

Analysis of deoxyribonucleic acid adducts in workers

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SZYFTER K, HEMMINKI K. Analysis of deoxyribonucleic acid adducts in workers. *Scand J Work Environ Health* 1992;18 Suppl 1:22–6. Repeated blood samples were obtained from volunteers who were occupationally exposed to polycyclic aromatic hydrocarbons in a Finnish iron foundry. Aromatic adducts were determined in white blood cell deoxyribonucleic acid of the subjects with the ³²P-postlabeling technique after nuclease P1 treatment and butanol extraction, which showed no major difference. When repeated samples were analyzed, it appeared that the oldest ones displayed the lowest adduct levels, probably due to the instability of the adducts upon storage at –20°C. The workers tended to maintain their adducts at a uniform level, a finding suggesting the contribution of host factors in the control of adduct levels.

Key terms: benzo[a]pyrene, binding to deoxyribonucleic acid, foundry workers, occupational exposure, ³²P-postlabeling.

Deoxyribonucleic acid (DNA) adducts of carcinogenic agents have recently been used as a dose monitor for exposed humans (1–5). The methods for adduct assay include the ³²P-postlabeling method (6–9), immunoassay (10–13), scanning fluorescence spectroscopy (14), and mass spectroscopy (15, 16). Qualitatively, the methods have been validated through comparisons of exposed populations with unexposed referents. The exposures studied for humans include tobacco smoke (11, 12, 17–19), polycyclic aromatic hydrocarbons (PAH) in the occupational environment (15, 16, 20–23), and cisplatin in cancer chemotherapy (13, 24). The validity of the ³²P-postlabeling study has been demonstrated in an interlaboratory comparison involving foundry workers (25).

In iron founding, PAH are formed during the thermal decomposition of the organic ingredients of the mold material, and the levels of PAH formed are dependent on the types of molds used (26–29). In the foundry where our study was carried out, the concentrations of benzo[a]pyrene (BaP) in the air samples varied between 10 and 50 ng · m^{–3}. In this report we present the results on DNA adducts of individual foundry workers followed over some years. In addition, two pretreatment methods, P1 nuclease digestion and butanol extraction (9, 30), are compared.

Materials and methods

Blood samples were obtained from healthy volunteers from a Finnish iron foundry. The sampling was carried out over a three-year period (1985–1988), and only those persons were included who had been sampled at least twice.

Blood (20–50 ml) was drawn into heparinized tubes and transported on ice to Helsinki. Cells were collected by centrifugation, and red blood cells were lysed by washing twice with 0.15 M ammonium chloride, each time followed by centrifugation at 1000 g for 5 min. Nuclei were isolated by washing the cells in 0.1 M saline solution, 0.5% polyethylene glycol p-isooctylphenyl ether (Triton X-100), and 10 mM tris(hydroxymethyl)aminoethanehydrochloride acid (TrisHCl) pH 7.4, followed by centrifugation. DNA was isolated from nuclei by treatment with pancreatic ribonuclease and proteinase K followed by extraction with phenol and chloroform:isoamyl alcohol (24:1), as described earlier (23).

The DNA samples (5 µg) were assayed by the nuclease P1 (9) or butanol extraction (30) version of ³²P-postlabeling as described earlier (31). The solvents in the thin-layer chromatography were 1.0 M sodium phosphate, pH 6.8 [direction 1 (D1)]; 4.8 M lithium formate, 7.8 M urea, pH 3.5 [direction 3 (D3)]; and 1.0 M sodium phosphate 7.2 M urea, pH 6.4 [direction 4 (D4)].

Results

The two pretreatment methods applied before the postlabeling, nuclease P1 digestion and butanol extraction, were tested with two aromatic standard compounds, the N²-deoxyguanosine-3'-monophosphate (N²-dGMP) adduct of BaP and the C-8-dGMP adduct of 4-aminobiphenyl (ABP). These are the main adducts of the respective carcinogens in DNA. The P1

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technique gave a slightly higher yield over butanol with the BaP standard, while the opposite was the case with the aminobiphenyl adduct (table 1). This finding is in accordance with the resistance of the N² adducts and sensitivity of the C-8 adducts towards nuclease P1 (30). The autoradiograms of the treated standard compounds are shown in figure 1.

The two techniques were compared with the use of 11 DNA samples from foundry workers. The total adduct levels were somewhat (20 %) higher after the butanol extraction procedure. The adduct patterns did not show marked differences (figure 2), a finding suggesting that a large proportion of the adducts were resistant to nuclease P1. The two distinct spots, the X spot (seen in a variety of animal species) and the spot comigrating with the BaP standard (marked B in figure 2), were also somewhat more abundant with the butanol extraction procedure.

