Chromosome damage induced by benzene after the use of conventional and FISH chromosome painting*

K. ŠIVIKOVÁ, B. HOLEČKOVÁ, J. DIANOVSKÝ

Institute of Genetics, e-mail: sivikova@uvm.sk, University of Veterinary Medicine, 041 81 Košice, Slovak Republic

Received April 26, 2004

The cytogenetic effects of *in vitro* exposure to benzene were investigated in cultured bovine peripheral lymphocytes. Stable and unstable chromosome aberrations (CA) and sister chromatid exchanges (SCE) were examined. Cultures of lymphocytes from two healthy donors were treated with benzene at concentrations of 5, 10, 50, 100, 500 and 1000 μmol.l^{-1} for the last 24 hours and for 2 hours of cultivation, both without and with metabolic activation (S9). In the CA assays with conventional Giemsa staining for 24 hours without metabolic activation, no dose-dependence in the induced chromosome aberration was achieved. A significant elevation in the induction of CA was found only after the application of benzene at a dose of 100 μmol.l^{-1} ($p<0.01$). Similar results were observed using fluorescence *in situ* hybridization (FISH) method for the detection both of non-stable and persistent chromatid-type aberrations and in the SCE assay for 24 hours without S9. All the lower and higher concentrations tested have no positive influence on CA and SCE induction. In experiments with S9 a significant increase in CAs, and a dose-dependence was achieved after exposure to benzene for 2 hours at concentrations ranging from 50 to 500 μmol.l^{-1} ($p<0.05$ and $p<0.01$, respectively).

Key words: benzene, bovine peripheral lymphocytes, chromosome aberrations, sister chromatid exchanges, FISH

Benzene is commonly used as a solvent in industry and is a ubiquitous contaminant of the environment as it has been detected in water, unleaded petrol and in cigarette smoke [19]. Exposure to benzene and its derivatives in the environment is associated with various adverse consequences on human or animal health; e.g. irritation of the stomach, vomiting, convulsions, rapid heart rate, tremors, headaches, coma, and death eventually were observed after exposure of people even to low dose of benzene.

Human epidemiology studies have indicated that a high level of benzene exposure may cause hematotoxicity and even acute myelogenic leukemia [11, 34]. It is suggested that a key step in expressing the hematotoxic, carcinogenic and/or genotoxic properties of benzene is dependent on the formation of active metabolites [12, 13, 14]. The main metabolites, e.g. phenol, hydroquinone, catechol, and 1, 2, 4-benzentriol have been found to be more effective in the expression of their carcinogenic properties than benzene itself [1, 8, 10].

Recently more sensitive methods for the evaluation of benzene carcinogenity have been established. LAGORIO et al [15] conducted a biomonitoring study with the aim of evaluating the association between the excretion of the modified base of guanin (8-hydroxydeoxyguanosine, 8-OHdG), which is a sensitive marker of DNA damage, and exposure to benzene among people occupationally at risk. The authors showed a dose-related response effect between personal exposure to benzene and urinary 8-OhdG concentrations.

Taking the leukemogenic properties into consideration, the genotoxic effect of benzene has been the object of numerous investigations. Endpoints as chromosome aberrations (CAs), sister chromatid exchanges (SCEs) and micronuclei (MNi) have been the most frequently examined. Primary attention has been focused on chromosome aberration related to cancer induction [2, 34]. In epidemiological studies BONASSI et al [6] showed that people with elevated frequencies of CA in their peripheral blood lym-

*The Ministry of Education and Science of the Slovak Republic (Grant No. 1/1271/04 and 1/1367/04) have supported this work.

phocytes have a significantly elevated risk of developing cancer. After occupational exposure of workers to low-level dose of benzene inconsistent results have been achieved; a slight increase in CAs and SCEs in human peripheral lymphocytes have been found by SASIADEK and JAGIELSKI [26], YARDLEY-JONES et al [31] and TOMPA et al [29] while no significant differences between exposed and control groups have been reported by other authors [7, 32, 35]. Further reports, e.g. SASIADEK [27] and MAJOR et al [16], have described significantly positive elevations in the CA and SCE frequency in workers chronically exposed to benzene for about 10–20 years.

