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Seasonal Variations in Levels of DNA Adducts and X-Spots in Human Populations Living in Different Parts of Poland

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White blood cell DNA adducts were measured in coke oven workers, in residents from the area next to the coke oven in Silesia, Poland (highly industrialized region), and in residents from the rural area of Poland using the ^{32}P-postlabeling technique. This method detected aromatic adducts including adducts formed by polycyclic aromatic hydrocarbons (PAHs). Highest levels of adducts in DNA were seen in the group of coke battery workers (6.9 adducts/10^8 nucleotides). Seasonal variations in levels of DNA adducts were observed both in residents of the district near the coke oven area and individuals from the rural area of Poland. Blood samples collected from people living near the coke oven in winter showed much higher levels of DNA adducts than blood samples obtained in summer (5.0 adducts/10^8 nucleotides in winter and 1.4 adducts/10^8 nucleotides in summer). The difference in the level of DNA adducts between winter and summer was smaller in the group of people living in the rural area (3.2 adducts/10^8 and 2.2 adducts/10^8, respectively). In most cases the levels of X-spots correlated with the levels of other DNA adducts. Correlation coefficients(r) between the levels of X-spots and other adducts ranged between 0.46 and 0.74 ($p < 0.05$), except for coke oven workers where no correlation was observed.

Introduction

Humans are exposed to polycyclic aromatic hydrocarbons (PAHs) from a wide variety of occupational (*1,2*), environmental (*3*), and dietary sources (*4*). Humans occupationally exposed to high concentrations of PAHs are known to be at an increased risk of developing lung cancer (*6*). The binding of chemicals to DNA is thought to be the critical initiating event in tumor formation. PAH–DNA adducts in white blood cells are then considered as internal dosimeters of human exposure to these compounds (*7–9*).

Silesia, a highly industrialized region in the south of Poland, is at present probably one of the most polluted areas in the world. The Silesian population is exposed to elevated levels of PAHs in the ambient air, with concentrations of the model compound benzo[*a*]pyrene (BaP) exceeding the permitted level (1 ng/m^3) by several times throughout the region (*10*). Pollution is caused by coal mines, coke ovens, steel mills, smelters, foundries, and chemical factories frequently using antiquated technological processes. In addition to industry, heavy automobile traffic and combustion of coal for cooking and heating homes contribute to the air pollution, especially in winter (*10*).

Materials and Methods

Groups of Volunteers Studied

One hundred thirty-three males were enrolled in the study between January and October 1990. Each person was asked about age, job titles, dietary habits, smoking, and use of medicines. Blood samples were obtained from healthy volunteers from coke battery workers in Gliwice, residents from the district of Gliwice located near the cokery, 12 children (both sexes, 7–12 years old) living in Bytom, and residents from the eastern Polish countryside 300 km away from Silesia. Blood samples from the same individuals living near the coke oven were drawn twice: in winter and in summer. Blood (20–50 mL) was drawn into heparinized tubes and transported on ice to the laboratory and DNA was prepared as described (*11*).

^{32}P-Postlabeling Assays

Coded DNA samples (4 μg) were assayed in duplicate using the postlabeling method as described previously (*12*) with slight modifications. The 7*R*,8*S*,9*S*-trihydroxy-10*R*-(N^2-deoxyguanosyl-3′-monophosphate)-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE-dG; 6.91 × 10^{-7} M) (Midwest Research Institute, Kansas City, MO) was used as a reference compound. DNA samples were digested with the nuclease P_1 procedure and postlabeled

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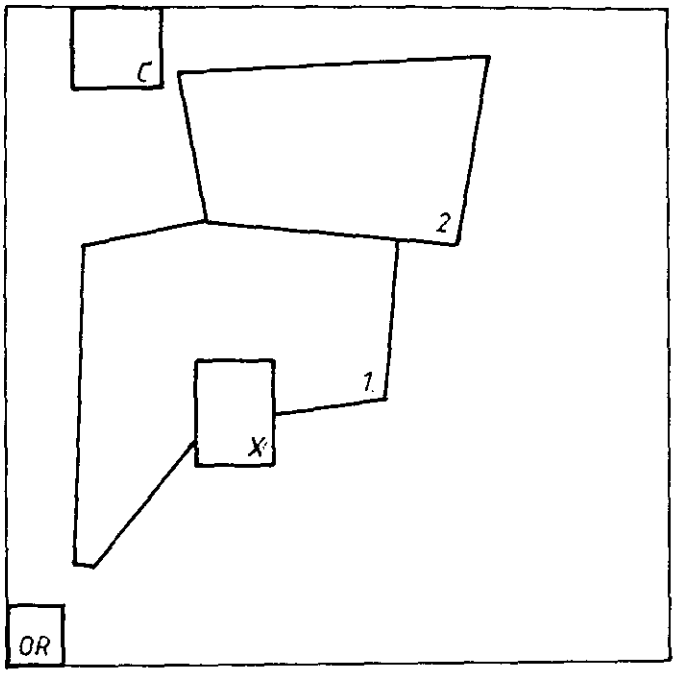

FIGURE 1. Four areas excised from the TLC plates. X, contained X-spot, 1 and 2 were DNA adducts, and area C was a blank plate for counting radioactivity. OR indicates the origin of sample application.

