CHROMOSOMAL ABERRATIONS BY FLUORESCENCE IN SITU HYBRIDIZATION (FISH) – BIOMARKER OF EXPOSURE TO CARCINOGENIC PAHS

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ABSTRACT

The fluorescence in situ hybridisation (FISH) technique with whole chromosome painting for chromosomes #1 and #4 was used to study the impact of air pollution containing higher concentrations of carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) in three European cities, Prague (Czech Republic), Kosice (Slovakia) and Sofia (Bulgaria). In each site an exposed group was followed consisting of police officers or bus drivers who work usually through busy streets for at least 8 h, and a control group, who spent more than 90% of their daily time indoors. In Prague, a significant increase was observed in all the studied endpoints in the police officers compared to the control population (P<0.05). This difference is most apparent between exposed and control nonsmokers (F_G/100=1.56±1.34 vs. 1.14±1.02, P<0.05). In Kosice, the exposed group differed from controls in the endpoint percentage of aberrant cells (% AB.C) (0.29±0.19 vs. 0.21±0.20, P<0.05) and t/1000 (3.91±3.14 vs. 2.84±3.10, P<0.05). In Sofia two exposed groups were followed: police officers and bus drivers. FISH endpoints were significantly higher in police officers compared to controls (F_G/100=1.60±0.99 vs. 0.82±0.79, P<0.01). In bus drivers compared to controls there was an increase in % AB.C. (0.25±0.18 vs.0.13±0.13, P<0.01) and t/1000 (3.24±2.28 vs. 2.13±2.05, P<0.05). This is the first study using the FISH method to analyse the impact of environmental air pollution. According to the original hypothesis it is expected that the most important group of chemicals responsible for the biological activity of air pollution represent c-PAHs.

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INTRODUCTION

Prospective cohort studies have shown that prolonged exposure to particulate air pollution may be associated with an increased rate of morbidity and mortality from respiratory and cardiovascular diseases in the general population (Dockery et al., 1993; Pope et al., 1995; 2002). High exposure is associated with an increased risk of cancer (Katsouyanni and Pershagen, 1997), and the presence of a wide variety of genotoxic compounds in environmental air pollution has been demonstrated. Binkova et al. (1999) observed that extracts of particulate matter were able to preferentially produce PAH-DNA adducts in calf thymus DNA. Distinct aromatic DNA adducts derived from carcinogenic polycyclic aromatic hydrocarbons (c-PAHs, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene (B[a]P), benzo[g,h,i]perylene, chrysene, dibenzo[a,h]anthracene and indeno[1,2,3-cd]pyrene) accounted for approximately 50% of the total radioactivity detected. Dejmek et al. (2000) demonstrated that the effect of exposure to particulate matter on intrauterine growth retardation (IUGR) may be at least partly explained by the presence of c-PAHs. It was also shown that IUGR is positively related to PAH-DNA adducts in placentas (Sram et al., 1999). Perrera et al. (1999) showed in studies in Poland that ambient air pollution was significantly associated with PAH-DNA adducts in both maternal and cord white blood cells. Newborns with elevated PAH-DNA adducts had significantly decreased birth weight compared to newborns with lower DNA adducts. All these results indicate that in some regions c-PAHs may be a major source of the genotoxic activity of organic mixtures associated with air pollution.

The genotoxicity of air pollution has repeatedly been observed using biomarkers of exposure. DNA adducts measured by a ^{32}P-postlabeling method have become the most popular of these biomarkers of exposure. For biomarkers of effect, the conventional cytogenetic analysis of peripheral blood lymphocytes has been accepted as a technique suitable for the monitoring of genetic damage in somatic cells since the early 1970s. Today, chromosomal aberrations in human peripheral lymphocytes are recognized as internationally standardized and validated biomarkers of effect (Carrano and Natarajan, 1988; Albertini et al., 2000). This method was widely used to evaluate the impact of occupational exposure to carcinogens (Sram et al., 2004a) and now it is generally accepted that a high frequency of chromosomal aberrations in peripheral lymphocytes is predictive of an increased risk of cancer (Hagmar et al., 1998; Bonassi et al., 2000; Smerhovsky et al., 2001).

