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Biological monitoring of foundry workers exposed to polycyclic aromatic hydrocarbons

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Abstract

This investigation describes benzo(a)pyrene (BP) serum protein adduct concentrations in 45 foundry workers and 45 matched non-occupationally exposed controls. High and low BP exposure groups were defined using breathing zone hygienic samples for both quartz and BP exposures. A newly developed enzyme linked immunosorbent assay detected benzo(a)pyrenediolepoxide-I binding to serum protein. Mean BP protein adduct concentrations (SD) for non-smoking (24·0 BP equivalents/100 μg protein (21·0)) and smoking (28·0 (18·2)) foundry workers were significantly higher than mean values for non-smoking (7·23 (8·72)) and smoking (14·2 (24·4)) controls. Foundry workers with high exposures to either quartz (28·4 (15·5)) or BP (30·7 (19·3)) had slightly raised mean adduct concentrations compared with foundry workers with low exposure for quartz (23·9 (23·1)) or BP (24·5 (19·4). Highest mean adduct concentrations were found among a small group of workers with simultaneous high exposures to both quartz and BP (39·2 (6·5)) suggesting an additive effect. These data support the ideas of a possible aetiological connection between an increased risk of lung cancer and BP exposure among foundry workers, and an additive effect between BP and quartz. Measurement of BP serum protein adduct concentrations appears to be a useful method by which groups exposed to BP may be biologically monitored.

Several studies have shown that foundry workers have an increased risk of lung cancer.[1–5] The reason for this is unknown. Air samples collected in foundries can contain mutagenic material[6] and several carcinogenic agents have been identified in contaminated foundry air—namely, polycyclic aromatic hydrocarbons (PAHs), nickel, chromium, aromatic amines, benzene, bis-chloromethyl ether, and quartz.[4 7] PAHs are produced from the incomplete combustion of organic material and have been considered as a possible cause of lung cancer among foundry workers. Benzo(a)pyrene (BP) is often measured as an indicator of PAH exposure and may be responsible for increased risk of lung cancer among smokers,[8] foundry workers,[9 10] and coke oven workers.[11]

It is now believed that the direct covalent binding of a carcinogenic agent to DNA to produce carcinogen DNA adducts is an essential step in the development of cancer,[12] and PAH DNA adducts have been detected in lung tissue from patients with lung cancer.[13] Target organ tissue, however, is not generally available for epidemiological studies, and carcinogen macromolecule—for example, DNA or haemoglobin—adduct concentrations in blood samples have been used as an estimation of biologically active dose[12]; BP DNA adducts in white blood cells have been identified in coke oven workers, roofers, and foundry workers.[10 14 15] The purpose of the present study was to measure BP protein adduct concentrations in the serum of foundry workers who were occupationally exposed to PAH. A possible influence of simultaneous exposure to quartz dust was also examined. The concentration of BP protein adducts in foundry workers was compared with that in a non-occupationally exposed control group.

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Subjects and methods

STUDY POPULATION AND EXPOSURE GROUPS

The study population consisted of 45 workers from an iron foundry located in a rural Danish town (population 3000) who were occupationally exposed to PAH. Forty five workers without occupational exposure to PAH, from a cotton plant located in a small Danish city (population 50 000), comprised the control group. These groups were matched for age, sex, and smoking habits (table 1).

The study population was subdivided into high and low exposure groups for PAH and quartz exposure based on breathing zone hygienic

measurements. Exposure groups were classified according to present job. Nine six hourly air samples were collected in August 1988 and May 1989 and were analysed for 16 types of PAH using high performance liquid chromatography. The high PAH exposure group (casting, hand moulding, shakeout, and oven workers) was exposed to a mean total PAH concentration of 6·41 $\mu g/m^3$ with a mean BP concentration of 0·04 $\mu g/m^3$. The low PAH exposure group (core making, machine moulding, and administrators) had a mean total PAH exposure of 0·46 $\mu g/m^3$ and undetectable BP. The high quartz exposed group (hand moulding, casting, shake out, and cleaning workers) had a minimum mean exposure of 1·50 mg/m^2 respirable dust. The low quartz exposed group (core making, machine moulding, and oven workers; administrators) had mean exposures of less than 0·61 mg/m^3. The quartz exposure groups were based on 93 measurements performed between 1981 and 1988.

Histories of smoking were obtained from self completed questionnaires and were checked during personal interviews. Pipe tobacco, cigars (5 g tobacco for each cigar), and cheroots (3 g tobacco for each cheroot) were translated to cigarettes (1 g tobacco for each cigarette) when calculating pack-years. Questions concerning alcohol consumption, use of coal tar salve, and respiratory symptoms were included in the questionnaire. Participants signed a consent form and the research protocol was approved by the local ethics committee.

