transferred to another work-site before completion of the survey and therefore was ineligible for participation. Of the remaining 13 roofers, 12 (93 %) agreed to participate. The matched comparison subjects were either employees of the Mount Sinai Medical Center or patients attending the Mount Sinai Occupational Health Clinical Center. Informed consent was obtained from all the study participants.

Field survey

The work process at the roofing site involved the removal or "tear off" of sequential sections of an old pitch roof followed by the replacement of each section with a new asphalt roof. The levels of occupational exposure to PAH among the individual roofers were assessed from air samples from the breathing zone of 10 individuals for two workdays while pitch tear-off and asphalt reapplication was being performed (day 1, Thursday, and day 2, the following Monday). In addition, pre- and postshift skin wipe samples for PAH were obtained from eight roofers on day 2 (Monday). These samples were obtained from a 9 cm² area of the forehead with a Whatman smear tab. Details of the industrial hygiene and skin wipe surveys have been published elsewhere (13).

On day 3 of the field survey (Tuesday), venous blood (35 ml) was collected in coded heparinized containers and transported on ice to Columbia University. All the study participants completed a self-administered ques-

tionnaire which was reviewed by a physician specialized in occupational medicine. The questionnaire obtained detailed information about occupational history (past and present); history of occupational, environmental, and dietary exposure to PAH, as well as to other carcinogens and mutagens; medical history; and history of prior and current use of cigarettes, pipes, and cigars.

Industrial hygiene sample analysis

The air and skin wipe samples were analyzed for individual PAH following method 5506 of the National Institute for Occupational Safety and Health (NIOSH). The individual PAH determined with high-performance liquid chromatography with fluorescence detection were anthracene, fluoranthene, pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, and benzo[ghi]perylene. No anthracene was found in the skin wipe samples. Total PAH, as reported for both the air and skin wipe samples, was the sum of the peaks listed. The personal air samples encompassed essentially the entire workday (excluding breaks), and the results have been reported as time-weighted averages for the period of sample collection.

³²P-postlabeling assay

White blood cells, plasma, and red blood cells were separated by centrifugation, and the samples were frozen at -70°C until used. DNA was isolated by standard phenol/chloroform and ribonuclease treatment procedures (14). The samples were analyzed essentially as described by Reddy & Randerath (15), except that lower levels (10 µCi) of ³²P were used. DNA (1 µg) in 2.5 µl of buffer (0.2 M sodium succinate, 0.1 M calcium chloride, pH 6.0) was digested with 2 µl of micrococcal nuclease (0.3 µg/µl, Sigma, St Louis, Missouri, United States) and 6 µl of spleen phosphodiesterase (0.8 µg/µl, Sigma) for 3.5 h at 37°C. The digests were further treated with 1.2 µl of nuclease P1 (5 µg/µl) after 3 µl of 0.25 M sodium acetate, pH 5.0, and 1.8 µl of 0.3 mM zinc sulfate had been added. After incubation at 37°C for 40 min, 2.4 µl of 0.5 M Tris-base[tris(hydroxymethyl)aminomethane] was added. For the labeling, 4.8 µl of labeling buffer (0.8 M bicine-sodium hydroxide, 0.4 M magnesium chloride, 0.4 M dithiothreitol, and 0.04 M spermidine, pH 9.6) and 10 µCi [³²P]adenosine 5'-triphosphate (1 µl of 10 mCi/ml, 5000 Ci/mmol, Amersham, Arlington Heights, Illinois, United States) was added followed by 3 µl (9 units) of cloned T4 polynucleotide kinase (US Biochemicals, Cleveland, Ohio, United States), and the mixture was incubated at 37°C for 30 min. The resolution of the adducts was carried out on laboratory-prepared (16) polyethyleneimine-cellulose sheets essentially as has already been described (15). Direction one (D-1) was run overnight onto a wick in 1 M sodium phosphate, pH 6.8. After each step the