The fluorescence in situ hybridisation (FISH) technique became available for public health purposes substantially later, in the middle of 1990s. In comparison with conventional cytogenetic analyses, which detect particularly unstable types of aberrations, FISH using whole chromosome painting was developed as a rapid and sensitive method of detecting structural rearrangements, especially reciprocal translocations (Swiger and Tucker, 1996; Pressl and Stephan, 1998). The FISH technique detects translocations, which are long lasting injuries likely transferred through many cell cycles. This means, that the FISH technique measures the type of chromosomal changes related to cancer and that these changes may circulate in peripheral blood lymphocytes (PBL) for a long period of time.
The FISH painting methods have been often applied in the field of ionising radiation research (Natarajan et al., 1996; Lindholm et al., 1998; Matsumoto et al., 1998). As far as the exposures to chemical carcinogens, the available data are scant. Only few reports were published on the effect of occupational exposure to carcinogens (Sram et al., 2004b).

We used the FISH technique with whole chromosome painting for chromosomes #1 and #4 to study the impact of air pollution containing higher concentrations of c-PAHs in three European cities, Prague (Czech Republic), Kosice (Slovakia) and Sofia (Bulgaria). In each site an exposed group was followed consisting of policemen or bus drivers, who work usually through busy streets for at least 8 h, and controls, who spent more than 90% of their daily time indoors.

MATERIALS AND METHODS

Study subjects

The study was undertaken in three European cities, Prague (Czech Republic), Kosice (Slovakia) and Sofia (Bulgaria). The exposed group in each site were male police officers (and in Sofia also bus drivers), who spent > 8 h outdoors. The control group was matched by age, gender and length of employment, spending > 90% of daily time indoors. The populations were followed in winter, as the highest exposure to PAHs occurs during this season at the selected sites. The personal monitoring was supplemented with data from HiVol samples from stationary air pollution monitors in those cities.

Air sample collection, extraction of EOM and chemical analysis

Personal monitoring was provided using a personal sampler from the U.S. Environmental Protection Agency (U.S. EPA, 1999; Watts et al., 1994). Respirable particles smaller than 2.5 μm were collected on Teflon-impregnated glass fiber filters. The sampler was connected to a pump and operated continuously during a shift (8-12 h). Particles from the filter were extracted (EOM, extractable organic matter) for quantitative PAH analysis.

PM_{10} air particles (particles <10 μm) were collected for three months at each city in the period when the population studies took place (during the months of biological sample collections and two months before). The samples were collected daily for a 24 h period with the exception of determinations of high particle concentrations in winter, when the sampling period was shortened to 12 h. HiVol air samplers (Anderson) equipped with 20 x 20 cm Pallflex filters (T60A20) were used. The filters with samples collected at the same location were pooled together (Binkova et al., 2003).

Quantitative chemical analysis of PAHs from all three cities was performed by HPLC with fluorimetric detection in the laboratories of the certified company Ecochem, a.s., Prague (EN ISO CSN IEC 17025). Ecochem also carried out the extraction of the filters. All procedures were performed according to US EPA methods (U.S. EPA, 1999). The carcinogenic PAHs (c-PAHs) analysed were benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene (B[a]P), benzo[g,h,i]perylene, chrysene, dibenzo[a,h]anthracene and indeno[1,2,3-cd]pyrene.

