Benzo(a)pyrene-albumin adducts in humans exposed to polycyclic aromatic hydrocarbons in an industrial area of Poland

E H Kure, Å Andreassen, S Øvrebø, E Grzybowska, Z Fiala, M Strózyk, M Chorazy, A Haugen

Abstract

Objectives—The interaction of benzo(a)pyrene with serum albumin was measured in an attempt to identify the actual exposure and to evaluate albumin adduct measurements as biomarkers for exposure monitoring.

Methods—Benzo(a)pyrene-diol-epoxide (BPDE)-albumin adducts were measured by competitive enzyme linked immunosorbent assay (ELISA) in plasma of coke oven plant workers from three plants and from people living in a highly industrialised area of Silesia in Poland. Due to the high air concentrations of polycyclic aromatic hydrocarbons (PAHs) in this area, a control group was selected from a rural non-industrialised area in Poland. Breathing zone air measurements of PAHs were collected from some of the participants.

Results—Coke oven plant workers and non-occupationally exposed people had similar concentrations of albumin adducts whereas the rural controls were significantly lower (2.74 fmol adducts/μg albumin (SEM 0.124)). The mean concentration of BPDE-albumin adduct in plasma of both the occupational and the environmental groups were significantly higher in the summer samples (4.34 fmol adducts/μg albumin (SEM 0.335) and 4.55 fmol adducts/μg albumin (SEM 0.296), respectively) than in the winter samples (3.06 fmol adducts/μg albumin (SEM 0.187) and 3.04 fmol adducts/μg albumin (SEM 0.184), respectively) even though the air measurements showed higher concentrations of PAHs in the winter. The statistical analysis did not show any effects of air exposures on concentrations of BPDE-albumin adduct.

Conclusions—A multiple regression analysis of the measured concentrations of BPDE-albumin adducts for all the groups, during both seasons, indicates that occupational exposures do not contribute significantly to the formation of adducts. In general, the concentrations of albumin adducts found vary within relatively small limits for the two seasons and between the various groups of participants. No extreme differences were found.

Keywords: albumin adducts; polycyclic aromatic hydrocarbons; enzyme linked immunosorbent assay

Polycyclic aromatic hydrocarbons (PAHs) are among the most prevalent air pollutants in Silesia, Poland.^{1,2} The main source of exposure to PAHs is the use of black coal for industrial purposes and domestic heating causing air concentrations of PAHs to be almost 10 times higher than in western Europe.² Humans are exposed to PAHs through the respiratory and digestive tract and the skin. In most settings the exposure to PAHs from food and tobacco is estimated to be much higher than from air and water.³ Thus, human exposure to PAHs is difficult to assess from air measurements alone. As serum albumin is synthesised in hepatocytes where microsomal oxidation takes place it is likely that highly reactive electrophiles of PAHs may react with serum albumin. Studies in rodents exposed to PAHs have shown that DNA adducts are formed in several tissues.^{4,5} However, the composition and persistence of the DNA adducts may differ in the various tissues.⁶⁻⁹ As genotoxic carcinogens form covalent bonds with DNA as well as with proteins some studies have also compared the degree of covalent binding of metabolites of benzo(a)pyrene with DNA and proteins.¹⁰ Contrasting results have been reported.¹¹ The assessment of benzo(a)pyrene-diol-epoxide (BPDE)-albumin adducts is considered as a potential marker of exposure to PAHs. There are few studies on measurements of BPDE-albumin adducts in populations.¹²⁻¹⁶

The primary aim of the study was to identify the relevance of using BPDE-albumin adducts as a measure of air exposure to PAHs. The concentration of BPDE-albumin adduct, measured in plasma by enzyme linked immunosorbent assay (ELISA), of occupationally and environmentally exposed people living in Silesia were compared with concentrations of adduct measured in a control group selected from a rural non-industrialised area of Poland. Breathing zone air measurements of PAHs were collected from some of the exposed groups.¹⁷ The general notion is that air concentrations of PAHs influence the concentration of DNA adducts in exposed people and this may also apply to albumin adducts.^{10,18}