plates were washed twice in water. Direction two was developed in the same direction as D-1 in 2.5 M ammonium formate, pH 3.5. Direction three, run in the opposite direction of D-1, was developed in 3 M lithium formate, 8.5 M urea, pH 3.5. Direction four was run after the plate was turned 90° in 0.6 M lithium chloride, 0.5 M Tris, 8.5 M urea, pH 8.0. A final chromatography was carried out in 1.7 M sodium phosphate, pH 6.0, to decrease the background levels of the radioactivity. The adduct spots were detected by autoradiography with intensifying screens and quantitated by scintillation counting. For positive samples with multiple adducts, the modification levels have been expressed as the sum of all adducts present. In all the assays a positive control, DNA modified by benzo[a]pyrene diol epoxide (BPDE-I-DNA) (17) and diluted with unmodified DNA from calf thymus to 1 adduct/10⁷ or 10⁸ nucleotides, was assayed. Unmodified DNA from calf thymus was routinely assayed as a negative control. For the mixing experiments, 1 µg of human DNA was mixed with 1 µg of benzo[a]pyrene 7β, 8α-dihydroxy-9α, 10α-epoxy 7,8,9,10 tetrahydrobenzo[a]pyrene (BPDE-I-DNA) at a modification level of 1 adduct/10⁷ nucleotides. All the samples were assayed at least twice, and many samples with detectable adduct levels were assayed more than twice.

Statistical analysis

For each subject, several replications were made of the ³²P-postlabeling assay (from 2 to 6). The assay results were not normally distributed, and therefore nonparametric test statistics were used. For the calculation of the median assay scores, undetectable levels were ascribed a value (1 × 10⁻⁹), which was the midpoint between zero and the limit of detection (2 × 10⁻⁹). Because of the small number of study subjects, an attempt was made to limit the number of statistical analyses performed. A matched pair analysis comparing the median levels of aromatic DNA adducts between the roofers and nonroofers was performed with Wilcoxon's signed rank test. To evaluate a possible association between cigarette smoking and adduct formation, we tested the results of the roofers and nonroofers separately. Among the roofers the adduct levels of the smokers were compared to the corresponding levels of the nonsmokers with the rank sum test for a comparison of independent samples. Among the 12 nonroofers the median adduct levels were the same for 10 individuals, and therefore no statistical tests were performed. The associations between measures of exposure to PAH and aromatic adduct levels were tested with Spearman's rank correlation coefficient. For the roofers, correlations were sought between the median adduct levels and (i) the duration of employment as a roofer, (ii) the levels of air and skin exposure to total PAH and benzo[a]pyrene, and (iii) dietary PAH consumption. Dietary PAH exposure was estimated from the addition of the number of servings of oven-broiled, flame-broiled, charcoal-broiled, or smoked foods con-

sumed in the month prior to the blood drawing, as reported on the questionnaire.

Demographic characteristics and nonoccupational exposure information

All the roofers and nonroofers were men. The median age of the roofers was 33.5 (range 19–45) years, and the median age of the nonroofers was also 33.5 (range 21–44) years. Eight (75 %) of the 12 roofers and eight of the nonroofers were current cigarette smokers. Among the current smokers, the median number of cigarettes smoked daily during the month prior to the blood collection was 25 (range 10–40) cigarettes/d for the roofers and 17.5 (range 10–20) cigarettes/d for the nonroofers. The median number of pack-years was 17.5 (range 6–30) for the currently smoking roofers and 8 (range 4–26) for the currently smoking nonroofers. All the current smokers inhaled and used filter cigarettes. One of the cigarette-smoking roofers also reported occasional pipe smoking; no other roofers or nonroofers reported smoking pipes or cigars. The median number of charcoal-broiled, oven-broiled, flame-broiled, and smoked food servings in the past month was eight (range 0–34) for the roofers and three (range 0–26) for the nonroofers. The roofers consumed a median of 1.5 (range 0–12) servings of alcoholic beverages per day, whereas the respective median of the nonroofers was 0 (range 0–2) per day.

Historical occupational exposure information

None of the nonroofers reported occupational exposure to PAH. Three (25 %) of the 12 roofers had been in the industry five years or less, five (42 %) had worked as roofers for 6–10 years, two (16.5 %) had worked as roofers for 11–15 years, and two (16.5 %) had been employed in roofing for 15–20 years. The median hours worked per week was 35 (range 25–42). The roofers were asked how many consecutive weeks they had worked with hot pitch or asphalt or performed pitch tear-off in the month prior to the blood drawing. Ten (83.3 %) of the 12 reported four weeks of such work, and two (16.7 %) reported three weeks.