The following levels of air pollution were recorded during the study period : Prague – from HiVol sampling PM_{10} 62.6 μg/m^3, c-PAHs 24.7 ng/m^3, B[a]P 3.50 ng/m^3, from personal monitoring for exposed population at the days of sampling c-PAHs 12.04±11.10 ng/m^3, B[a]P 1.79±1.67 ng/m^3, for controls c-PAHs 6.17±3.48, B[a]P 0.84±0.60 ng/m^3; Kosice - from HiVol sampling PM_{10} 58.0 μg/m^3, c-PAHs 11.9 ng/m^3, B[a]P 1.37 ng/m^3, from personal monitoring for exposed population at the days of sampling c-PAHs 21.72±3.12 ng/m^3, B[a]P 2.94±1.44 ng/m^3, for controls c-PAHs 6.39±1.56, B[a]P 1.07±0.66 ng/m^3; Sofia - from HiVol sampling PM_{10} 89.9 μg/m^3, c-PAHs 36.4 ng/m^3, B[a]P 4.84 ng/m^3, from personal monitoring for exposed population at the days of sampling c-PAHs 45.19±25.41 ng/m^3, B[a]P 4.31±2.60 ng/m^3 (policemen) and c-PAHs 34.46±33.22 ng/m^3, B[a]P 5.40±3.18 ng/m^3 (bus drivers), for controls c-PAHs 24.14±16.82 ng/m^3, B[a]P 1.96±1.53 ng/m^3 (Farmer et al., 2003).

Cell cultivation

Whole venous blood cultures were established within 24 h after blood collection in sodium-heparinised tubes, according to the method described by Sorsa et al. (1994). Lymphocyte cultures were set up in the tissue culture flasks, each culture containing 0.6 ml of whole blood and 7.5 ml of medium (cultivation medium for one culture was composed of RPMI 1640 Sevac 1.06 ml, calf serum Opavac 1.80 ml, distilled water 4.24 ml, glutamine 0.10 ml, $NaHCO_3$ (7.5%) 0.16 ml, PHA HA-15 Murex, U.K. 0.10 ml).

The cultures for the FISH were cultivated at 37^{o}C and harvested after 72 h of incubation to obtain a sufficient number of mitoses. Colchicine (Fluka) was added to a final concentration of 0.5 μg/ml 2 h before the end of the incubation. The cells were collected by centrifugation, re-suspended in pre-worm (37^0 C) hypotonic solution (0.075 M KCl) for 10 min and fixed in acetic acid/methanol according to the standard protocol The cell suspensions were stored at -20^0 C. Slides were always prepared using the air-dry method on the day before painting by FISH, randomly numbered and after the painting scored “blind” in numerical order.

FISH

Fresh slides were prepared by dropping the fixed pellet of metaphase cells onto slides, which were stored in ethanol with 1 % ether at -4ºC. The protocol used to perform the FISH with whole chromosome probes for chromosomes #1 and #4 was adapted from the protocol provided by Cambio (Cambridge, UK). The counterstain, following the washes, was DAPI mixed with mountant Vectashield (Vector Laboratories, Burlingame, CA) in final concentration 0.24 μg/ml.

Analysis of FISH slides was performed using a Zeiss microscope equipped with a triple filter for simultaneous visualization of DAPI (blue), FITC (green) and Cy-3 (red) signals (Rubes et al. 1998). Color images were

collected using a computer-controlled Zeiss axioskop with monochrome CCD camera (JAI Corporation, Japan) and ISIS 4.4.16 software (MetaSystems GmbH, Germany). 1000 metaphases per subject were analyzed.

All aberrant cells were classified according to the Protocol for Aberration Identification and Nomenclature (PAINT) (Tucker et al., 1995). The Protocol defines translocations as follows: translocation (t) is a rearranged chromosome with a single centromere and is to be counted as an aberration; the translocated chromosome must exhibit at least two colors, reciprocal translocation (rcp) is the exchange of genetic material between the two chromosomes of a different color, dicentric chromosome (dic) contains two centromeres from the chromosomes of different colors, acentric fragment (ace) is a linear part of red or green painted chromosome without centromere, insertion (ins) is an acentric chromosomal material inside the chromosome of another color. Other analyzed parameters were percentage of aberrant cells (%AB.C.) and the number of color junctions (NCJ). All cells with color junction or painted acentric fragment were counted as aberrant cells.

Genomic frequencies (F_G) of stable chromosome exchanges were calculated according to Lucas et al. (1993) using the equation : $F_G = F_{rg} /2.05 [f_r (1-f_r) + f_g (1 - f_g) -f_r f_g]$. F_{rg} is the translocation frequency measured by FISH after two-color painting, f_r and f_g are the fractions of the genome painted red and green, respectively.