BLOOD COLLECTION

Blood samples (10 ml) were collected from exposed subjects at least five weeks after their summer vacation on two Friday mornings in September 1988 at the foundry. The blood was allowed to coagulate overnight at 4°C. The coagulum was removed and the serum was centrifuged at 3000 × rpm to remove remaining cells.

CHEMICALS

Benzo(a)pyrenediolepoxide (BPDE)-I was obtained from Chemsyn Science Laboratories, Lenexa, Kansas, and a racemic mixture of BP tetrols was formed by aqueous hydrolysis at 37°C overnight. Dextran-78500 and p-nitrophenyl phosphate were obtained from Sigma, St Louis, Missouri. Normal swine serum and affinity purified rabbit antimouse and goat antirabbit immunoglobulins conjugated with alkaline phosphatase were obtained from Dakopatts, Copenhagen. Analytical grade tetrahydrofuran (THF) was obtained from Ferak, West Berlin. Antibody 8E11 recognising BPDE modified protein was generously donated by Dr Santella, Columbia University, New York.

Table 1 Comparison of mean values (SD) for control and exposed groups

	Control group (cotton factory workers) (n = 45)	*Exposed group (foundry workers) (n = 45)*
	Smokers	
Sex	Seven women, 19 men	Six women, 20 men
Age (y)	43·2 (11·6)	43·5 (11·8)
Age range (y)	20–58	20–58
Pack-years	24·6 (13·3)	27·2 (19·2)
	Non-smokers	
Sex	Four women, 15 men	Four women, 15 men
Age (y)	36·3 (13·5)	36·5 (13·6)
Age range (y)	19–64	19–63

PREPARATION OF ANTIGEN FOR ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Dextran-78500 was resuspended in THF (500 mg/5 ml), BPDE (5 mg) dissolved in THF (1 mg/ml) was added, and the mixture incubated overnight at room temperature while shaking. The resulting precipitate was isolated by centrifugation, washed twice with THF (5 ml), and dried in a vacuum. The precipate was redissolved in H_2O (10 ml).

PREPARATION OF COMPETITIVE ANTIGEN PROTEIN ADDUCT

Human serum (2 ml) was mixed with saturated ammonium sulphate (2 ml) and kept overnight at 4°C with shaking. The precipitate was isolated by centrifugation at 4000 × rpm for two × 20 minutes. The pellet was washed once with ethyl acetate/acetone (2 ml; 1:1) to remove PAH not covalently bound to the serum proteins. The air dried proteins were redissolved in 10 mM Tris/1·0 mM EDTA buffer (1 ml; pH 8·0), and the protein concentration determined by the Lowry method using bovine serum albumin as the standard. The solution was diluted with buffer to give 10 mg protein/500 μl. HCl (IN; 50 μl) was added and the mixture incubated for three hours at 96°C.[16] The hydrolysate was neutralised with NaOH and diluted with water and methanol to a final concentration of 10%. This mixture was applied to a prewet C18-Sep Pak cartridge (Waters, Milford, Maryland), and washed with 5% methanol (5 ml). The PAH metabolites were eluted with 80% methanol (5 ml) and the eluate was evaporated to 500 μl in a vacuum.

COMPETITIVE ELISA

Polystyrene 96 microwell plates (NUNC immuno plates, Nunc, Roskilde, Denmark) were coated with BPDE modified dextran (150 μg in 100 μl 50 mM carbonate buffer, pH 9·6) and incubated overnight at 4°C. Control wells were coated with unmodified dextran. The plates were washed five times with PBS, and were then incubated with 1% swine serum (100 μl/well) diluted with phosphate buffered saline

(PBS) for one hour at room temperature followed by washing five times with PBS. The competitive antigen was diluted 1:10 and 1:100 in PBS, and 100 μl/well (five wells for each dilution) were added, followed by the primary antibody (8E11, 100 μl/well) diluted 1:250 000 in PBS. A series of different concentrations of hydrolysed BPDE (0·1 fM to 1·0 pM, four wells for each concentration) was included on each plate. The plates were incubated overnight at 4°C, washed five times with PBS/0·05% Tween 20, and incubated with alkaline phosphatase conjugated rabbit antimouse immunoglobulin diluted 1:5000 in PBS/1% normal swine serum for 90 minutes at room temperature. After five washes with PBS/0·5% Tween 20, alkaline phosphatase substrate (100 μl, 1 mg/ml) dissolved in 1·0M diethanolamine (pH 9·0) was added and the plates were incubated for 45–60 minutes. The absorbance was read at 405 nm (EIA reader; Bio-tek instruments). The level of modification was estimated from the standard curve with the 1:1000 dilution of the competitive antigen, and was expressed as fM BP equivalents/100 μg serum protein. Adduct concentrations were quantifiable in all exposed individuals. In five of the controls adduct concentrations were less than 0·1 BP equivalents/100 μg protein. These five concentrations were equated with 0·1 BP equivalents for statistical purposes. Further details of the competitive assay have been previously described.[17]