Results

Field survey (industrial hygiene)

The results of the personal air sampling performed Thursday (day 1) and Monday (day 2) and of the pre- and postshift skin wipe samples collected on Monday (day 2) are presented in table 1. On Monday (the day before the blood samples were collected) the median level of benzo[a]pyrene in the personal air samples was 0.79 (range 0.60–1.39) µg/m³, and the median total PAH in the personal air samples was 8.32 (range 6.0–13.77) µg/m³. Neither the total PAH nor the benzo[a]pyrene levels in the personal air samples were correlated with those of the same individual on the fol-

Table 1. Polycyclic aromatic hydrocarbon (PAH) exposure among roofers^a.

Roofers	PAH in air ($\mu\text{g}/\text{m}^3$) ^b				PAH in skin wipes (ng)			
	Thursday		Monday		Preshift		Postshift	
	Benzo[a]-pyrene	Total	Benzo[a]-pyrene	Total	Benzo[a]-pyrene	Total	Benzo[a]-pyrene	Total
8	2.03	21.74	1.39	13.77	3.8	30.2	15.8	106.3
3	0.53	8.09	0.90	9.87	.	.	.	.
4	1.46	16.61	0.83	8.42	5.2	41.2	58.0	674.9
14	1.54	18.37	0.60	6.24	.	.	.	.
16	1.03	23.07	0.65	8.28	4.4	28.5	40.3	385.3
12	0.97	14.22	.	.	.	.	.	.
17	.	.	0.61	6.05	0.6	4.4	13.7	158.7
5	1.78	32.35	0.62	7.81	2.7	26.4	44.7	392.1
13	1.71	19.80	.	.	12.9	97.9	62.8	782.9
15	0.82	20.53	0.92	12.32	15.6	121.4	159.3	1691.9
18	.	.	0.85	8.33	.	.	.	.
7	1.30	16.12	0.75	7.67	6.1	57.4	46.7	771.5

^a Thursday was six days, Monday one day, prior to blood collection. Skin wipes were taken on Monday.^b Time-weighted average.**Table 2.** ³²P-postlabeling assay for aromatic DNA adducts among the roofers and nonroofers.

	Number of samples	Age (years)	Smoking ^a	DNA adducts/10 ⁷	
				Median	Range ^b
Roofers					
Number 8	4	34	0	0.08	0.02—0.09
Number 3	3	29	0	0.12	0.12—0.40
Number 4	6	21	0	0.19	0.07—0.23
Number 14	2	19	0	0.27	0.14—0.43
Number 16	3	32	30	0.01	ND—ND
Number 12	3	45	40	0.01	ND—ND
Number 17	4	41	40	0.10	0.06—0.21
Number 5	4	36	10	0.13	0.09—0.20
Number 13	2	33	40	0.15	0.13—0.16
Number 15	3	36	20	0.24	0.19—0.62
Number 18	3	32	10	0.24	0.21—0.52
Number 7	3	37	20	0.96	0.88—1.28
Nonroofers					
Number 1	2	35	0	0.01	ND—ND
Number 2	2	35	0	0.01	ND—ND
Number 3	2	30	0	0.01	ND—ND
Number 4	2	27	0	0.01	ND—ND
Number 5	2	22	10	0.02	ND—0.02
Number 6	2	44	20	0.01	ND—ND
Number 7	2	39	20	0.01	ND—ND
Number 8	2	27	20	0.01	ND—ND
Number 9	2	32	10	0.01	ND—ND
Number 10	2	36	15	0.01	ND—ND
Number 11	2	21	10	0.01	ND—ND
Number 12	2	37	20	0.03	ND—0.05

^a Cigarettes per day.^b ND (not detectable); less than 0.02 adducts per 10⁷ DNA nucleotides. All of the ND values were replaced by 0.01 in the calculation of the medians.

lowing Monday. However, the meteorological conditions were different on the two days and not all individuals performed the same job on both days. The levels of benzo[a]pyrene in the postshift skin wipes on Monday ranged from 13.7 to 159.3 ng with a median level of 45.7 ng, and the range of the levels of total PAH in the postshift skin wipes was 106.3—1692 (median 533) ng.