In the present study the results on CAs and SCEs in bovine blood lymphocytes exposed to benzene *in vitro* are reported. Conventional chromosome analysis was completed with the data on stable and unstable chromosome aberrations using fluorescent *in situ* hybridization (FISH) technique. There is no information about the possible effect of benzene exposure to domestic animals although adverse consequences of various environmental pollutants on animal health are widely described [25, 4, 22]; data of the spontaneous level of stable chromosome aberrations in cattle have been documented [23].

Material and methods

Benzene (>99% purity, Lachema, Brno, Czech Republic) was dissolved in dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO, USA) and applied to all experimental cultures at concentrations of 5, 10, 50, 100, 500 and 1000 μmol.l^{-1}. The highest dose of benzene was determined on the basis of a preliminary study in which a sufficient number of metaphases could be analysed. The final DMSO concentration in both treated and control cultures was 0.1%.

For conventional CA assays, experiments were performed in both the presence and absence of an S9 fraction. A freshly prepared S9 fraction (10% of the v/v culture) from Aroclor 1254 (Supelco, Bellefonte, PA, USA) induced rats was prepared according to the MARON and AMES method [17] and applied to the control and experimental cultures. Glucose-6-phosphate (Aldrich, Milwaukee, WI, USA) and NADP (Sigma, St. Louis, MO, USA) were used as cofactors.

Ethylmethanesulphonate (EMS, Sigma, St. Louis, MO, USA, 250 μg.ml^{-1}), mitomycin C (MMC, Sigma, St. Louis, MO, USA, 0.4 μmol.l^{-1}) and cyclophosphamide (CP, Jenapharm, Ankerwerk, Rudolstadt, Germany, 0.1 mmol.l^{-1}) were used as positive control agents in the assays in the absence and presence of the metabolic activation (S9 mix).

Experiments were carried out on two healthy cow donors (Slovak spotted cattle, one year old).

Whole blood specimens (0.5 ml) were cultivated for 72 hours at 38 °C in 5 ml of RPMI 1640 medium supplemented with L-glutamine, 15 μmol.l^{-1} HEPES (Sigma, St. Louis, MO, USA), 15% foetal calf serum (Sigma, Chemical Co., St. Louis, MO, USA), antibiotics (penicillin 250 U/ml – Biotika, Slovenská Ľupča, Slovak Republic, and streptomycin 250 μg.ml^{-1} – Antibiotic Co., Bulgaria), and phytohaemagglutinin (PHA, 180 μg.ml^{-1}, Welcome, Dartford, England).

Lymphocyte cultures were exposed to benzene for the last 24 hours and for 2 hours, without and with S9, respectively. After metabolic activation of S9 in a serum-free medium, the cells were removed twice with PBS and replaced by a completely fresh culture medium. Slides were prepared by the standard cytogenetic method.

FISH technique using the whole chromosome-painting probes was performed for the detection of chromatid- or chromosome-type aberrations. On the basis of previous CA results, two benzene concentrations (50 and 100 μmol.l^{-1}) were selected. Spectrum green labelled whole chromosome painting probe, specific for the bovine chromosome 1 (Veterinary Research Institute, Brno, Czech Republic) was used for hybridization. The painting probe in hybridization mixture (50% formamide, 2xSSC, 10% dextran sulphate, salmon sperm DNA, competitor DNA) was denatured at 72 °C for 10 min and reannealed at 37 °C for 80 min. The denaturation of slides was performed in 70% formamide, 2xSSC (pH 7.0) at 72 °C for 2 min and followed by dehydration procedure (70, 80, 96% ethanol, –20 °C). After overnight hybridization at 37 °C, the slides were washed in 50% formamide, 2xSSC (pH 7.0) at 42 °C, in 0.1 SSC (pH 7.0) at 42 °C and in TNT (Tris-NaCl-Tween 20 buffer, pH 7.0) at 42 °C. The slides were counterstained in DAPI/Antifade (4',6'-diamino-2-fenolindol, Q-BIOgene, Middlesex, UK).