STATISTICAL ANALYSIS

The data are presented using box plots.[18] Statistical methods comprised Student's *t* test, non-parametric testing of paired data,[19 20] and multiple regression analysis.[21] All tests were two tailed unless stated otherwise.

Results

Figure 1 gives a summary of all BP protein adduct measurements. Mean adduct concentrations (SD) for both non-smoking (24·0 fM BP equivalents/100 μg protein (21·0)) and smoking (28·0 (18·2)) foundry workers were significantly higher than mean values for non-smoking (7·23 (8·72)) and smoking (14·2 (24·4)) controls (smoking controls *v* smoking foundry workers, <0·05; non-smoking controls *v* non-smoking foundry workers, $p < 0·005$; Student's *t* test). The difference between non-smoking and smoking controls was not significant ($p > 0·20$). Analysis using non-parametric testing of paired data showed statistically significant differences between control and exposed subjects (mean of paired differences, $p < 0·005$; Wilcoxon signed rank test, $p < 0·01$)).

Figure 2 shows BP protein adduct data for all foundry workers divided into low and high PAH exposure groups. No significant differences among groups could be demonstrated. Table 2 gives BP

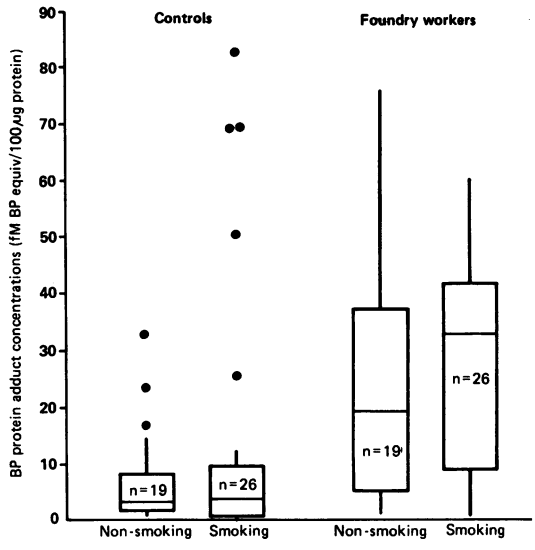

Figure 1 Box plots of BP serum protein adduct concentrations in non-smoking and smoking foundry workers and controls.

protein adduct concentrations for controls and foundry workers classified according to PAH and quartz exposures. All mean values for exposure groups were significantly higher than mean values for the controls. Mean adduct concentrations from high exposure groups were only slightly higher compared with low exposure groups and these did not differ significantly.

A possible additive effect of PAH and quartz exposure was examined (fig 3). Foundry workers with high exposure to both PAH and quartz had significantly raised mean adduct concentrations (39·2, 95% confidence level (CI) 32·4–46·0) compared with foundry workers with mixed exposures (24·3, 95% CI 17·8–30·8; $p = 0·002$) and with controls (11·2, 95% CI 5·4–17·1; $p < 0·001$). Foundry workers with mixed exposures also had higher mean adduct concentrations than controls ($p = 0·003$). Multiple regression analysis was also performed using adducts and log adducts as the dependent variables. Only PAH and quartz exposure were significantly ($p < 0·05$) correlated with adduct concentrations. Sex, age, duration of employment, and tobacco consumption (daily or pack-years) did not correlate with adduct concentrations. No correlation between BP DNA and BP protein adduct concentrations could be demonstrated in a subgroup; this has been reported in detail elsewhere.[17]

Discussion

The hygienic environment from the present foundry has been fairly well characterised by measurement of