³²P-postlabeling assay

Ten (83 %) of the 12 roofers and two (17 %) of the 12 nonroofers had detectable levels of aromatic DNA adducts in the ³²P-postlabeling assays. These results are presented in table 2, and examples of the ³²P-postlabeled DNA isolated from the white blood cells of the roofers are shown in figure 1. The majority of the samples from the roofers showed one predominant

Figure 1. Autoradiogram of covalent DNA adducts in human peripheral white blood cells as revealed by 2-D PEI-cellulose thin-layer chromatography of ^{32}P -labeled DNA digests. (a = roofer number 7, b = roofer number 8, c = roofer number 17, d = mixing experiment in which DNA from roofer number 17 was mixed with BPDE-I-DNA before the analysis)

spot on the autoradiograph and varying levels of other minor spots. Roofer number 7 had the highest total adduct level (figure 1a) with three distinct adduct spots detectable. In another case (roofer number 8), two distinct spots were detectable (figure 1b), while in a number of other samples only a single spot was visible (eg, roofer number 17, figure 1c). Of the samples from the unexposed nonroofers, only two contained detectable

adducts, both of them near the sensitivity limit for the assay in our laboratory (2 adducts/ 10^9 nucleotides). The adducts detected in the human DNA samples migrated in the same region of the plate as the major DNA adduct of benzo[a]pyrene diol epoxide. To determine whether any of the adducts present in the roofer samples were related to this adduct, DNA from two roofers (numbers 7 and 17) were mixed with

BPDE-I-DNA before the digestion and labeling. The adducts in the human samples did not cochromatograph with the major BPDE-I guanine N2 adduct in either sample. Data for sample number 17 are shown in figure 1d.

A comparison of the median adduct levels of the matched pairs of roofers and nonroofers showed that the roofers had significantly higher adduct levels than the nonoccupationally exposed subjects ($P < 0.01$, two-tailed). Among the roofers, no association was found between the adduct levels and current cigarette smoking ($P > 0.10$). Among the nonroofers, the two individuals with detectable adduct levels were both smokers; the remaining six smoking nonroofers all had undetectable adduct levels.

The correlations between the measures of exposure to PAH and the median aromatic adduct levels were evaluated for the roofers with Spearman's rank correlation coefficient. The results of this analysis are summarized in table 3. The adduct levels of the roofers were not correlated with the levels of benzo[a]pyrene nor the total PAH in the personal air samples from either Thursday or Monday. Among the eight roofers for whom the skin wipe data were available, the median adduct levels correlated with both the postshift skin levels of the total PAH (Spearman rank correlation coefficient 0.81, $P < 0.05$) and with the postshift skin levels of benzo[a]pyrene (Spearman rank correlation coefficient 0.74, $P < 0.05$). The preshift skin levels of PAH and benzo[a]pyrene were modestly correlated with the median adduct levels; these results were not, however, statistically significant. The adduct levels of the roofers were not correlated with the duration of employment as a roofer or with the dietary consumption of PAH.

Table 3. Spearman rank correlation coefficients for the median of the aromatic DNA adduct levels and the exposure measures among the roofers. (DNA = deoxyribonucleic acid, PAH = polycyclic aromatic hydrocarbons, BaP = benzo[a]pyrene)

Exposure measures	Rank correlation coefficient r_s	P-value
PAH in personal air, Thursday ^a	-0.16	0.66
PAH in personal air, Monday ^a	-0.24	0.50
BaP in personal air, Thursday ^b	0.07	0.84
BaP in personal air, Monday ^b	-0.13	0.73
PAH in preshift skin wipe samples ^c	0.69	0.06
BaP in preshift skin wipe samples ^b	0.67	0.07
PAH in postshift skin wipe samples ^c	0.81	0.01
BaP in postshift skin wipe samples ^d	0.74	0.04
Years employed as a roofer	-0.18	0.56
Dietary PAH ^e	-0.20	0.52

^a Total polycyclic aromatic hydrocarbon $\mu\text{g}/\text{m}^3$ (time-weighted average); the Monday samples were the more recent.