For the SCE determination bromodeoxyuridine (8 μg.ml^{-1}, BUdR, Sigma, St. Louis, MO, USA) was applied to all cultures 24 hours after the initiation of division; then the slides were stained using the FPG technique to differentiate cell cycles [21]. Cell proliferation was stopped in metaphases by colchicine (Merck, Darmstadt, Germany) at a concentration of 5 μg.ml^{-1} two hours before harvesting.

One hundred well-spread metaphases were analyzed for the conventional detection of CAs for each concentration and donor; chromatid, isochromatid breaks and also gaps; and chromatid and isochromatid exchanges (CB, CE, IB and IE) were determined. Five hundred metaphases were scored for chromatid fragments and chromatid-type aberrations using FISH technique according to TUCKER et al [30]. Fluorescent microscope Nikon-Labophot 2A/2, equipped with dual band pass filter DAPI/FITC was used for visualization.

Fifty differentially stained metaphases for SCE were analyzed per donor and concentration.

The statistical analysis of results was performed using the chi-square tests to estimate the induction of stable and unstable CAs. Gaps were reported but excluded from the total

number of CAs and considered separately. A simple analysis of variance (ANOVA), and subsequently the Student's t test were used for the evaluation of SCE occurrence between treated and untreated groups.

Results

In Tables 1 and 2, the results of benzene induced chromosomal aberration frequencies in bovine peripheral lymphocyte *in vitro* in both samples with and without metabolic activation are shown. Multiple micromolar concentrations of benzene were tested; the mean values ± SD in each group and concentration are given.

Table 1. The frequency of CAs in cultured bovine lymphocytes exposed to benzene for 24 h without S9

Dose	Types of chromosomal aberrations					% breaks (±SD)
	G	CB	IB	CE	IE	
Control (DMSO)	8	4	1	–	–	2.5±0.15
Benzene (μmol.l^{-1})						
5	9	5	–	–	–	2.5±0.15[a]
10	10	6	–	–	–	3.0±0.17[a]
50	11	6	–	–	–	3.0±0.17[a]
100	31	17	1	–	–	9.0±0.28[**]
500	15	7	3	–	–	5.0±0.22[a]
1000	17	5	2	–	–	3.5±0.18[a]
250 μg.ml^{-1}, EMS	23	13	4	7	–	15.5±0.36[***]

200 well-spread metaphases of each group were determined. [**], [***], statistical significance (p<0.01 and p<0.001, respectively) – χ^2 test; [a] – no statistical significant data; CB, IB – chromatid, isochromatid breaks; CE, IE – chromatid, isochromatid exchange; G – gaps not included in statistical data.

Table 2. The frequency of CAs in cultured bovine lymphocytes exposed to benzene for 2 h with S9

Dose	Types of chromosomal aberrations					% breaks (±SD)
	G	CB	IB	CE	IE	
Control	4	2	–	–	–	1.0±0.10
Control (DMSO)	8	3	–	–	–	1.5±0.12
Benzene (μmol.l^{-1})						
5	9	8	–	–	–	4.0±0.19[a]
10	10	8	–	–	–	4.0±0.19[a]
50	21	10	–	–	–	5.0±0.22[*]
100	21	11	1	–	–	6.0±0.24[*]
500	12	13	2	–	–	7.5±0.26[**]
1000	13	9	–	–	–	4.5±0.21[a]
0.1 mmol.l^{-1} CP	23	19	12	–	–	15.5±0.29[***]

200 well-spread metaphases of each group were determined. [*], [**], [***], statistical significance (p<0.05, p<0.01 and p<0.001, respectively) – χ^2 test; [a] – no statistically significant data; CB, IB – chromatid, isochromatid breaks; CE, IE – chromatid, isochromatid exchange; G – gaps not included in statistical data.