The secondary aim of the study was to investigate any seasonal effects on the concentration of BPDE-albumin adducts. Recent data on DNA adducts in lymphocytes of people

Department of
Toxicology, National
Institute of
Occupational Health,
PO Box 8149 Dep,
N-0033 Oslo, Norway
E H Kure
Å Andreassen
S Øvrebø
Z Fiala
A Haugen

Department of Tumor
Biology, Institute of
Oncology, 44-100
Gliwice, Poland
E Grzybowska
M Strózyk
M Chorazy

Correspondence to:
Dr Elin H Kure, Department
of Occupational and
Environmental Medicine,
Telemark Central Hospital,
N-3710 Skien, Norway.

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Table 1 Characterisation of the study groups

Location	Age (y)			Smokers (%)	Subjects (n)
	Mean	Range	SD		
Coke oven plants:					
Plant B	45.1	30-57	8.51	33.3	15
Plant D	46.7	30-59	8.51	78.6	14
Plant E	38.1	22-59	10.89	76.7	30
All plants	41.9	22-59	10.37	66.1	59
Environmentally exposed groups:					
Gliwice	38.8	24-57	10.96	84.6	13
Bytom	38.6	19-55	12.14	56.5	23
Swietochlowice	51.0	17-76	22.03	16.7	12
All groups	41.7	17-76	15.57	54.2	48
Rural controls:					
Biala Podlaska	34.8	22-57	9.37	62.2	45

Table 2 Linear regression models for the analysis of concentrations of BPDE-albumin adducts (dependent variable) in occupationally exposed, environmentally exposed, and rural control subjects

Model	Variables in model	Regression coefficient	(95% CI)	P values
1	Control	-1.7239	(-2.3093 to -1.1385)	< 0.001
	Smoking	0.7643	(0.1837 to 1.3450)	0.01
	Constant	3.9877	(3.4890 to 4.4864)	< 0.001
2	Season*	-1.4359	(-1.9132 to -0.9586)	< 0.001
	Age	-0.0192	(-0.0368 to 0.0015)	0.03
	Constant	5.2744	(4.4240 to 6.1247)	< 0.001
3	Season*	-1.3399	(-2.0385 to -0.6413)	< 0.001
	Age	-0.0298	(-0.0605 to 8.39×10 ⁻⁴)	0.06
	Constant	-5.6321	(4.1993 to 7.0649)	< 0.001
4	Season*	-1.0941	(-1.9997 to -0.18841)	< 0.02
	Constant	4.1682	(3.4904 to 4.8459)	< 0.001

* Season = winter.

living in this region^{19,20} have shown a seasonal effect which has been linked to higher air exposure of PAHs in the winter season. It is of interest to investigate whether this also applies to BPDE-albumin adducts.

This study contributes to determination of the relevance of the use of albumin adducts to assess the total exposure to PAHs in a human population and the contribution of exposures from air.

Materials and methods

STUDY SUBJECTS AND SAMPLE COLLECTION

The subjects were male workers from three coke oven plants in the Silesian area and environmentally exposed people living in close vicinity to the plants. The members of the rural control group lived in Biala Podlaska, an area of Poland without heavy industry. Each participant completed a questionnaire on lifestyle factors (including smoking), medical history, age, and workplace description. Blood was collected by venepuncture in heparinised sterile tubes. Plasma was separated from the whole blood and transported frozen. Samples of PAHs in the breathing zone air of the workers were collected during the same day as the blood sampling took place. Personal sampling of PAHs of non-occupationally exposed people was performed in one of the Silesian environmental groups (Gliwice). For practical reasons, the other two environmental groups and the rural controls were not measured by personal sampling. However, stationary air sampling was performed in Biala Podlaska during the summer season and blood samples were available for the rural controls only for this season. Except for these limitations, air and blood were sampled twice; winter (February 1992) and

summer (September 1992). Rural control blood samples were collected only in the summer of 1993. If exposures to airborne PAHs from general heating affected the concentrations of albumin adduct it would be expected that adduct concentrations in the summer would be lower than in the winter and therefore be a better control group if there was only the option of sampling once. Table 1 shows the characteristics of the study groups.