^b Benzo[a]pyrene ($\mu\text{g}/\text{m}^3$) (time-weighted average).

^c Total polycyclic aromatic hydrocarbons (ng) obtained Monday.

^d Benzo[a]pyrene (ng) obtained Monday.

^e Total number of servings of charcoal broiled, oven-broiled, flame-broiled, and smoked food servings consumed in the month prior to the blood collection.

Discussion

In this pilot study, we found that aromatic DNA adducts were detectable in the white blood cells of 83 % of a sample of roofers with occupational exposure to PAH but were detected, at very low levels, in only 17 % of matched, nonoccupationally exposed subjects. The DNA adduct levels in the white blood cells of the roofers were significantly correlated with the levels of total PAH and benzo[a]pyrene in the postshift skin wipe samples. Neither the total PAH nor the benzo[a]pyrene levels of the personal air samples correlated with the levels of the aromatic DNA adducts. DNA adducts hold great promise for use as biomarkers of human exposure to occupational and environmental carcinogens. Previous studies of populations exposed to PAH in industry (such as roofers, coke oven workers, and foundry workers) utilizing immunoassay techniques with antibodies to BPDE-I-DNA have demonstrated the presence of white blood cell adducts (3–5). Adducts have also been demonstrated in coke oven workers and foundry workers with fluorescence methods (3, 5).

In this study, we utilized ^{32}P -postlabeling methods, which can detect effects of exposure to complex mixtures of bulky aromatic compounds such as PAH and thus are ideal for monitoring workers who are exposed to complex mixtures of PAH. Aromatic DNA adducts have been detected by ^{32}P -postlabeling methods in a number of human tissues, including human placenta, buccal mucosa, bone marrow, lung, and peripheral white blood cells (18–22). More recently, foundry workers, who sustain occupational exposure to a mixture of PAH, have been studied with ^{32}P -postlabeling methods (23). With the use of historical (nonconcurrent) industrial hygiene data and job title, foundry workers were classified as belonging to high (benzo[a]pyrene $\geq 0.2 \mu\text{g}/\text{m}^3$), medium ($0.05 \mu\text{g}/\text{m}^3 < \text{benzo[a]pyrene} < 0.2 \mu\text{g}/\text{m}^3$), or low (benzo[a]pyrene $< 0.05 \mu\text{g}/\text{m}^3$) exposure groups. Aromatic DNA adducts were found to be present in the white blood cell DNA in three out of four samples from highly exposed workers, in eight out of 10 samples from the medium exposure group, in four out of 18 samples from the low exposure group, and in one out of nine samples from the nonoccupationally exposed subjects. The adduct levels among the foundry workers ranged from undetectable to 1 adduct/ 10^7 nucleotides. Our work corroborates the findings of this study and indicates that ^{32}P -postlabeling methods provide a useful bioassay of internal exposure to carcinogenic PAH in the workplace.

This study controlled for age, sex, and cigarette smoking, one of the major nonoccupational sources of exposure to PAH. Environmental sources of exposure to PAH were carefully characterized, and both air and dermal levels of occupational exposure to PAH were measured.

The levels of total PAH and benzo[a]pyrene in the personal air samples did not correlate with the adduct

Figure 2. Total polycyclic aromatic hydrocarbons (PAH) in postshift skin wipe samples from roofers versus the median of the white blood cell levels of aromatic DNA adducts as determined by ³²P-postlabeling assay.

levels. Nevertheless, the air sampling data provide useful information about exposure ranges at which adduction occurs and, hence, provide information which may be useful in human risk assessment. The roofers in this study were exposed to levels of benzo[a]pyrene which would be classified as high in the scheme utilized in the foundry worker study (23) and which would be classified as fairly high in the scheme proposed by Linstedt & Sollenberg in 1982 (24).

