Interphase Cytogenetics of Workers Exposed to Benzene

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Fluorescence *in situ* hybridization (FISH) is a powerful new technique that allows numerical chromosome aberrations (aneuploidy) to be detected in interphase cells. In previous studies, FISH has been used to demonstrate that the benzene metabolites hydroquinone and 1,2,4-benzenetriol induce aneuploidy of chromosomes 7 and 9 in cultures of human cells. In the present study, we used an interphase FISH procedure to perform cytogenetic analyses on the blood cells of 43 workers exposed to benzene (median = 31 ppm, 8-hr time-weighted average) and 44 matched controls from Shanghai, China. High benzene exposure (> 31 ppm, $n = 22$) increased the hyperdiploid frequency of chromosome 9 ($p < 0.01$), but lower exposure (≤31 ppm, $n = 21$) did not. Trisomy 9 was the major form of benzene-induced hyperdiploidy. The level of hyperdiploidy in exposed workers correlated with their urinary phenol level ($r = 0.58$, $p < 0.0001$), a measure of internal benzene dose. A significant correlation was also found between hyperdiploidy and decreased absolute lymphocyte count, an indicator of benzene hematotoxicity, in the exposed group ($r = -0.44$, $p = 0.003$) but not in controls ($r = -0.09$, $p = 0.58$). These results show that high benzene exposure induces aneuploidy of chromosome 9 in nondiseased individuals, with trisomy being the most prevalent form. They further highlight the usefulness of interphase cytogenetics and FISH for the rapid and sensitive detection of aneuploidy in exposed human populations. — Environ Health Perspect 104(Suppl 6):1325–1329 (1996)

Key words: benzene, aneuploidy, hyperdiploidy, chromosome aberrations, leukemia

Introduction

Benzene is an important industrial chemical and environmental pollutant. It causes leukemia, aplastic anemia, and other bone marrow disorders in humans (*1,2*). However, the mechanisms involved in its leukemogenesis remain unknown. In contrast to many chemical carcinogens, benzene and its phenolic metabolites exhibit poor DNA-binding ability and are weakly active or inactive in standard gene mutation assays (*3–5*). By using the glycophorin A (GPA) gene mutation assay, we recently showed that occupational exposure to benzene induces gene-duplicating mutations at the GPA locus in humans (*6*). Benzene also causes chromosome aberrations, mostly breaks and gaps, and sister chromatid exchanges in lymphocytes of exposed workers (*7–10*). These results suggest that chromosome abnormalities and mitotic recombination may be the most likely mechanisms by which benzene induces genetic alterations that lead to leukemias in humans.

Previous studies have shown that benzene causes cytogenetic changes in specific chromosomes, especially of the C-group chromosomes 6–12 and X (*11–14*). For example, trisomy C, an extra chromosome of the C group, was frequently detected in bone marrow and blood of several patients suffering from benzene-induced myelogenous leukemia (*12,15,16*), myelodysplastic syndrome, and pancytopenia (*14*). In one of these patients, a clonal expansion of trisomy 9 was observed in all cells examined (*12*). A nonclone of trisomy 9 was also found in another benzene-poisoned patient (*14*). The t(9;22) translocation, known as the Philadelphia chromosome, was also observed in a case of leukopenia due to chronic exposure to benzene for 4 years (*17*).

Rowley et al. (*18*) speculated that changes in the C-group genetic material coupled with a transforming event could perturb normal hematopoiesis, resulting in a greater risk for leukemia. Trisomy of C-group chromosomes has been detected in myeloproliferative disorders (*19*) and in metaplasia with possible leukemia (*20*). Therefore, numerical and structural alterations of C-group chromosomes may play an important role in the leukemogenesis induced by benzene.