ANALYSIS OF BENZO(a)PYRENE-ALBUMIN ADDUCTS IN PLASMA

Globulins were precipitated from plasma with saturated ammonium sulphate. After removal by centrifugation, albumin was precipitated by acidification of the supernatant with acetic acid and redissolved in Tris-EDTA buffer (10 mM Tris/1.0 mM EDTA, pH 8.0).¹³ The concentration of albumin was measured by the Lowry assay²¹ before acid hydrolysis. The digest was applied to a prewet C18-Sep-Pak cartridge (Waters, Milford, ML). The cartridge was washed with 5% methanol (5 ml) to remove small peptides and amino acids. The PAH metabolites were eluted with 80% methanol (5 ml), evaporated to 1 ml in a vacuum, and assayed directly with competitive ELISA with monoclonal antibody 8E11 as described elsewhere.^{14,22,23} Briefly, microtitre plates were coated with BPDE modified calf thymus DNA in phosphate buffered saline (PBS) by drying. The plates were washed with PBS-Tween and then incubated with 2% fetal calf serum in PBS. A standard curve was made of serial dilutions of benzo(a)pyrene tetrols from 0.2–220 nM (50 µl each) and mixed with 50 µl antibody 8E11 (diluted 1:20 000), before addition to the wells. After incubation overnight at 4°C and washing of the plates, 100 µl goat anti-mouse IgG conjugated with alkaline phosphatase (diluted 1:750) was added and incubated for 1.5 hours. Finally, 100 µl *p*-nitrophenyl phosphate in 1 M diethanolamine (pH 8.6) was added. After incubation the absorbance at 405 nm was measured with a Titertek Multiscan Pluss microplate reader. Samples were analysed twice with triplicate wells. The values are the mean of all assays and expressed as fmol equivalents of BPDE-I adducts due to a significant cross reactivity with several PAH metabolites.¹⁴

MEASUREMENTS OF PAH EXPOSURE

For the assessment of exposure to PAHs 12 compounds were selected as outlined by the National Institute of Occupational Safety and Health (fluoranthene, pyrene, benz(a)anthracene, chrysene/triphenylene, benzo(e)pyrene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)-anthracene, benzo(ghi)perylene, and benzo(a)fluoranthene (two isomers)).²⁴

Sampling of particulate PAH was performed with Casella AFC 123 (Casella, London, England) and DuPont S2500 (DuPont, Largo, Florida) pumps at a flow rate of 2 l air per minute for six to eight hours as previously described.¹⁷ The standard 25 mm sampling cassette Nucleopore filter (Pleasanton, California) was made of polyethylene with carbon black

Table 3 Mean (SEM) concentrations of BPDE-albumin adducts for the three groups

Location	BPDE-albumin adducts (fmol adducts/μg albumin)					
	February 92			September 92		
	Mean	(SEM)	n	Mean	(SEM)	n
Combined groups plants	3.06	(0.187)	59	4.34	(0.335)	38
Combined environmental groups	3.04	(0.184)	48	4.55	(0.296)	36
Rural controls*	—	(—)	—	2.74	(0.124)	45

*September 1993.