Repeated samples were obtained from all of the workers. Figure 3 shows an example of a worker sampled at different times. The most apparent finding is the higher level of adducts in the most recent samples.

The total DNA adduct levels obtained by the P1 modification of the ³²P-postlabeling technique are plotted in figure 4 for all of the workers sampled according to the sampling time. The figure shows relatively large interindividual variation. It is striking that with one or two exceptions the adduct levels appear to increase with time. When the data are plotted on a time axis (figure 5), the individual consistency of the adduct levels is revealed, those with low levels staying at a low level, and there is limited crossing of the lines for individual adduct levels.

Table 1. Comparison of P1 and butanol extraction with adduct standards. (BaP = benzo[a]pyrene, dGMP = deoxyguanosine-3'-monophosphate, ABP = 4-aminobiphenyl)

Adduct	Relative labeling ^a (%)
BaP-dGMP	
Direct labeling	100
Nuclease P1	97
Butanol extraction	79
ABP-dGMP	
Direct labeling	100
Nuclease P1	1.4
Butanol extraction	72

^a Means of three experiments.

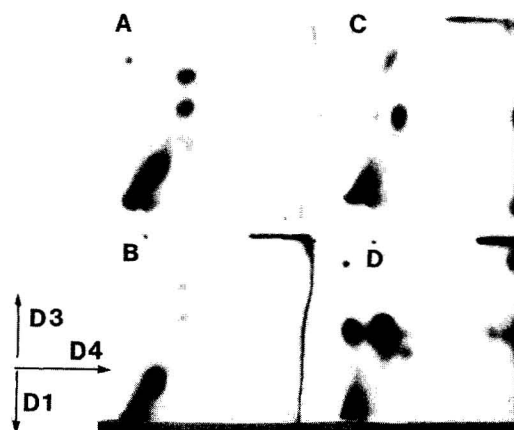


Figure 1. Autoradiograms of benzo[a]pyrene-N²-deoxyguanosine-3'-monophosphate (A and B) and 4-aminobiphenyl-C8-deoxyguanosine-3'-monophosphate (C and D) after nuclease P1 treatment (A and C) and butanol extraction (B and D). (D1 = direction 1, D3 = direction 3, D4 = direction 4)

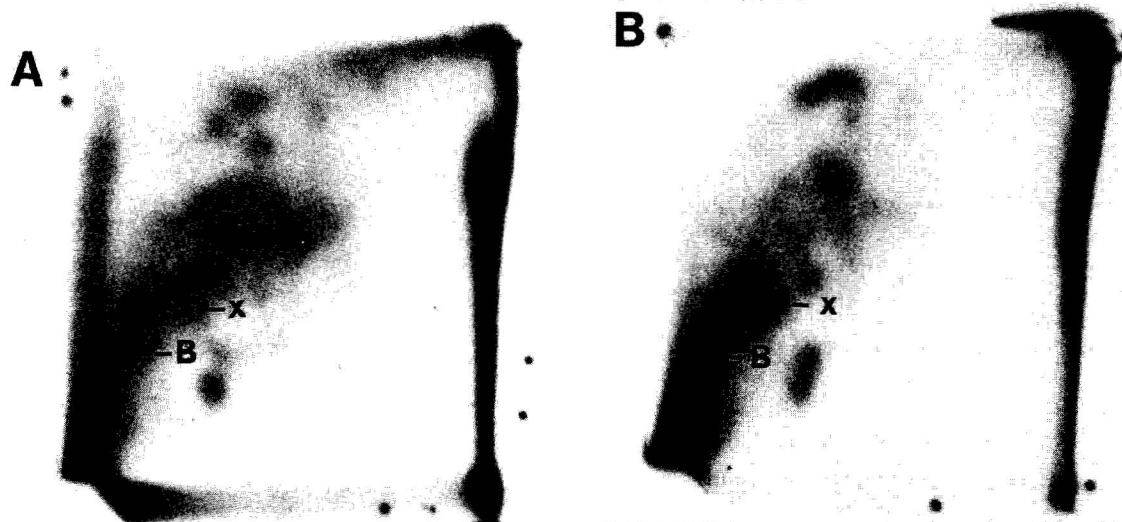


Figure 2. P1 nuclease treatment (A) and butanol extraction (B) of a deoxyribonucleic acid sample from a foundry worker. Two commonly found spots "X" and "B" are marked.