The findings described above were obtained using conventional cytogenetic analysis or chromosome-banding techniques. These standard techniques could only examine a relatively small number of well-spread metaphase cells. Thus, the majority of cells that remain in interphase are excluded from analysis. These traditional assays are too time consuming to score a large number of cells, and rare events may therefore not be detected. Recently, fluorescence *in situ* hybridization (FISH) has made it possible to detect numerical and structural chromosomal changes in large numbers of both interphase nuclei and metaphase spreads (*21*). This molecular cytogenetic method uses *in situ* hybridization with DNA probes specific to blocks of repetitive DNA sequences on defined regions of specific

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Abbreviations used: FISH, fluorescence *in situ* hybridization; GPA, glycophorin A; TWA, time-weighted average.

chromosomes (*22,23*). Aneuploidy is determined by simply counting the number of label regions representing a particular chromosome of interest within the isolated nucleus.

A FISH procedure was used to test whether polyphenolic metabolites of benzene induce aneuploidy of chromosome 9 in human cells. Our results showed that 1,2,4-benzenetriol increased the hyperdiploid frequency of chromosome 9 in the human myeloid HL60 cell line (*24*). Others showed that hydroquinone induced aneuploidy of chromosome 9 in cultured human lymphocytes (*25*). Trisomy 9, not tetrasomy, accounted for the majority of the hyperdiploidy induced by these benzene metabolites.

From these *in vitro* studies and the early case reports, two conclusions can be made: benzene metabolites induce aneuploidy of chromosome 9 in human cells, and trisomy 9 is detected in patients poisoned with benzene or with benzene-induced leukemia. It is not known, however, if benzene induces aneuploidy of chromosome 9 in nondiseased individuals. To answer this question, we have applied interphase FISH technology in a biomarker study of an exposed Chinese population. We demonstrate that high levels of benzene exposure produce hyperdiploidy of chromosome 9 in the lymphocytes of exposed workers.

Materials and Methods

Subject Enrollment and Exposure Assessment

Eighty-eight study subjects were enrolled in Shanghai, China. Blood and urine samples from 87 of these subjects were used for this study because the blood from one subject was not cultured successfully. Subject enrollment has been described in detail earlier (*26*). Briefly, 44 healthy workers who were currently exposed occupationally to a wide range of benzene concentrations but had minimal exposure to toluene and other aromatic solvents and no exposure to other known marrow-toxic chemicals or ionizing radiation were enrolled. Forty-four controls frequency-matched to the exposed subjects on age (5-year intervals) and gender were selected from the same geographic area. These controls had no history of occupational exposure to benzene or other chemical carcinogens. Exclusion criteria for all subjects included history of cancer, therapeutic radiation, or chemotherapy.

Current benzene exposure for each subject was monitored by organic vapor passive dosimetry badges (3M, St. Paul, MN). Each worker wore the badge for the full work shift on 5 continuous days prior to phlebotomy. An 8-hr time-weighted average (TWA) benzene exposure level, along with an exposure level to toluene and xylene, was calculated as the geometric mean of the five air measurements. Only trace amounts of other aromatic solvents were detected in the samples. All exposure assessment was performed blinded with respect to aneuploidy detection by FISH.

Personal benzene air levels in these factories were much higher than had been expected based on historical area monitoring data. As a direct result of this study, remedial action was taken at the two workplaces with the highest benzene exposures. Actions taken included substitution of toluene for benzene, enclosure of reaction vessels, and improvement in ventilation.

Urine Collection and Phenol Measurement

Forty-three of 44 benzene-exposed workers provided a spot urine sample at the end of a work shift or at the end of the high exposure period during their work shift. The control subjects provided a spot urine sample during their blood collection. Urine samples were immediately aliquoted and frozen on dry ice, and stored at −70°C. Urinary phenol was measured using a modification of a previously described isotope dilution gas chromatography–mass spectroscopy (GC–MS) assay, which employed a ^{13}C-label analog of phenol as the internal standard (*27*). Concentrations of phenol in urine were determined by calibrating against a primary standard solution. Urine samples were diluted with deionized water (1:1) because of the high levels of metabolites. We added 100 µl of each internal standard solution and concentrated HCl into 400 µl diluted urine samples in vials. The vials were capped and incubated at 100°C for 60 min, and allowed to cool. Following extraction, drying, and decantation, the sample aliquots in ethyl acetate were combined and evaporated to 200 µl under nitrogen. The samples were derived with 100 µl of bistrimethysily–trifluoracetamide, incubated at 60°C for 30 min, and analyzed by GC–MS.