Table 3. The frequency of chromatide-type aberrations detected by whole chromosome painting probe for 24h

Dose	Number of cells scored	Chromatid-type of aberrations (%)	Acentric fragments (%)	Chromatid exchanges
Control (DMSO)	500	0.2±0.04	0.2±0.04	0.0±0.00
Benzene (μmol.l^{-1})				
50	500	0.8±0.09[a]	0.8±0.09[a]	0.0±0.00
100	500	2.2±0.15[**]	1.8±0.13[*]	0.6±0.08[a]

500 well-spread metaphases of each concentration were determined. [*], [**] – statistical significance (p<0.05 and p<0.01, respectively) according to χ^2 test; [a] – no statistically significant data (p>0.05).

Table 4. The frequency of SCEs in cultured bovine lymphocytes exposed to benzene for 24 h without S9

Dose Treatment for 24 h	SCE/cell (±SD)
Control (DMSO)	6.61±1.57
Benzene (μmol.l^{-1})	
5	6.56±1.35[a]
10	6.66±1.74[a]
50	6.96±2.18[a]
100	8.26±2.78[***]
500	7.04±2.32[a]
1000	6.87±1.97[a]
Positive control, 0.4 μmol.l^{-1} MMC	9.64±2.30[***]

100 second-division metaphases of each group were analyzed for SCE, if it was possible. [***] – statistically significant data (p<0.001, ANOVA, Student's t test); [a] – no statistical significance.

In the CA assay with conventional Giemsa staining for 24 hours without S9 no dose-related effect was found. Chromatid breaks were the most frequent types of structural aberration; no chromosomal rearrangements and dicentric chromosomes were found. Although chromosomal aberrations were slightly elevated in all the concentrations tested, there was a significant difference in CA induction obtained only after exposure to a dose of 100 μmol.l^{-1} (p<0.01, χ^2 test) in comparison with its concomitant control.

The clastogenic effect of benzene was achieved in the cultures for 2 hours with S9. A significant increase of induced chromosome damage was seen when exposure to concentrations ranged from 50 to 500 μmol.l^{-1} (p<0.05, and 0.01, respectively, χ^2 test). Treatment with the later dose caused elevation in the frequency of induced chromatid breaks four-fold greater than those seen in the corresponding controls. With respect to this dose, the highest concentration (1000 μmol.l^{-1}) showed a decrease in the frequency of chromosome breaks.

The results of FISH technique using whole chromosome painting probes are shown in Table 3. Only chromatide-type aberrations, such as acentric fragments and chromatid exchanges were detected. No chromosome-type aberrations were obtained. A significant increase in the frequency of chromatide-type aberrations was observed after the exposure to benzene at a concentration of 100 μmol.l^{-1} in agreement with findings described for conventional CA technique ($p<0.01$, χ^2 test). Only weak clastogenic effect ($p<0.05$) was determined for acentric fragments.

The ability of benzene to induce sister chromatid exchange frequencies is summarized in Table 4. In conformity to the CA assay, a 24 hour exposure to benzene in the absence of S9 showed a statistically significant elevation in the mean of SCEs at a concentration of 100 μmol.l^{-1} ($p<0.001$, ANOVA and Student's t test), but no dose relation effect has been found.

Discussion

Benzene has been documented as inducing chromosome damage in both human and animal somatic cells. Many cytogenetic investigations have dealt with the occupational exposure of workers to low-level doses of benzene. Either a significant increase in the frequency of chromosome aberrations or sister chromatid exchanges [27, 16, 5, 13, 35] or slight or no differences between occupationally exposed workers or control groups have been described [7, 32, 34].