Table 1. Adduct levels from white blood cells of studied populations collected in summer.

Population	n	Adducts/10^8 nucleotides[a]	SD
Gliwice, coke oven workers	37	6.85	10.67
Gliwice, residents	25	1.42	2.21
Bytom, children	12	1.28	0.62
Biala Podlaska, residents	27	2.13	2.22

[a]Data are presented as arithmetic means.

as described (*13*). The ^{32}P-labeling procedure was performed in kinase buffer (pH 9.8) supplemented with 33 pmole/μL of cold ATP. The final kinase mixture, containing 30 μCi of [γ-^{32}P] ATP (3000 Ci/mmole; Amersham, UK), 33 pmole cold ATP, and 4 U of T_4 polynucleotide kinase, was added to the samples and incubated for 40 min at 37 °C.

Adducts were resolved on polyethyleneimine (PEI)–cellulose thin layers (Macherey-Nagel, Germany). Solvents used were as follows: 1 M sodium phosphate, pH 6.8 (dimension 1), 4.8 M lithium formate, 7.8 M urea, pH 3.5 (dimension 3), and 1 M sodium phosphate, 7.2 M urea, pH 6.4 (dimension 4) (*14*). Adduct spots were detected by autoradiography with intensifying screens and quantitated by Cerenkov counting of excised areas of the autoradiograms. Four zones were cut out from the TLC plates, as shown in Figure 1. X-Spots were always calculated separately.

Results

Summary data of the DNA adduct levels (arithmetic mean values) in white blood cells collected in summer from coke battery workers in Gliwice, residents of the district of Gliwice

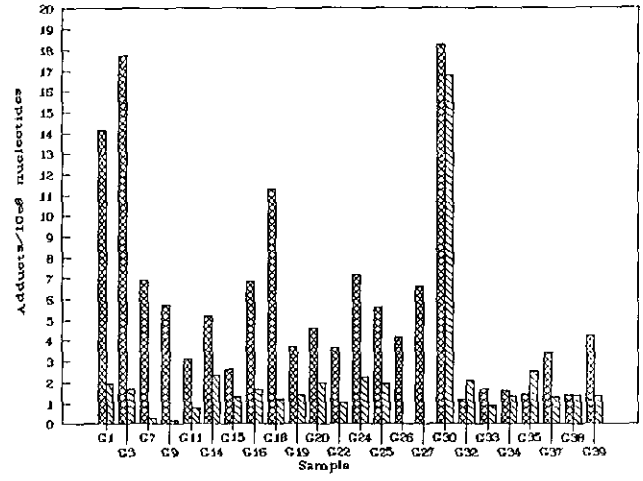

FIGURE 2. Seasonal variation in the level of DNA adducts from white blood cells of Gliwice residents. (▨) Blood sample collected in winter; (▧) blood sample collected in summer. Individuals G26 and G27 had no adducts in summer, and individuals G32 and G35 had more adducts in summer than in winter.

Table 2. Seasonal variations in DNA adduct levels in Gliwice and Biala Podlaska residents.

Population	Sampling time	Average temperature of air, °C	n	Adducts/10^8 nucleotides[a]	SD
Gliwice residents, winter	January	0.6			
	February	4.5			
	March	6.6	36	5.03	3.56
Gliwice residents, summer	September	11.2			
	October	9.0	25	1.42	2.21
Biala Podlaska residents, winter	March	5.8	21	3.09	2.40
Biala Podlaska residents, summer	October	8.9	27	2.13	2.22

[a]Data are presented as arithmetic means.

located in the vicinity of the coke oven, children from Bytom (town located 25 km from Gliwice), and residents from the rural, eastern part of Poland (Biala Podlaska), are shown in Table 1. Coke battery workers had the highest level of DNA adducts (6.9 per 10^8 nucleotides). The other groups had lower levels of adducts (1.4 per 10^8 nucleotides for residents from Gliwice, 1.3 for children, and 2.1 for individuals living in Biala Podlaska). These groups had 4.8-fold and 3.2-fold, respectively, lower levels of adducts than the group of people working in the coke battery.