Blood Cultures and Slide Preparation

The heparin-anticoagulated whole blood was cultured in RPMI-1640 medium supplemented with 15% fetal bovine serum, 1% penicillin–streptomycin, 1% L-glutamine (Gibco, Grand Island, NY), and 1% phytohemagglutinin-P (Pharmacia, Piscataway, NJ). The cultures were incubated at 37°C in a 5% CO_2 moist atmosphere and lymphocytes harvested 72 hr after initiation. After hypotonic treatment (0.075 M KCl) for 30 min at 37°C, the cells were fixed three times with freshly made Carnoy's solution (methanol:glacial acetic acid = 3:1). The fixed cells were then dropped onto prelabeled glass slides, allowed to air dry, and stored at −20°C under a nitrogen atmosphere.

Fluorescence *in Situ* Hybridization

Detailed procedures for FISH with repetitive DNA probes have been described previously (*24,28*). Briefly, the biotinylated human centromeric probe (classical satellite) specific for chromosome 9 (Oncor, Gaithersburg, MD) was mixed with sonicated salmon sperm carrier DNA in 55% formamide (Fluka, Buchs, Switzerland) with 10% dextran sulfate. The probe DNA was denatured at 70°C for 5 min, and cellular DNA was denatured in 70% formamide in 2×SSC (0.3 M sodium chloride and 0.03 M sodium citrate, pH 7.0) at 72°C for 2 min. The denatured probe of chromosome 9 was then hybridized with target DNA overnight at 37°C in a humidified chamber. The hybridized region can be detected with fluorescein isothiocyanate-conjugated avidin antibody (Vector, Burlingame, CA) after postwash in 50% formamide/2×SSC for 3 times at 45°C. After three washes in phosphate buffer, the hybridization signal was amplified using a biotinylated goat antiavidin antibody (Vector, Burlingame, CA). The red fluorescent dye propidium iodide (0.5 µg/ml) in a mounting medium (Vector, Burlingame, CA) was used to counterstain DNA. The hybridization signals were viewed by a Nikon fluorescence microscope equipped with epifluorescent illumination and a 100× oil immersion lens. A filter for fluorescein (excitation at 450–490 nm, dichroic at 510 nm, emission at 520 nm) was used. The nuclei appeared red with bright green–yellow spots indicating the hybridization regions.

Statistical Analyses

Arithmetic mean ± standard deviation (SD) and median with range are used to summarize data, which are presented as number of cells with an aneuploidy event detected out of 1000 cells scored. The Wilcoxon rank sum test was used to test for differences in aneuploidy rates between exposed subjects, as a single group, and

controls. Subsequent analyses were performed on study subjects divided into three groups: controls, subjects exposed to ≤31 ppm benzene (the median TWA exposure level), and subjects exposed to >31 ppm benzene. Analysis of covariance was used to test for group differences in aneuploidy. Correlation analyses were performed with Pearson's product-moment correlation. For these analyses, 0.5 was added to each aneuploidy value (since some subjects had no detectable events) and transformed by the natural logarithm to improve normality.

Variables tested for potential confounding were age, gender, current and lifelong tobacco use status, current alcohol intake, and body mass index, a measure of obesity calculated as weight (kg)/height (m)^2. Since results were minimally changed after including these variables, results are presented adjusted only for age and gender, the original matching variables. Two-sided p-values were calculated throughout: p-values < 0.05 were considered adequate to reject the null hypothesis. All statistical analyses were performed using SAS for personal computer, version 6.04 (SAS Institute Inc., Cary, NC).

Results

Age, smoking, and alcohol use patterns were similar among the subjects exposed to benzene and controls (Table 1). The hyperdiploid frequency (mean ± SD) of chromosome 9 was 0.007 ± 0.004 in controls (n = 44) and 0.009 ± 0.008 in exposed workers (n = 43). This difference was not statistically significant (p = 0.29). There was a difference, however, by level of exposure. Because the median exposure level in the benzene-exposed group was 31 ppm, this group was divided into two exposure categories: a lower exposed group, with exposures ≤31 ppm (n = 21), and a higher exposed group, with exposures >31 ppm (n = 22). Compared to controls, the hyperdiploidy of chromosome 9 was significantly increased (p = 0.008) in the highly exposed workers (0.013 ± 0.009) but not in the lower exposed group (0.006 ± 0.004, p = 0.1) (Figure 1; Table 2). This suggests that chromosome 9 may only be sensitive to the aneuploidogenic effects of benzene at higher exposure levels.