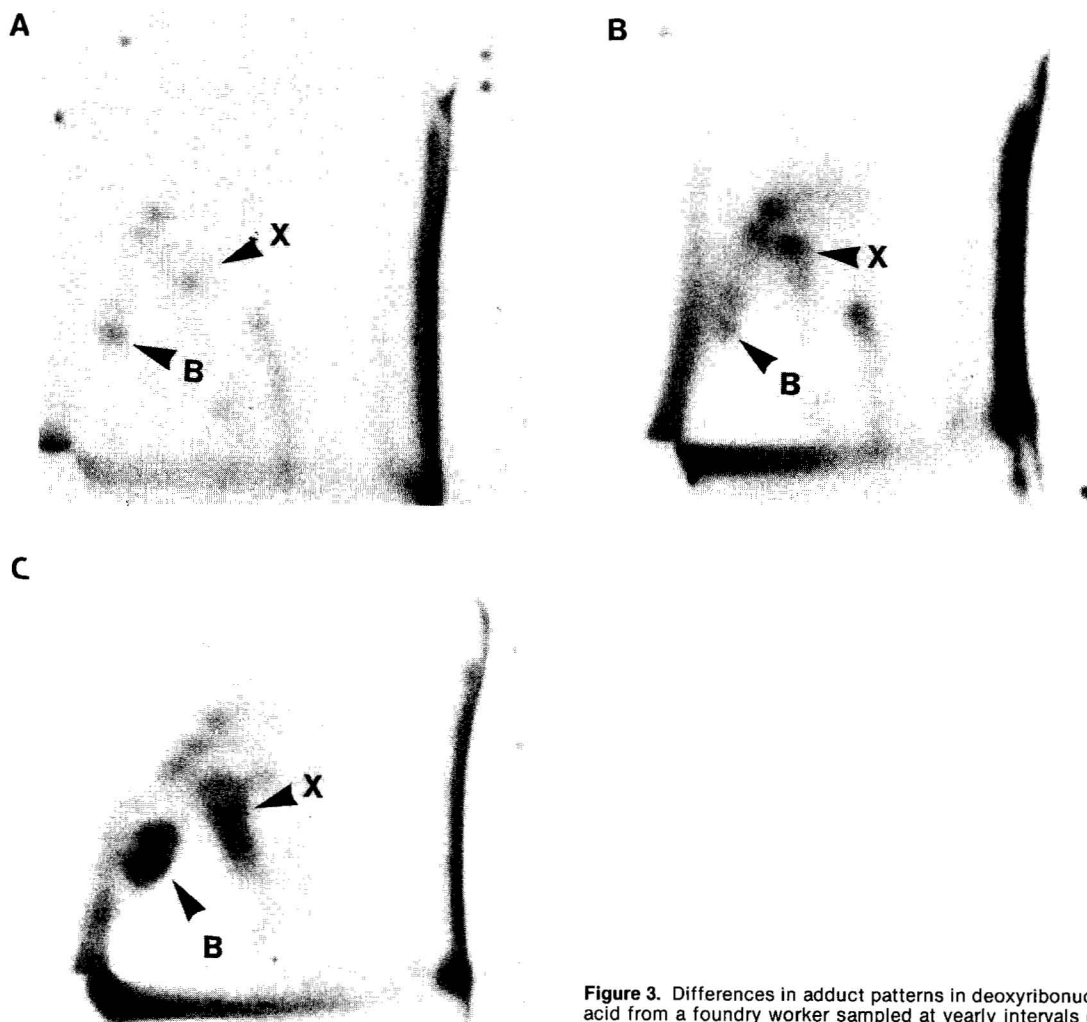


Figure 3. Differences in adduct patterns in deoxyribonucleic acid from a foundry worker sampled at yearly intervals (A = 1986, B = 1987, and C = 1988)

Discussion

DNA adduct measurements have been used in occupational settings where PAH exposures are high (12, 14–16, 20–23, 25, 32–35). Many of the studies have found a difference between the workers and referents, including classification into exposure categories (12, 32, 35). However, large interindividual variations in the levels of adducts have been noted in all of the studies. These variations have been explained by differential exposure and host factors, including individual metabolic capacities. This study investigated the contribution of the host factors in selecting individuals staying in the same foundry job but sampled at different times. The nuclease P1 technique was selected for the main study since no main difference was observed in the quantity or pattern of adducts with this method or the more tedious butanol extraction technique.