Table 4 PAH and benzo(a)pyrene air measurements (μg/m³) from personal sampling*

Location	September 92				February 92			
	PAH		B(a)P		PAH		B(a)P	
	Mean	(SEM)	Mean	(SEM)	Mean	(SEM)	Mean	(SEM)
Combined plants	4.40	(1.75)	0.58	(0.24)	7.03	(2.10)	0.83	(0.25)
Environmental, Gliwice	0.13	(0.07)	0.01	(0.01)	0.28	(0.08)	0.03	(0.01)
Biala Podlaska†	—	—	0.02	—	—	—	—	—

* Concentrations of PAH and benzo(a)pyrene (B(a)P) have previously been published by Øvrebø et al.¹⁷

† Stationary sampling.

to minimise the effect of static electricity. The filters were extracted with cyclohexane after addition of internal standards as described elsewhere.¹⁷

STATISTICAL METHODS OF ANALYSIS

The statistical analysis of the data set was performed with multiple regression analysis (SPSS, Student version). Due to the limitations in data collection four different multiple regression models were applied to answer the aims of the study. Due to the low number of participants in each subgroup the study has not enough power to analyse effects within and between these subgroups of workers and environmentally exposed people. The statistical analysis was performed on the combined groups of workers and the combined groups of environmentally exposed people except in one case (model 4).

To analyse any differences between the concentrations of BPDE-albumin adduct in the three groups, only the data for the summer season had all the information needed. The two exposed groups (workers and environmental groups) were combined and compared with the rural control group. This subset of the data was adjusted for any confounding effects of smoking and age. Table 2 shows the final regression model 1.

The effect of season (and group) on the level of BPDE-albumin adduct could only be tested on the two exposed groups as the rural controls only were sampled during one season. The subset of the data was adjusted for any confounding effects of smoking and age. Table 2 model 2 shows the final regression model.

The effect of air exposures (PAHs and benzo(a)pyrene), and season on the concentrations of BPDE-albumin adduct were analysed for the occupationally exposed group as just one of the environmental subgroups had air measurements for both seasons. The data set was adjusted for any confounding effects of smoking and age. Table 2 model 3 shows the final regression model.

The last analysis focused on the effect of air measurements (PAHs and benzo(a)pyrene), and group on the concentration of BPDE-albumin adduct in the environmental subgroup of Gliwice and the occupational group working in plant D for the two seasons. The subset of the data was adjusted for any confounding effects of smoking and age. Table 2 model 4 shows the final model. A detailed statistical analysis on air exposures of these groups has previously been published by Øvrebø et al.¹⁷

Results

Table 3 shows the mean concentrations of BPDE-albumin adducts in plasma collected during summer and winter seasons of coke oven plant workers, environmentally exposed people (living in the vicinity of the plants), and rural controls (from Biala Podlaska).

The mean concentration of BPDE-albumin adducts of the rural controls was 1.72 fmol adducts/μg albumin lower than for the other two groups for the summer season having controlled for smoking status ($P < 0.001$, table 2 model 1). The environmental groups had concentrations of BPDE-albumin adducts similar to those of coke oven plant workers in both the summer and the winter season. However, season had a significant effect on the mean concentration of BPDE-albumin adducts (1.44 fmol adducts/μg albumin higher in the summer samples than in the winter samples after controlling for age ($P < 0.001$, table 2, model 2)).

Air measurements of PAHs and benzo(a)pyrene presented in this study have previously been published by Øvrebø et al (table 4).¹⁷ The data have been used in this study for comparing the air concentrations of PAHs and benzo(a)pyrene with the concentrations of BPDE-albumin adducts of the occupationally exposed groups for the two seasons. The statistical analysis did not show any effects of air exposures on the concentrations of BPDE-albumin adducts. Although the air concentrations of PAHs and benzo(a)pyrene were much higher in the winter season; the concentrations of adducts showed the opposite effect with lower concentrations in the winter. The only significant effect found in the analysis was for season. The concentrations of BPDE-albumin adduct for the occupationally exposed groups were on average 1.34 fmol adducts/μg albumin higher in the summer samples than in the winter samples after controlling for age ($P < 0.001$, table 2 model 3). A seasonal effect only was found when analysing effects of air measurements of PAHs and benzo(a)pyrene on the concentrations of BPDE-albumin adducts in the environmental subgroup of Gliwice and the occupational group working on the plant in this area for the two seasons. The mean concentration of BPDE-albumin adducts was 1.09 fmol adducts/μg albumin higher in the summer than in the winter samples ($P < 0.02$, table 2 model 4).