DNA adducts in blood samples collected twice from the same persons living in the district of Gliwice near the coke oven showed a 3.5-fold difference between the winter (5.0 adducts per 10^8 nucleotides) and summer samples (1.4 adducts per 10^8 nucleotides) (Fig. 2). This effect was observed for all but two individuals ($r = 0.54$, $p = 0.0055$). In one case (individual coded G3), the level of DNA adducts in winter was 10 times higher than in summer.

Table 2 shows seasonal variation in the adduct levels (mean values) in people living in Gliwice and in Biala Podlaska. In both groups the winter samples had a higher level of adducts than the

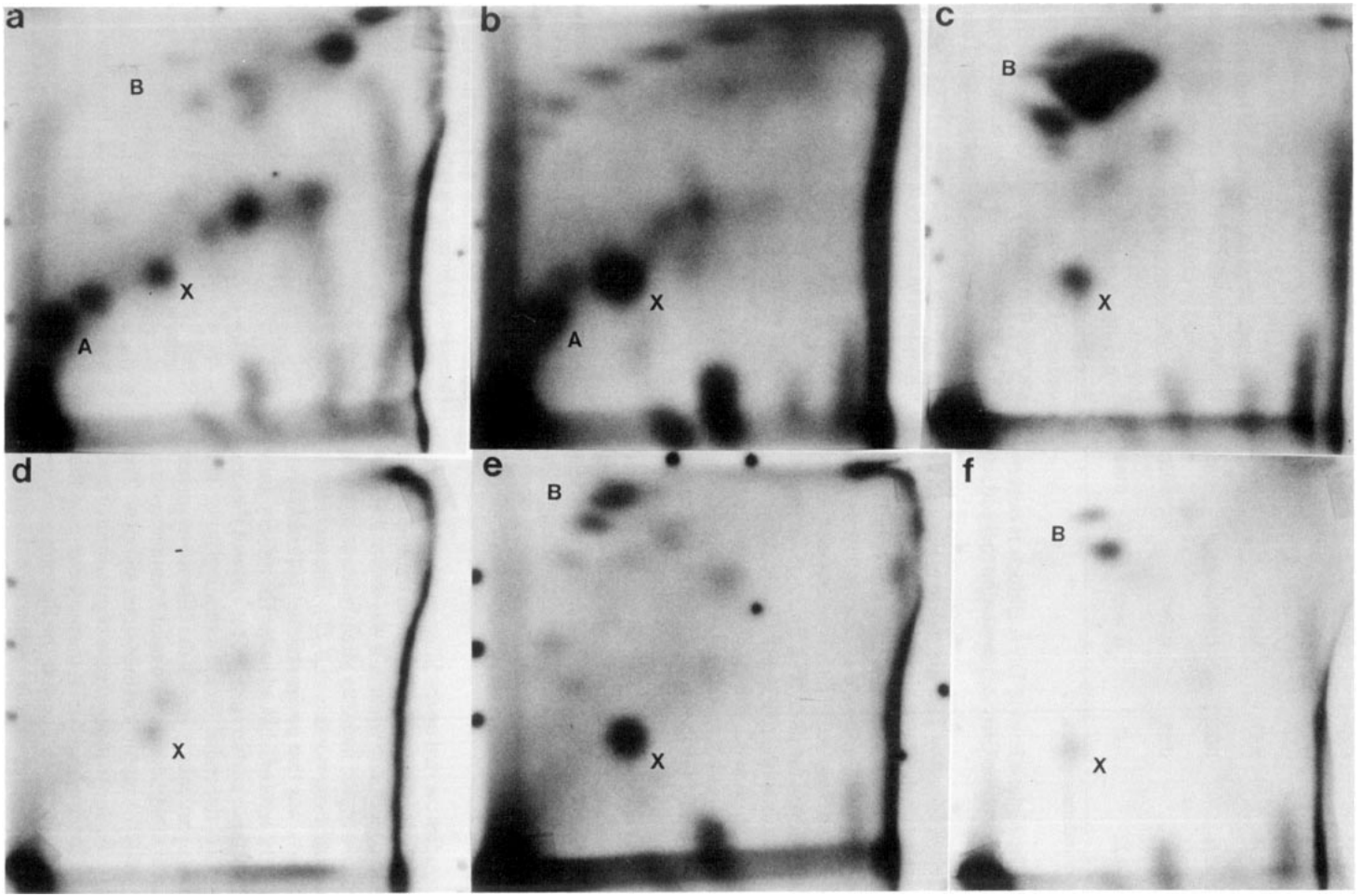

FIGURE 3. Autoradiograms of PEI-cellulose maps of P_i-nuclease enhanced ^{32}P-postlabeled DNA (4 μg) digests from white blood cells of (*a*) coke worker, summer sample; (*b*) Gliwice resident, winter sample; (*c*) Gliwice resident, summer sample; (*d*) child from Bytom, summer sample; (*e*) Biala Podlaska resident, winter sample; (*f*) Biala Podlaska resident, summer sample. The adducts were resolved by four-dimensional chromatography (see Materials and Methods). Autoradiography was carried out by exposing the Kodak XAR-5 film at -70 °C for 5 days.