Numerous *in vivo* cytogenetic studies have documented that benzene exposure induces a dose and time-dependent elevation of chromosome aberrations and SCEs in the bone marrow of experimental animals [14, 12]. CHUNG et al [8] and RESS et al [24] have reported a marked increase in MNi induced by benzene metabolites in mice and human lymphocytes *in vitro*. FARRIS et al [11] have confirmed these *in vivo* findings concerning the hematological consequences of benzene inhalation in B6C3F1 mice exposed to 1, 5, 10, 100 and 200 ppm benzene for 6 hours a day, 5 days a week (followed by two days without dosing) for 1, 2, 4 or 8 weeks and the recovery group. The exposure of mice to 100 and 200 ppm benzene reduced the total bone marrow cells, progenitor cells and most blood parameters. No significant effects on hematopoetic parameters were seen from an exposure to 10 ppm or less.

It is assumed that the mechanisms by which benzene expresses its genotoxic effects in the bone marrow has been complicated by involving multiple metabolites. This is supported by evidence that benzene metabolites, such as phenol, hydrochinone, catechol, results in cytotoxicity and genotoxicity potential in the bone marrow of treated mice. EASTMOND et al [9] supposed that increased chromosomal breakage, occurring in euchromatin regions, might be due to an inhibitory effect of benzene metabolites on enzymes involved in DNA replication and repair. In human bone marrow there was a derived leukemia cell line and *in vivo* in the bone marrow of treated mice, it was shown that benzene inhibits topoisomerase II enzyme activity. Topoisomerase II enzymes are thought to play an important role during replication, recombination, and DNA repair [3]. Interference with normal topoisomerase II activity at critical stages of the cell cycle can lead to chromosome breaks, induction of aneuploidy or the death of cells [9].

The aim of the present study was to demonstrate the cytogenetic effects of benzene in cultured bovine lymphocytes *in vitro*. Based upon our data chromosome aberrations and sister chromatid exchanges for 24 hours, a significant increase in induced chromosome damage (FISH and conventional Giemsa staning) was found at 100 μmol.l^{-1} of benzene ($p<0.01$ and $p<0.001$, respectively). Both higher doses tested (500 μmol.l^{-1} and 1000 μmol.l^{-1}) have been shown to cause a decrease in the frequency of the CA and SCE.

Our results correspond to those of MORIMOTO and WOLFF [20] in human lymphocytes cultures. The authors have reported that benzene does not induce either sister chromatid exchanges or affect the cell cycle kinetics over a range of doses. On the other hand, positive results in SCE induction were obtained with benzene metabolites, e.g. catechol and hydroquinone, indicating the need for metabolic activation of benzene.

The lack of genotoxic activity has been indicated by ZARANI et al [33] in whole blood cultures that were treated with several organic solvents, e.g. toluene, benzene and acetone *in vitro* for 48 hours at the concentrations ranging from 0.1 to 5 mmol.l^{-1}.

A dose-related response in CA induction was observed in the bovine cultures after benzene administration for 2 hours with metabolic activation. A positive clastogenic effect of benzene was seen at concentrations ranging from 50 to 500 μmol.l^{-1}. The highest dose (1000 μmol.l^{-1}) showed a decrease in the CA frequency in comparison to the later active dose.

In contrast to conventional cytogenetic methods, FISH is considered as a rapid and more sensitive technique to detect the large spectra of chromosome aberrations. It allows recognizing stable chromosome aberrations that could be transmitted from one to the next cell generation [28].

The evaluation cytogenetic effects of benzene under used experimental conditions in our study has a limitation. Considering that no dose dependence in the assays for 24 hours without S9 was obtained, the statistically significant elevations in the CA and SCE frequencies at a dose of 100 μmol.l^{-1} have no biological significance. A positive dose-related effect seen in the CA assay for 2 hours confirmed the effectiveness of modification in the benzene metabolic activation. A decrease in CAs at the highest concentration with S9 could be assumed to be the effect of the inhibition of further metabolism of phenol [18].

References

[1] ANGELOSANTO FA, BLACKBURN GR, SCHREINER CA, MACKERER CR. Benzene induces a dose-responsive increase in the frequency of micronucleated cells in rat zymbal glands. Environ Health Perspect 1996; 104 Suppl 6: 1–11.