Subjects with suspected clonality were excluded from the final evaluation.

Statistical analysis

The Student t-test was used to analyze the differences between the groups. The influence of independent variables (like c-PAHs exposure, age and smoking) on FISH endpoints was estimated by multiple regression model.

RESULTS

According to personal monitoring, the air pollution during the 8 h shift before the blood sampling to PAHs and B[a]P seems to be in the order Sofia>Kosice>Prague. As air pollution may vary daily, the exposure during the last three months characterized from HiVol sampling seems to be more important for the relationship between c-PAHs and biomarker of effect. Then the exposure in three cities is in the order Sofia>Prague>Kosice.

In Table 1 are summarized all the cytogenetic endpoints for exposed and control groups divided further according to smokers and nonsmokers. Probably the most important endpoints are $F_G/100$, % AB.C. and t/1000 (translocations per 1000 cells).

In Prague was observed a significant increase in all studied endpoints in the police officers ($P<0.05$). This difference is the most apparent between exposed and control nonsmokers ($F_G/100=1.56\pm1.34$ vs. 1.14 ± 1.02, % AB.C.$=0.29\pm0.20$ vs. 0.22 ± 0.16, t/1000$=4.21\pm3.61$ vs. 3.05 ± 2.73, $P<0.05$, respectively) (Figure 1). A similar effect of air pollution was not observed in smokers.

In Kosice the exposed group differed from controls in the endpoints % AB.C. (0.29 ± 0.19 vs. 0.21 ± 0.20, $P<0.05$) and t/1000 (3.91 ± 3.14 vs. 2.84 ± 3.10, $P<0.05$). No differences were observed between exposed and control nonsmokers (Figure 2) or exposed and control smokers.

In Sofia two exposed groups were followed: police officers and bus drivers. FISH endpoints were significantly higher in police officers compared to controls ($F_G/100=1.60\pm0.99$ vs. 0.82 ± 0.79, % AB.C.$=0.25\pm0.14$ vs. 0.13 ± 0.13, t/1000$=4.19\pm2.65$ vs. 2.13 ± 2.05, $P<0.01$, respectively). All these parameters were also significantly higher in exposed nonsmokers (Figure 3, $P<0.05$) as well as in exposed smokers ($P<0.05$). In bus drivers compared to controls were increased % AB.C. (0.25 ± 0.18 vs.0.13 ± 0.13, $P<0.01$) and t/1000 (3.24 ± 2.28 vs. 2.13 ± 2.05, $P<0.05$). In bus drivers nonsmokers vs. control nonsmokers all endpoints were significantly increased (Figure 3, $P<0.05$ or $P<0.01$). No difference was observed between smokers from both groups.

Comparing three cities, there was no difference between Prague and Kosice, all groups, nonsmokers and smokers, respectively. Similarly Kosice and Sofia did not differ. Comparing Prague and Sofia, FISH % AB.C. was higher in Prague in exposed smokers (0.39 ± 0.32 vs. 0.26 ± 0.14), in control smokers (0.40 ± 0.23 vs. 0.17 ± 0.13) and control nonsmokers (0.22 ± 0.16 vs. 0.09 ± 0.13, $P<0.05$ respectively) and t/1000 in control smokers (5.50 ± 4.04 vs. 2.69 ± 1.93, $P<0.05$).

Table 1. Effect of air pollution on chromosomal aberrations in peripheral lymphocytes.