Table 3. X-Spot levels in studied populations.

Population	X-spots/10^8 nucleotides[a]	SD	Correlation coefficient, r, between X-spots and other adducts	p
Gliwice, coke oven workers	0.53	0.43	0.0540	0.7509
Gliwice residents, winter	0.37	0.46	0.6094	0.0001
Gliwice residents, summer	0.16	0.15	0.7476	0.0001
Bytom, children summer	0.14	0.08	0.6874	0.028
Biala Podlaska residents, winter	0.27	0.28	0.7397	0.0001
Biala Podlaska residents, summer	0.22	0.12	0.4642	0.0147

[a]Data are presented as arithmetic means.

summer samples. The highest level of DNA adducts is observed in the winter samples of the Gliwice population (5.0 adducts per 10^8 nucleotides).

The adduct patterns resolved by TLC are shown in Figure 3 for each of the groups analyzed. The X-spot is seen in each chromatogram. Generally, the autoradiograms consisted of two other groups of spots: one diagonally arranged in the center of the plate and another one that often consisted of four distinct spots (labeled B) and migrated to the top of the plate (Fig. 3*a,c,e,f*). In sample G30, collected in winter from the resident of Gliwice, the strong spot (labeled A in Fig. 3*a,b*) in the bottom of the chromatogram is seen. This spot co-migrated in two chromatographic systems with the synthetic adduct BPDE-N^2 dGMP used as a standard, and one spot was observed in mixing experiments.

X-Spots, found in most samples (Fig. 3) were quantitated separately in each chromatogram. Table 3 contains the results of these calculations. Generally, X-spots had less radioactivity than the other DNA adducts, but the two covaried in a significant manner, except in the group of coke oven workers.

Discussion

In the previous studies carried on the Silesian population exposed environmentally to high levels of PAHs in the ambient air (blood samples were collected in winter), it was shown that people living in the Silesia region had only slightly lower levels of DNA adducts than coke oven workers (*3,11,15*). The most striking aspect of this study was that environmental pollution contributed markedly to the level of DNA adducts. The present study generally confirmed these earlier data, but it showed that the levels of DNA adducts depended on the sampling time. The seasonal variation was particularly high in the Gliwice residents. In summer the level of DNA adducts decreased 3.5 times as compared to the levels of DNA adducts in the winter samples. The difference is likely to be due to the intensive combustion of coal for domestic heating in winter, as coal is the most common fuel used for heating in Silesia. An earlier study on the mutagenic activity of ambient air samples of this region also found the seasonal variation (*16*). Fukino et al. (*17*) reported that the mutagenicity of the airborne particles was related to atmospheric BaP concentrations, known in Silesia to be higher in winter than in summer (*10*). The seasonal variation in DNA binding may also be related to the fluctuation of the aryl hydrocarbon hydroxylase activity (*18–20*).

Large interindividual variation in the levels of adducts were observed. These differences were about 100-fold between the coke workers and about 20-fold between the other groups of adult volunteers. The smallest variation was noted in the group of children from Bytom. Differences in exposure and host factors such as metabolic activation, deactivation, and DNA repair capacity of PAHs may be the sources of variation in the DNA adducts levels (*2,21,22*). Studies *in vitro* also reported an extensive interindividual variation in the binding of BaP to DNA in human tissue cultures (*23*). The small variation in DNA adduct levels observed in children may reflect more uniform activities of the detoxification and repair enzymes. Children lack the exposure periods, smoking, etc., that affect adduct levels in adults.

Another notable aspect of this study was the analysis of X-spot levels in respect to other DNA adducts. Calculation of mean values of X-spots in each group of persons analyzed (Table 3) showed that the levels of X-spots in each group are related to the level of other DNA adducts; moreover, they reflect seasonal variations observed in these groups. The correlation coefficients between the X-spots and the other DNA adducts were statistically significant in groups other than coke workers, who showed high levels of occupationally derived adducts. Phillips et al. (*14*) suggested that this spot was unlikely to be a molecule covalently bound to DNA, but it could serve as a reference point for the mobilities of adduct spots in different experiments. The X-spot is observed in DNA samples as a strong background spot appearing in chromatograms regardless of tissue or species origin (*24,25*). Another spot was frequently seen in coke worker DNA and winter samples from Gliwice. It co-migrated with the labeled BPDE-N^2dGMP, which may signal the presence of PAH adducts in that area of the TLC plates.

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