[2] ANWAR WA. Biomarkers of human exposure to pesticides. Environ Health Perspect 1997; 105 Suppl 4: 801–806.

[3] AU WW, OBERHEITMANN B, HARMS C. Assessing DNA damage and health risk using biomarkers. Mutat Res 2002; 509: 153–163.

[4] BÍREŠ J, DIANOVSKÝ J, BARTKO P, JUHÁSOVÁ Z. Effects on enzymes and the genetic apparatus of sheep after administration of samples from industrial emissions. Bio Metals 1995; 8: 53–58.

[5] BOGADI-SARE A, BRUMEN V, TURK R, KARACIC V, ZAVALIC M. Genotoxic effects in workers exposed to benzene: With special reference to exposure biomarkers and confounding factors. Ind Health 1997; 35: 367–373.

[6] BONASSI S, HAGMAR L, STROMBERG U, MONTAGUT AH, TINNERBERG H et al. Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. Cancer Res 2000; 60: 1619–1625.

[7] CLARE MG, YARDLEY-JONES A, MACLEAN AC, DEAN BJ. Chromosome analysis from peripheral blood lymphocytes of workers after an acute exposure to benzene. Br J Med 1984; 41: 249–253.

[8] CHUNG HW, KANG SJ, KIM SY. A combination of the micronucleus assay and a FISH technique for evaluation of the genotoxicity of 1, 2, 4-benzenetriol. Mutat Res 2002; 516: 49–56.

[9] EASTMOND DA, SCHULER M, FRANZ CH, HONGWEI CH, PARKS R et al. Characterization and mechanisms of chromosomal alterations induced by benzene in mice and humans. In: Characterization and Mechanisms of Chromosomal Alterations Induced by Benzene in Mice and Humans. Research report. Health Effect Institute, Cambridge M.A., 2001, Printed at Capital City Press, Montpelier, pp. 1–19.

[10] FABIANI R, DE BARTOLOMEO A, ROSIGNOLI P, SCAMOSCI M, LEPORE L, MOROZZI G. Influence of culture conditions on the DNA-damaging effect of benzene and its metabolites in human peripheral blood mononuclear cells. Environ Mol Mutagen 2001; 37: 1–6.

[11] FARRIS GM, ROBINSON SN, GAIDO KW, WONG BA, WONG VA et al. Benzene-induced hematotoxicity and bone marrow compensation in B6C3F1 mice. Fundam Appl Toxicol 1997; 36: 119–129.

[12] KANEKO T, WANG PY, SATO A. Benzene-associated leukemia and its risk assessment. J Occup Health 1997; 39: 159–178.

[13] KAŠUBA V, ROZGAJ R, ŠENTIJA K. Cytogenetic changes in subjects occupationally exposed to benzene. Chemosphere 2000; 40: 307–310.

[14] KENYON EM, KRAICHELY RE, HUDSON KT, MEDINSKY MA. Differences in rates of benzene metabolism correlate with observed genotoxicity. Toxicol Appl Pharmacol 1996; 136: 49–56.

[15] LAGORIO S, TAGESSON C, FORASTIERE F, IAVARONE I, AXELSON O, CARERE A. Exposure to benzene and urinary concentrations of 8-hydroxydeoxyguanosine, a biological marker of oxidative damage to DNA. Occup Environ Med 1994; 51: 739–743.

[16] MAJOR J, JAKAB M, KISS G, TOMPA A. Chromosome aberration, sister-chromatid exchange, proliferative rate index, and serum thiocyanate concentration in smokers exposed to low-dose benzene. Environ Mol Mutagen 1994; 23: 137–142.

[17] MARON DM, AMES BN. Revised methods for the Salmonella mutagenicity test. Mutat Res 1983; 113: 173–215.

[18] MEDINSKY MA, KENYON EM, SCHLOSSER PM. Benzene: a case study in parent chemical and metabolite interactions. Toxicology 1995; 105: 225–233.