The data from the postshift skin wipe samples for PAH and benzo[a]pyrene were significantly correlated with the adduct levels of the roofers (figure 2). The finding of a correlation between the adduct levels and the skin levels of PAH and benzo[a]pyrene but not between the adduct levels and the air exposure data may be an aberration caused by the small numbers since exposure data were missing for some individuals. Alternatively, white blood cell aromatic DNA adduct levels may prove particularly useful as indicators of dermal exposure to PAH. PAH are absorbed percutaneously, and skin has been demonstrated to have significant arylhydrocarbon hydroxylase activity. Pohl et al (25) have shown that benzo[a]pyrene metabolites, including diol epoxides, can be formed in skin. More recently, ³²P-postlabeling methods have been used to demonstrate the presence of DNA adducts in mouse skin treated with benzo[a]pyrene, benz[a]anthracene, and benzo[g,h,i]perylene (26), as well as in mouse skin treated with coal tar or asphalt (27). Work by Schocket et al (28) has also demonstrated that the treatment of adult and fetal skin with coal tar or asphalt in short-term culture results in the formation of DNA adducts which are detectable by ³²P-postlabeling. However, although suggestive, the skin wipe technique is relatively new, and it is not known how it relates to actual dermal or respiratory absorption. Therefore, proper interpretation of the use of skin wipes as a measure of exposure requires further study and validation.

The preshift skin wipe levels of PAH and benzo[a]pyrene were modestly, but not significantly, correlated

with the median of the adduct levels (table 3). These preshift wipes, obtained on a Monday morning, presumably reflect levels of PAH retained in the skin over the weekend despite temporary cessation of occupational exposure.

We did not find that cigarette smoking was associated with elevated adduct levels in peripheral white blood cells in the ³²P-postlabeling assay. Other studies investigating the relationship between adduct levels in peripheral white blood cells and cigarette smoking have yielded similar results (21, 23). However, a relationship between adducts and cigarette smoking has been found in other tissue types, such as placenta and lung (18, 19, 22). Although the roofers in our study tended to smoke more heavily than their matched counterparts, it is unlikely that this differential distribution affected the findings of this pilot study since cigarette smoking does not appear to be associated with increased adduct levels. We also found no effect of dietary PAH consumption on the median adduct levels among the roofers and therefore different levels of dietary PAH consumption is unlikely to have resulted in confounding.

The identity of the adducts present in this study remains unknown, although they are likely to be aromatic DNA adducts. In the two samples evaluated in a mixing experiment, the adducts did not appear to be the major BPDE-1-guanine N2 adduct; however these results cannot be generalized to the samples from the other study participants.

While ³²P-postlabeling was able to discriminate between the exposed and unexposed subjects of our study, some limitations of the assay must be noted. The quantitative data presented in table 2 have been based on the assumption that the DNA was completely digested. While this assumption is true for a number of types of carcinogen-modified DNA, some unknown adducts may be resistant to nuclease digestion and therefore not detected by the assay. In addition, other adducts originally present may not be resistant to

nuclease P1 and thus would not be labeled. In the procedure used in this study, neither are alkylated bases that chromatograph with the normal nucleotides detected. Therefore, the adduct levels given in table 2 should be considered the minimum amount present.

Nonetheless, although this study is limited by its small sample size, it suggests the utility of incorporating ^{32}P -postlabeling assay into studies of industrial populations as a marker of internal exposure to PAH and as an adjunct to traditional methods of exposure assessment. Incorporation of this biomarker into traditional epidemiologic studies holds promise both for improving the ability to discern the degree of risk posed by occupational and environmental exposures to carcinogens and putative carcinogens and for elucidating disease mechanisms.

Acknowledgments

This work was supported by grant ES 00928 from the National Institute of Environmental Health Science. Dr Herbert was a Charles A Dana Foundation Fellow in Environmental Epidemiology at the time of the study.

We thank Mr C DeBaise and Mr P Dolce of Local 8 of the United Union of Roofers, Waterproofers and Allied Workers for arranging and facilitating the field survey, Ms C Dellenbaugh for assisting with the field survey, Ms D Monahan for the specimen collection and the recruitment of comparison subjects, and Ms AM Sanchez for the manuscript preparation.

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Received for publication: 4 August 1989