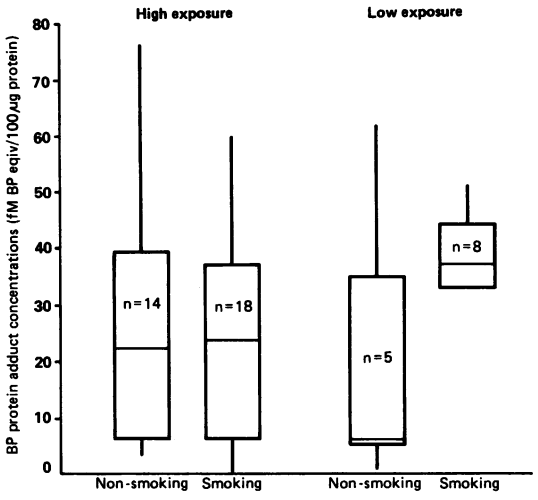

Figure 2 Box plots of BP serum protein adduct concentrations in non-smoking and smoking foundry workers classified according to low and high PAH exposure.

breathing zone air samples. Additional sampling, however, would have allowed us to define more clearly PAH exposure. BP concentrations in air samples from our high exposure group (0·04 $\mu g/m^3$) were considerably lower than corresponding concentrations in a recent Finnish study that quoted concentrations of greater than 0·2 $\mu g/m^3$.[10] The Finnish values were based on air sampling carried out in 1979–80. Our systematic measurement of samples for quartz shows significant improvements since 1981. It is thus possible that PAH contamination has decreased in a parallel fashion in this period. The high BP exposure at the foundry is about four times greater than the highest level of BP detected in air in Copenhagen (0·009 $\mu g/m^3$),[22] and 66 times higher than that found in rural air in Denmark (0·0006 $\mu g/m^3$).[23] Coke oven workers can be exposed to higher BP levels (7·3 $\mu g/m^3$).[24]

Table 2 Mean benzo(a)pyrene serum protein adduct concentrations (SD) for controls and foundry workers classified according to PAH and quartz exposures

	Control group	*Low exposure group*	*High exposure group*
	PAH		
No of subjects	45	32	13
BP protein adduct (fM BP equiv/100 μg protein (SD))	11·2 (19·6)	24·5 (19·4)**	30·7 (19·3)**
	Quartz		
No of subjects	45	21	24
BP protein adduct (fM BP equiv/100 μg protein (SD))	11·2 (19·6)	23·9 (23·1)*	28·4 (15·5)**

*p < 0·05; **p < *0·005 (exposed *v* control; two tailed *t* test).

The antibody used to detect BP binding to serum protein is specific for BPDEs that are released by acid hydrolysis of blood proteins. The possibility of crossreaction with BP triols and other PAH tetrols cannot be excluded, however.[25] Therefore, the results are expressed as BP equivalents corresponding to the same amount of BPDE required to give the same level of inhibition; it is not known how other PAHs which we measured in foundry air (naphthalene, fluorene, phenanthrene, anthracene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, and benzo(ghi)perylene) might have affected our results.

Our control population appeared to be well matched to the exposed group. One man was incorrectly matched with a woman. They were excluded from calculations concerning paired data. Mean pack-years for foundry workers was slightly higher than the mean value for the control group. As the present study and similar studies[10 26] concerning BP DNA adducts could not show any correlation between tobacco consumption and adduct concentrations, this minor difference in pack-years is probably unimportant. PAH hygienic samples were not obtained from the control cotton factory. There was, however, no reason to suspect that these workers should be occupationally exposed to PAH. The

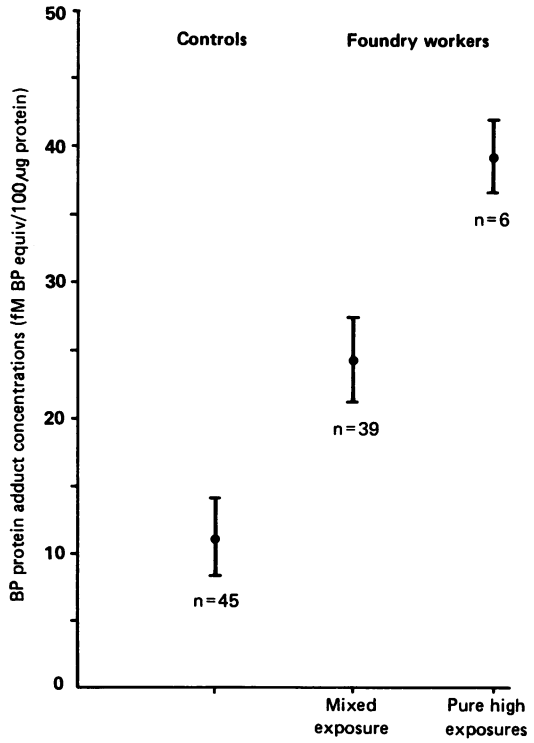

Figure 3 Mean BP serum protein adducts (SEM) in controls and foundry workers.

control plant is located in the middle of a small Danish city which probably has more air pollution than the small town where the iron foundry is located. Thus it is improbable that higher levels of BP contaminated air outside the foundry biased this study.