[19] MEDINSKY MA, KENYON EM, SEATON MJ, SCHLOSSER PM. Mechanistic considerations in benzene physiological model development. Environ Health Perspect 1996; 104 Suppl 6: 1399–1404.

[20] MORIMOTO K, WOLFF S. Increase of sister chromatid exchanges and perturbations of cell division kinetics in human lymphocytes by benzene metabolites. Cancer Res 1980; 40: 1189–1193.

[21] PERRY P, WOLFF S. New Giemsa methods for differential staining of sister chromatids. Nature 1974; 251: 156–158.

[22] RASZYK J, TOMAN M, GAJDŮŠKOVÁ V, NEZVEDA K, ULRICH R et al. Effects of environmental pollutants on the porcine and bovine immune systems. Vet Med – Czech 1997; 42: 313–317.

[23] ŘEZÁČOVÁ O, KUBÍČKOVÁ S, ČERNOHORSKÁ H, RUBEŠ J. Comparison of spontaneous backround genomic aberration frequencies among cattle, pig and humans using dual-colored FISH. Chromosome Res 2003; 11: 715–724.

[24] RESS NB, WITT KL, XU J, HASEMAN JK, BUCHER JR. Micronucleus induction in mice exposed to diazoaminobenzene or its metabolites, benzene and aniline: implications for diaminobenzene carcinogenicity. Mutat Res 2002; 521: 201–208.

[25] RUBEŠ J, HOŘÍNOVÁ Z, GUSTAVSSON I, BORKOVEC L, URBANOVÁ J. Somatic chromosome mutations and morphological abnormalities in sperms of boars. Hereditas 1991; 115: 139–143.

[26] SASIADEK M, JAGIELSKI J. Genotoxic effects observed in workers occupationally exposed to organic solvents. Pol J Occup Med 1990; 3: 103–108.

[27] SASIADEK M. Nonrandom distribution of breakpoints in the karyotypes of workers occupationally exposed to benzene. Environ. Health Perspect 1992; 97: 255–257.

[28] STRONATI L, FARRIS A, PACCHIEROTTI F. Evaluation of chromosome painting to assess the induction and persistence of chromosome aberrations in bone marrow cells of mice treated with benzene. Mutat Res 2004; 545: 1–9.

[29] TOMPA A, MAJOR J, JAKAB MG. Monitoring of benzene-exposed workers for genotoxic effects of benzene: improved-working-condition-related decrease in the frequencies of chromosomal aberrations in peripheral blood lymphocytes. Mutat Res 1994; 304: 159–165.

[30] TUCKER JD, MORGAN WF, AWA AA, BAUCHINGER M, BLAKEY D et al. A proposed system for scoring structural aberrations detected by chromosome painting. Cytogenet Cell Genet 1995; 68: 211–221.

[31] YARDLEY-JONES A, ANDERSON D, LOVELL DP, JENKINSON

PC. Analysis of chromosomal aberrations in workers exposed to low level benzene. Br J Ind Med 1990; 45: 48–51.

[32] YARDLEY-JONES A, ANDERSON D, JENKINSON PC, LOVELL DP, BLOWERS SD, DAVIES MJ. Genotoxic effects in peripheral blood and urine of workers exposed to low level benzene. Br J Ind Med 1988; 45: 694–700.

[33] ZARANI F, PAPAZAFIRI P, KAPPAS A. Induction of micronuclei in human lymphocytes by organic solvents in vitro. J Environ Pathol Toxicol Oncol 1999; 18: 21–28.

[34] ZHANG L, EASTMOND DA, SMITH MT. The nature of chromosomal aberrations detected in humans exposed to benzene. Crit Rev Toxicol 2002; 32: 1–42.

[35] ZHU CQ, LAM TH, JIANG CQ. Lymphocyte DNA damage in bus manufacturing workers. Mutat Res 2001; 491: 173–181.