Hyperdiploidy can be separated into two categories: trisomy and tetrasomy. The effect of benzene on chromosome 9 was not statistically significant for trisomy or tetrasomy (p = 0.38 and 0.75, respectively) in exposed workers compared with controls. However, when the exposed group was separated into two categories, as described above, a dose-dependent effect was observed. Trisomy of chromosome 9 was significantly increased (p = 0.02) in the higher exposed workers (>31 ppm), and an increase of borderline significance (p = 0.05) in tetrasomy 9 was also observed in this group. No increase in trisomy and tetrasomy was detected in the ≤ 31 ppm exposure group (Table 2). Thus, high benzene exposure induces hyperdiploidy of chromosome 9 with trisomy being more prevalent.

Phenol is the principal metabolite of benzene. Its concentration in the urine of exposed workers can be used as an index of internal dose because the urinary level is highly correlated with external benzene exposure. Figure 2 shows the strong correlation between hyperdiploidy of chromosome 9 and levels of urinary phenol in the exposed workers (r = 0.58; p < 0.0001).

Previously we found that decreased absolute lymphocyte count was the most sensitive indicator of benzene exposure of all the hematological parameters examined in this population (*26*). We therefore looked

Table 1. Demographic characteristics by exposure category (means ± SD).

	Age, years	Cigarettes/day		Alcoholic drinks/week
		Males, n=23	Females, n=21	
Controls, n=44	35.4 ± 7.3	13.5 ± 13.7	0.0	1.4 ± 2.2
Exposed, n=44	35.3 ± 7.8	11.0 ± 7.8	0.0	1.5 ± 3.2

Table 2. Hyperdiploidy of chromosome 9 in benzene-exposed workers and matched controls (means ± SD).

Benzene exposure	Hyperdiploidy	Trisomy	Tetrasomy
Controls, n=44	0.007 ± 0.004	0.0054 ± 0.0029	0.0017 ± 0.0018
≤ 31 ppm, n=21	0.006 ± 0.004	0.0046 ± 0.0037	0.0010 ± 0.0014
> 31 ppm, n=22	0.013 ± 0.009**	0.0086 ± 0.0055*	0.0040 ± 0.0043†

*p<0.05 versus controls; **p<0.01 versus controls; ***p<0.001 versus controls; †0.05<p<0.1 versus controls by analysis of covariance, adjusted for age and gender, using ln-transformed aneuploidy rates.

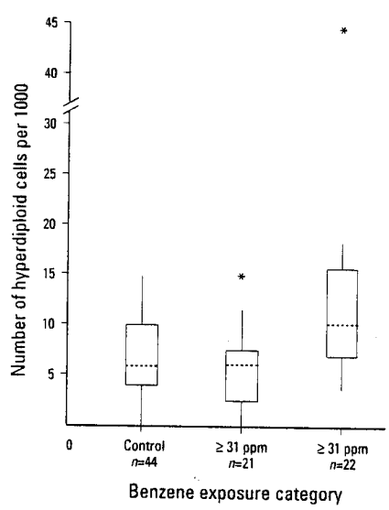

Figure 1. Hyperdiploidy of chromosome 9 in Chinese workers exposed to benzene and in matched controls. Dashed lines represent the median. Upper and lower edges of box represent 25 percentile and 75 percentile of data (the interquartile range), respectively. Whiskers include remaining data except for outliers. Outliers, represented by stars, are defined as values greater than 1.5× the interquartile range.