Site	Group	N	Age	F_G/100	% AB.C.	t	rcp	dic	ace	ins	NCJ
Prague	Exposed	50	31.8	1.72±1.57*	0.33±0.25*	4.62±4.21*	1.74±1.84*	0.16±0.42	0.72±1.20	0.12±0.39*	5.16±4.62**
	SM	17	33.3	2.02±1.95	0.39±0.32	5.41±5.21	1.88±2.06	0.18±0.53	1.12±1.69	0.18±0.53	6.29±5.72
	NS	33	31.0	1.56±1.34*	0.29±0.20*	4.21±3.61*	1.67±1.74*	0.15±0.36	0.52±0.80	0.09±0.29	4.58±3.91*
	Controls	48	29.4	1.25±1.11	0.24±0.18	3.35±2.99	1.21±1.32	0.08±0.35	0.44±0.68	0.00±0.00	3.42±2.96
	SM	6	35.5	2.05±1.51	0.40±0.23	5.50±4.04	2.17±1.72	0.00±0.00	0.83±0.75	0.00±0.00	5.50±4.04
	NS	42	28.5	1.14±1.02	0.22±0.16	3.05±2.73	1.07±1.22	0.10±0.37	0.38±0.66	0.00±0.00	3.12±2.71
Kosice	Exposed	47	32.0	1.52±1.17	0.29±0.19*	3.91±3.14*	1.26±1.21	0.30±0.59*	0.98±1.59	0.09±0.28	4.70±3.55*
	SM	23	32.7	1.69±1.17	0.33±0.21*	4.43±3.12	1.39±1.20	0.26±0.45	1.30±1.92	0.00±0.00	5.13±3.91
	NS	24	31.2	1.36±1.18	0.26±0.16	3.42±3.15	1.13±1.23	0.33±0.70	0.67±1.17	0.17±0.38	4.29±3.20
	Controls	45	35.3	1.12±1.30	0.21±0.20	2.84±3.10	0.93±1.16	0.13±0.34	0.56±0.94	0.11±0.32	3.33±3.41
	SM	21	37.1	1.22±1.48	0.21±0.18	2.95±3.22	0.90±1.09	0.14±0.36	0.67±1.06	0.10±0.30	3.48±3.60
	NS	24	33.8	1.03±1.14	0.21±0.22	2.75±3.05	0.96±1.23	0.13±0.34	0.46±0.83	0.13±0.34	3.21±3.31
Sofia	Police-men	26	31.3	1.60±0.99**	0.25±0.14**	4.19±2.65**	1.46±1.07**	0.08±0.27	0.23±0.65	0.08±0.27	4.42±2.86
	SM	17	31.5	1.69±1.06*	0.26±0.14*	4.47±2.83*	1.65±1.06*	0.12±0.33	0.24±0.75	0.00±0.00	4.59±2.94
	NS	9	31.0	1.42±0.88*	0.23±0.13*	3.67±2.35*	1.11±1.05	0.00±0.00	0.22±0.44	0.22±0.44	4.11±2.85
	Drivers	25	37.7	1.22±0.85	0.25±0.18**	3.24±2.28*	1.24±1.01*	0.28±0.68	0.64±0.99	0.04±0.20	3.64±2.83
	SM	15	38.3	1.11±0.79	0.25±0.20	2.93±2.12	1.07±0.88	0.40±0.83	0.80±1.08	0.00±0.00	3.47±2.92
	NS	10	36.8	1.38±0.95*	0.26±0.16**	3.70±2.54*	1.50±1.18*	0.10±0.32	0.40±0.84	0.10±0.32	3.90±2.81
	Controls	23	38.4	0.82±0.79	0.13±0.13	2.13±2.05	0.70±0.76	0.09±0.29	0.22±0.60	0.09±0.29	2.35±2.46
	SM	13	40.4	1.04±0.74	0.17±0.13	2.69±1.93	0.85±0.80	0.08±0.28	0.31±0.75	0.08±0.28	3.00±2.55
	NS	10	35.8	0.54±0.79	0.09±0.13	1.40±2.07	0.50±0.71	0.10±0.32	0.10±0.32	0.10±0.32	1.50±2.17

* P < 0.05, ** P < 0.01, N – number of subjects, SM – smokers, NS – nonsmokers, F_G/100 – genomic frequency of translocations/100 cells, % AB.C. – percentage of aberrant cells, t – number of translocations/1000 cells, rcp – number of reciprocal translocations/1000 cells, dic – number of dicentric chromosomes/1000 cells, ace – number of acentric fragments/1000 cells, ins – number of incertions/1000 cells, NCJ – number of color junctions/1000 cells