A surprising finding was an apparent increase in adduct levels with time. Although we cannot exclude some accumulation of adducts into long-lived lymphocytes (31), it is likely that the chemical stability of the adducts is not infinite, in spite of storage at -20°C . In addition, the older samples had been thawed more often for various types of assays. As we observed a similar trend in another set of DNA samples, one has to be concerned with sampling the study and reference groups close in time. The results supported the contribution of host factors in controlling the level of adducts. Individuals with high adduct levels tended to maintain high adduct levels in repeated samples. As most of the workers belonged to the “medium” exposure category (12), it is unlikely that the exposures could have been systematically different over the study period so as to explain constant differences in the adduct levels. The present data are unable to single out individual host factors such as metabolic parameters,

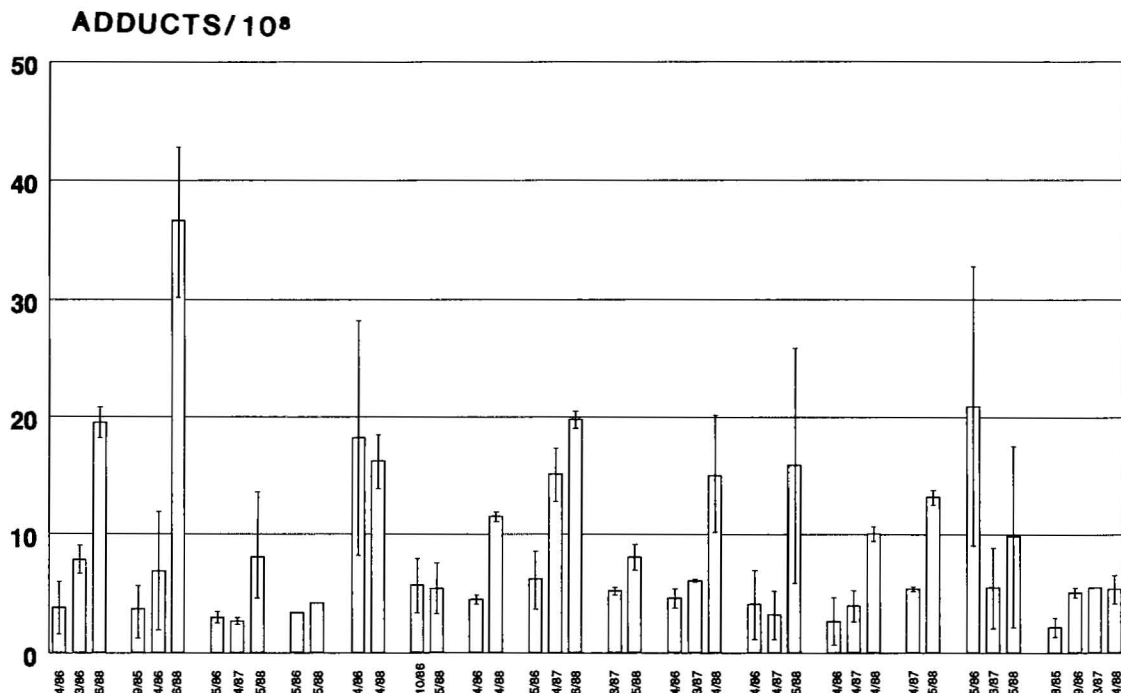


Figure 4. Adduct levels of individual foundry workers (SD, N=3) sampled at different times.

DNA repair, or life-times in white blood cell populations, which have to be addressed in future studies.

Acknowledgments

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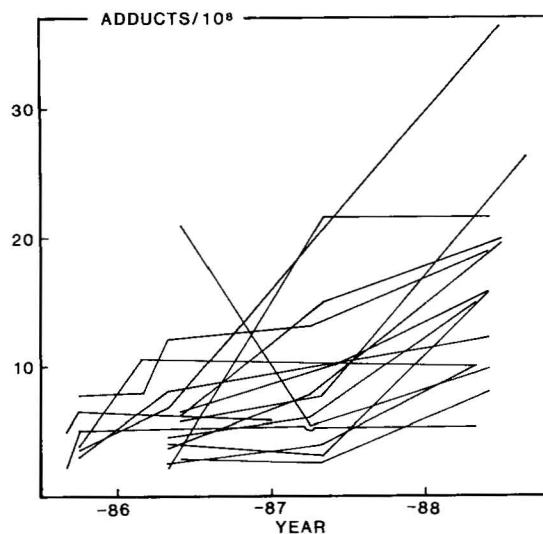


Figure 5. Adduct levels from individual foundry workers sampled more than twice.

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