The suggested difference between exposed groups and controls (fig 1) was confirmed by non-parametric testing. Although occupationally related PAH exposure may well be responsible for the statistically significant difference in mean BP protein adduct concentrations, several potentially confounding factors must be taken into consideration. Multiple regression analysis showed no effect of either age or sex, although higher DNA adducts in women compared with men[27] as well as an inverse correlation between age and lung DNA adducts[26] have been described. Duration of employment for our cases and controls was unrelated to adduct concentrations.

Coal tar salve is another potential confounder. Psoriatic patients under treatment with coal tar have increased urinary excretion of PAH metabolites.[28] None of our controls and only three of the exposed group were being treated with coal tar when blood specimens were taken. This treatment apparently did not affect our results as the mean adduct concentration for the three cases (9·8 fM BP equivalents/100 μg protein) was less than the mean value for all controls (11·2).

With 20–40 ng (mainstream) and 68–136 ng (sidestream) BP per cigarette,[29] tobacco is probably the most significant confounder. BP DNA adducts were not detected in mice exposed to cigarette smoke or products from cigarette smoke despite the induction of aryl hydrocarbon hydroxylase activity.[16] We found no correlation between adduct concentration and tobacco consumption. Passive smoking was investigated among exposed individuals and had no apparent effect. A study of Finnish foundry workers reached the same conclusion concerning DNA adducts and smoking.[10] There are, however, other studies which suggest a relation between smoking and haemoglobin adducts[27] or DNA adducts.[15 24 26 27 30 31] Thus the relation between adduct formation and smoking is not well defined, but it seems possible that occupational exposure to BP can overshadow the effects of tobacco.

The half life of adducts has not been sufficiently studied. Four foundry workers had an eight fold fall in DNA adduct concentrations after four weeks vacation.[10] Our blood samples from foundry workers were obtained at least five weeks after the completion of summer vacation to avoid such a possible effect.

Several potentially important confounding variables were not controlled for. BP can be found in both drinking water and food.[9 32] Our study did not control for diet. One study could not show any effect of diet on DNA adduct concentrations[26] and a possible protective effect of charcoal broiled food has also been described,[31] but essentially, dietary effects on adduct formation have not been investigated in detail. Alcohol intake was defined and had no apparent effect. The presence of liver disorders that might affect protein state was not investigated. The metabolism of PAHs is complex with inducibility of aryl hydrocarbon hydroxylase activity probably playing an important part,[33] and persons have shown a more than 100 fold variation in the metabolic conversion of BP to the molecular species that binds to serum proteins.[34] An assessment of metabolic variability was not examined in the present study.

A possible effect of seasonal variation has been sporadically investigated.[26] Contamination of foundries with BP can decrease 10-fold during the summer months compared with winter[35]; our hygienic samples and adduct concentrations may, therefore, only represent late summer or early fall.

In addition to well defined differences in adduct concentrations between exposed workers and controls, there are other aspects of our data which suggest an occupationally related effect. Although not statistically significant, there was a tendency for workers with higher exposures to either PAH or quartz to have higher adduct concentrations (table 2). There also appeared to be an additive effect when both PAH and quartz exposures were taken into consideration; workers with high exposures to both substances had significantly higher adduct concentrations than workers with mixed exposures (fig 3). Intracellular absorption of BP is facilitated in rats when BP is absorbed onto quartz particles.[36] In an in vitro system ingestion of fibres coated with BP resulted in an enhancement of BP uptake and metabolism in hamster tracheal explants.[37] Thus BP may be adsorbed to quartz particles in foundry environments and this could explain the observed additive effect.

The new assay for protein adducts may have several advantages over assays for DNA adducts. We were able to identify BP protein adducts in all subjects. The ability to detect DNA adducts varies but is often under 50%[10 15 24 26 27] and the protein adduct assay therefore appears to be more sensitive. Another advantage is that only small amounts (5 ml) of blood are required. Which method better reflects the carcinogenic mechanism in target organs is unknown.

In conclusion our data have shown a raised mean BP protein adduct in serum concentration from foundry workers compared with a non-occupationally exposed control group; the hypothesis that there is no difference in serum BP protein adduct concentrations between foundry workers and controls was rejected. The data suggest a dose response relation between both PAH and quartz exposure and adduct concentrations. An additive effect between PAH and

quartz exposure was also shown. The present investigation and another recent study[10] support the theory that PAH exposure in foundry workers is aetiologically related to an increased risk of lung cancer.

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