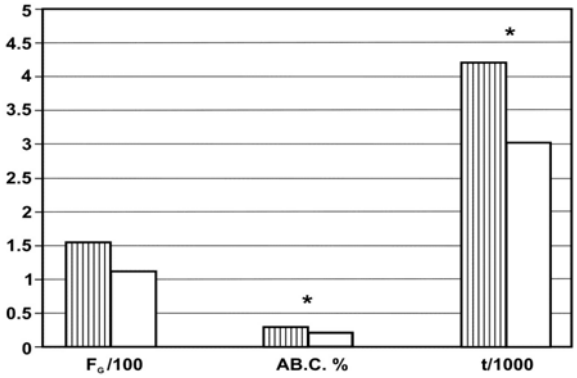

Figure 1: Effect of air pollution determined by FISH on nonsmokers in Prague, Czech Republic. (P < 0.05; shaded columns – police officers; empty columns – controls; F_G/100 – genomic frequency of translocations/100 cells; % AB.C. – percentage of aberrant cells; t – number of translocations/1000 cells).

According to the FISH results genetic injury of chromosomes seems to be higher in Prague than in Sofia; Kosice does not differ from these two cities.

Multiple regression analysis (Table 2) indicates the significant effect of exposure to c-PAHs at Kosice and Sofia, of age in Prague and Sofia, and of smoking in Prague, on FISH endpoints.

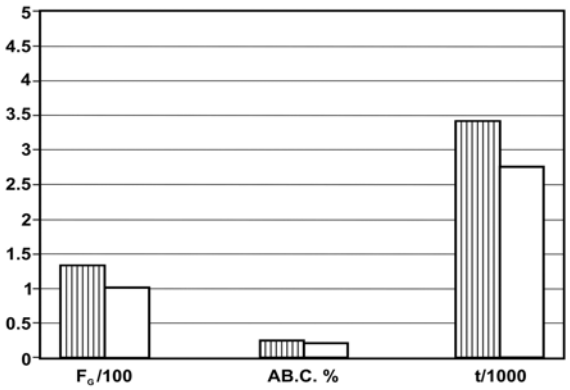

Figure 2: Effect of air pollution determined by FISH on nonsmokers in Kosice, Slovakia. (P < 0.05; shaded columns – police officers; empty columns – controls; F_G/100 – genomic frequency of translocations/100 cells; % AB.C. – percentage of aberrant cells; t – number of translocations/1000 cells).

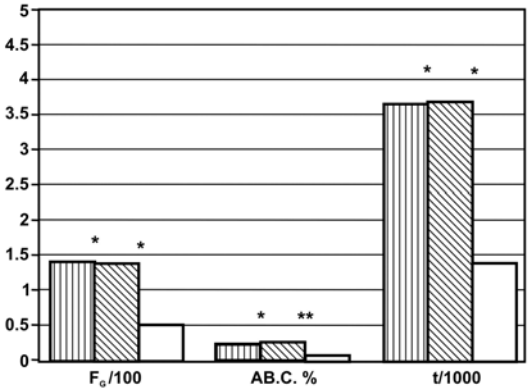

Figure 3: Effect of air pollution determined by FISH on nonsmokers in Sofia, Bulgaria. (P < 0.05; oblique shaded columns – police officers; shaded columns – bus drivers; empty columns – controls; F_G/100 – genomic frequency of translocations/100 cells; % AB.C. – percentage of aberrant cells; t – number of translocations/1000 cells).

Table 2. The results of multivariate analysis for FISH endpoints.