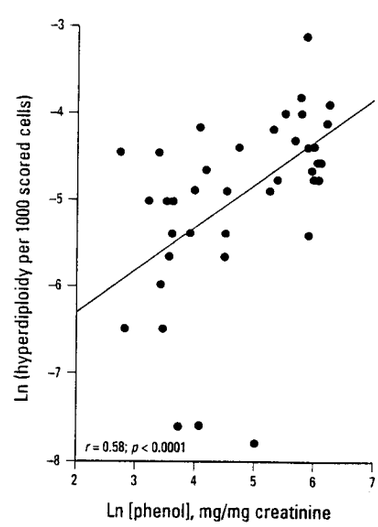

Figure 2. Correlation between hyperdiploidy of chromosome 9 and phenol levels in the urine of Chinese workers exposed to benzene. The vertical axis presents natural logarithm of hyperdiploid cells per 1000 scored interphase lymphocytes. The horizontal axis presents natural logarithm of urinary levels of phenol.

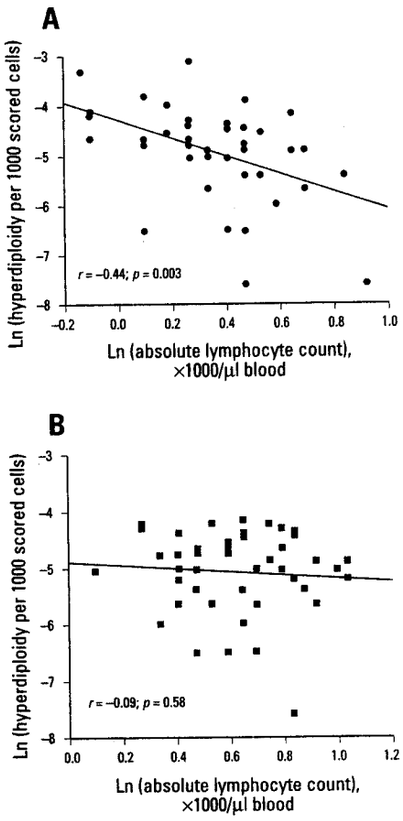

Figure 3. Correlation between hyperdiploidy of chromosome 9 and peripheral absolute lymphocyte counts in (*A*) exposed ($n = 43$) and (*B*) control ($n = 44$) subjects. The vertical axis presents natural logarithm of hyperdiploid cells per 1000 scored interphase lymphocytes. The horizontal axis presents natural logarithm of absolute lymphocyte counts.

for a correlation between hyperdiploidy of chromosome 9 and absolute lymphocyte count in both the exposed and control groups. Hyperdiploidy was significantly correlated with the lymphocyte count of the exposed workers ($r = -0.44$; $p = 0.003$) (Figure 3A), but the correlation was not found in the control group ($r = -0.09$; $p = 0.58$) (Figure 3B). Thus, hyperdiploidy of chromosome 9 is highly correlated with both the dose and hematotoxic effect of benzene.

Discussion

The ability of benzene to induce aneuploidy in humans has been a matter of some debate. Aneuploidy of specific chromosomes has been detected in benzene-poisoned individuals and in cases of benzene-induced leukemia (*1,13*). However, most previous studies of currently exposed workers have not observed significant increases in aneuploidy levels (*7,9,29*). These previous studies were limited to examining chromosome aberrations in relatively few (25–100) metaphase spreads by conventional cytogenetics. In the current study, we used FISH technology to examine the ploidy status of the C-group chromosome 9 in at least 1000 interphase blood cells per subject in 43 benzene-exposed workers and 44 unexposed controls. Our results demonstrate that benzene exposure increases the hyperdiploidy frequency of chromosome 9 at exposures above 31 ppm, but not below this level. The hyperdiploidy of chromosome 9 was strongly correlated with measured air levels of benzene and urinary levels of phenol, a marker of internal dose. It also correlated strongly with decreased lymphocyte count in the exposed workers. Thus, benzene appears to have a clear dose-dependent effect on the aneuploidy of chromosome 9.