The background concentrations of albumin adducts in the rural controls are relatively high and it is suspected that they cannot be explained by the air measurements alone as

stationary monitoring of environmental air in Biala Podlaska, September 1993 was only 20.4 ng/m³ benzo(a)pyrene.¹⁷

In general, the concentrations of BPDE-albumin adducts found vary within relatively small limits for the two seasons and between the various groups of participants. No extreme differences were found.

Discussion

In this study environmental controls and coke oven plant workers had similar concentrations of BPDE-albumin adducts whereas the rural controls had significantly lower concentrations. Although the rural control samples were collected a year later than the samples from the exposed groups this would not be expected to be of any importance to the study as the concentrations only reflect relatively short term exposures related to the half life of albumin. Our results from the linear regression analysis show that the concentrations of albumin adduct are not influenced significantly by air concentrations of PAHs. The measured air concentrations of PAHs were higher in the winter than in the summer with a wide range in exposures between the different people participating in the study. The measurements were based on one sampling of each person on one particular day (mean six to eight hours). The measurements should for this reason only be viewed as point estimates and the group means do not reflect the true means of these populations. The results from the regression analysis could be influenced by this. However, this does not explain the fact that the mean concentrations of BPDE-albumin adducts in both the workers and the environmental groups were significantly higher in the summer samples than in the winter samples. Other sources could contribute to the higher concentrations in the summer season.

This also contrasts with what was found on groups from this region analysed by postlabelling and ELISA on PAH-DNA adducts in lymphocytes.^{19,20} However, no seasonal variation was found in DNA adducts of granulocytes measured by the ³²P postlabelling assay¹⁹ and ELISA (unpublished results) in coke oven plant workers and environmental groups. This is probably due to the short lifespan of granulocytes.²⁵

Hemminki *et al* also found that the patterns of hydrophobic adducts in DNA of lymphocytes were similar in coke oven plant workers and environmental groups but not in rural controls.²⁶ They concluded that people living in the vicinity of the plants were highly exposed to PAHs and that the concentrations of aromatic adducts in white blood cell DNA did not linearly relate to ambient air concentrations of PAH. They suggested that other sources might contribute to the high background concentrations.

The concentrations of albumin adducts reflect integrated exposure over a period of time related to the half life of albumin (20–25 days). The lymphocytes are thought to have an active DNA repair system.¹⁹ The kinetics of albumin adducts are linear whereas lym-

phocytes may have dose dependent non-linear kinetics as well as cell turnover. The decrease in concentrations of DNA adduct of lymphocytes indicate that they have an active DNA repair with a half life of one to two months which is only slightly longer than for albumin.¹⁹ Therefore, other sources of exposure may contribute to the concentration of albumin adducts in these people especially during summer. The PAHs in the environment may contaminate locally grown vegetables and fruits.^{27,28} The inhabitants of Silesia tend to supplement their diet with locally grown produce during the summer season and this may contribute to the higher concentrations of albumin adducts found in the summer. In the general population, the oral intake of PAH may be higher than the inhaled.^{28,29} The effects of PAH ingested in the diet on concentrations of DNA adducts in lymphocytes are not well known.²⁶ Some studies have shown an increased concentration of DNA adducts in humans after ingestion of charcoal broiled meat but the data are limited.²⁹ As albumin is synthesised in the hepatocytes where microsomal oxidation takes place highly reactive electrophiles may react with albumin. Perhaps PAH through diet may contribute more than airborne PAH to the concentration of protein adducts than to that of lymphocytic DNA adducts.^{10,18}

In summary, albumin adducts as a biomarker of exposure may be a sensitive end point measure for PAHs when various routes of absorption contribute to the total body burden.

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