Site	FISH parameter	Intercept	Exposure	Age	Smoking status
Prague	F_G/100	1.459	0.215 (p=0.39)	**0.078 (p=0.0000)**	0.312 (p=0.30)
	% AB.C	0.259	0.047 (p=0.26)	**0.010 (p=0.0001)**	***0.089 (p=0.0743)***
	t	3.913	0.598 (p=0.37)	**0.210 (p=0.0000)**	0.813 (p=0.31)
Kosice	F_G/100	0.915	**0.572 (p=0.0245)**	**0.053 (p=0.0009)**	0.136 (p=0.58)
	% AB.C	0.187	**0.111 (p=0.0047)**	**0.009 (p=0.0002)**	0.004 (p=0.92)
	t	2.361	**1.502 (p=0.0198)**	**0.133 (p=0.0010)**	0.283 (p=0.65)
Sofia	F_G/100	0.741	**0.562 (p=0.0191)**	-0.000 (p=0.99)	0.194 (p=0.36)
	% AB.C	0.117	**0.113 (p=0.0055)**	-0.000 (p=0.94)	0.038 (p=0.28)
	t	1.952	**1.505 (p=0.0180)**	-0.008 (p=0.83)	0.515 (p=0.36)

F_G/100 – genomic frequency of translocations/100 cells, %Ab.C – percentage of aberrant cells, t- number of translocations/1000 cells.

When we analyzed the impact of exposure to B[a]P (as the representative of c-PAHs, groups of nonsmokers) to F_G/100 in all three cities, the outcome was similar for data/group by personal monitoring (Figure 4 A) or HiVol sampling (Figure 4 B). But using data from individual personal monitoring, the effect of B[a]P exposure to F_G/100 is not very significant (Figure 5).

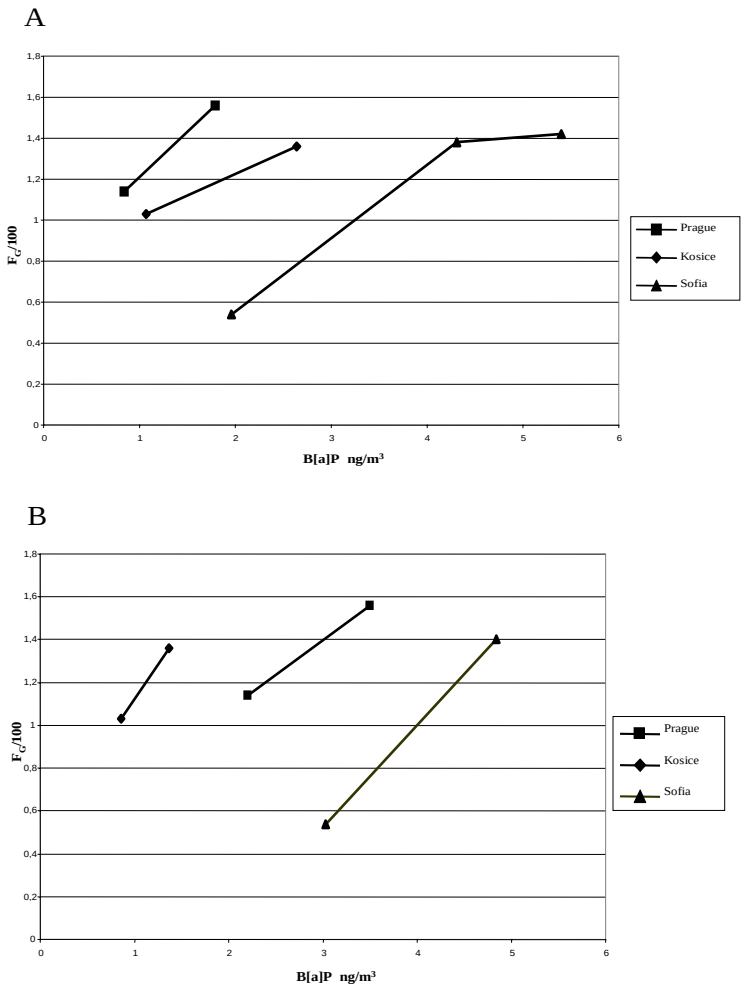

Figure 4: A – The relationship between B[a]P exposure by personal monitoring and F_G/100, exposed vs. control in Prague, Kosice and Sofia. B - The relationship between B[a]P exposure by HiVol sampling and F_G/100, exposed vs. control in Prague, Kosice and Sofia.

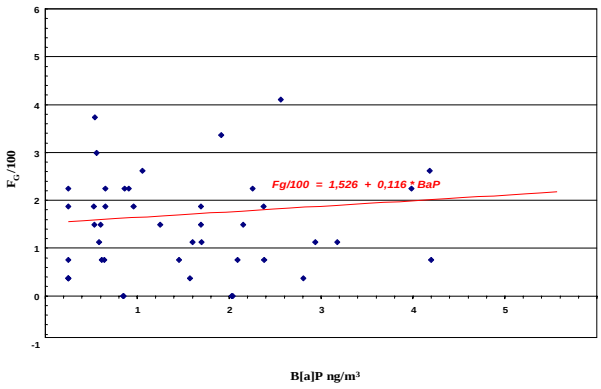

Figure 5: The relationship between B[a]P exposure by personal monitoring and F_G/100 – data for individual subjects in Prague.

DISCUSSION

The obtained results indicate a significant genotoxicity of organic compounds adsorbed onto ambient air particles, which induce stable translocations determined in peripheral lymphocytes. It corresponds to genotoxicity of extracted organic matter from air particles as was proved by Binkova et al. (2003) for the sample from Prague. We may conclude that FISH analysis indicates that police officers in Prague, Kosice and Sofia as well as bus drivers in Sofia represent a group with increased genotoxic risk.

In both the cities Prague and Sofia, traffic represents a significant source of pollution, and this may also be affected by the age of used cars as well as their technical quality (e.g. diesel emissions from trucks). Genetic damage observed in all three cities Prague, Kosice and Sofia seems to be important as a marker of possible health injury during the next decades, especially likely to affect future pregnancies, cardiovascular diseases and cancer. The differences in genetic effects observed between the occupationally exposed and control populations in this study reflect the longer exposure to polluted air experienced by the former population. It seems to be appropriate to study the air pollution in all three cities more thoroughly as other non-occupationally exposed city dwellers experience long-term environmental exposure to similarly polluted air.

The basic difference between the studied exposed groups and controls is that the police officers usually walk through busy streets in 8-12 h shifts, but controls spent usually more than 90% of their time indoors. Usually it is calculated that in a non-smoking house the concentration of $PM_{2.5}$ and c-PAHs is 50-60 % of outdoor pollution. It means that the police officers may be exposed to approximately twice the concentration of $PM_{2.5}$ and c-PAHs than the controls during their workshifts. In all three cities the level of air pollution to c-PAHs significantly increased the genomic frequency of translocations during the winter period.

This is the first study when the FISH method was used to analyze the impact of environmental air pollution. According to the original hypothesis it is expected that the most important group of chemicals responsible for the biological activity of air pollution represent c-PAHs. Using biomarkers of exposure, an increased level of DNA adducts in exposed groups compared to controls has usually been observed (Peluso et al., 1998). Using cytogenetic endpoints as biomarkers of effect, no increase of chromosomal aberrations by the conventional method, SCE or micronuclei by air pollutants was observed in Europe (Bolognesi et al., 1997a, b; Binkova et al., 1996). Zhao et al. (1998) observed an increase of SCE and micronuclei in traffic policemen compared to controls, but the PAH exposure was not determined. We may postulate that the FISH method seems to be more sensitive than other cytogenetic endpoints, and it may be concluded as well that the increase of genomic translocations represent a more significant health risk for their carriers, especially for the process of carcinogenesis.

It will be necessary to analyze different factors, which could affect genomic frequency of translocations such as for example life style, antioxidant vitamin levels or genetic polymorphism of metabolic genes. Preliminary results from Prague indicate that the frequency of translocations determined by FISH was associated with B(a)P-like DNA adducts (corresponding to the exposure of c-PAHs) (Sram et al., 2004b). This result supports the idea to use FISH whole chromosome painting as a new sensitive biomarker of effect to evaluate not only occupational exposure to carcinogens, but also environmental exposure to much lower concentrations as detected in air pollution, and to establish how to translate (use) these data for risk assessment.

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