In previous studies, it was shown that metabolites of benzene-induced kinetochore-positive micronuclei in human lymphocytes (*30,31*). This suggested a potential aneuploidogenic effect of hydroquinone and other benzene metabolites. As a follow-up to these studies, it was shown that the benzene metabolites hydroquinone and 1,2,4-benzenetriol induced aneuploidy of chromosome 9 (*24,25*). These studies employed interphase FISH technology to investigate the ploidy status of chromosome 9 in interphase nuclei from cultured human cells. The aneuploidogenic effects of benzene observed in the present study are therefore consistent with previous *in vitro* findings.

Hyperdiploidy of chromosome 9 has been observed in two patients with bone marrow toxicity from benzene (*12,14*). Hyperdiploidy of C-group chromosomes has also been detected in patients with benzene-induced myelogenous leukemia. In the present study, we have demonstrated that benzene induces aneuploidy of the C-group chromosome 9 in nondiseased individuals. This is the first demonstration to our knowledge of a chromosome-specific aneuploidogenic effect of benzene in currently exposed humans.

One of the metabolites of benzene, 1,4-benzoquinone, has been shown to be a potent inhibitor of microtubule assembly (*32,33*). The metabolites hydroquinone and 1,2,4-benzenetriol have also been shown to produce microtubule disruption, in addition to aneuploidy, in intact human cells (*24*). This is probably the result of their oxidation to quinones, which then covalently bind to tubulin. The most likely mechanism for the benzene-induced aneuploidy observed in this study is therefore the conversion of benzene to quinonoid metabolites in the blood and bone marrow, which bind to and disrupt the microtubules of the mitotic spindle and cause malsegregation of chromosomes at anaphase. Our findings in exposed people are therefore consistent with this mechanism of benzene-induced aneuploidy, and we suggest that chromosome-specific aneuploidy plays a key role in benzene-induced leukemia.

Finally, our study shows the value of combining laboratory analyses with carefully documented exposures and measures of internal dose in epidemiologic studies. It also highlights the usefulness of interphase cytogenetics and FISH for the rapid and sensitive detection of aneuploidy in exposed human populations. The recent studies from Scandinavia and Italy (*34,35*), which show a strong link between chromosome aberrations and future cancer risk, especially hematopoietic malignancy (*35*), support the further development and application of FISH to rapidly detect chromosome aberrations in populations exposed to potential chromosome-damaging agents. Furthermore, by using FISH to characterize a large number of cells and to selectively study aberrations of clinical importance, it may be possible in the future to estimate an individual's risk for hematologic and perhaps other cancers. FISH is therefore likely to have an increasingly important role in studies of cancer etiology.

REFERENCES

1. Aksoy M. Benzene carcinogenicity. In: Benzene Carcinogenicity (Aksoy M, ed). Boca Raton, FL:CRC Press, 1988;113–151.
2. IARC. Benzene (Group 1). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol 42, Suppl 7. Lyon:International Agency for Research on Cancer, 1987;120–122.
3. Dean BJ. Recent findings on the genetic toxicology of benzene, toluene, xylenes and phenols. Mutat Res 154:153–181 (1985).

4. Reddy MV, Schultz SC, Blackburn GR, Mackerer CR. Lack of DNA adduct formation in mice treated with benzene. Mutat Res 325:149–155 (1994).
5. Waters MD, Stack HF, Brady AL, Lohman PH, Haroun L, Vainio H. Use of computerized data listings and activity profiles of genetic and related effects in the review of 195 compounds. Mutat Res 205:295–312 (1988).
6. Rothman N, Haas R, Hayes RB, Li GL, Wiemels J, Campleman S, Quintana PJ, Xi LJ, Dosemeci M, Titenko-Holland N, Meyer KB, Lu W, Zhang L, Bechtold W, Wang YZ, Kolachana P, Yin SN, Blot W, Smith MT. Benzene induces gene-duplicating but not gene-inactivating mutations at the glycophorin A locus in exposed humans. Proc Natl Acad Sci USA 92:4069–73 (1995).
7. 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 23:137–42 (1994).
8. Sasiadek M, Jagielski J. Genotoxic effects observed in workers occupationally exposed to organic solvents. Pol J Occup Med 3:103–108 (1990).
9. 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 304:159–165 (1994).
10. Turkel B, Egeli U. Analysis of chromosomal aberrations in shoe workers exposed long term to benzene. Occup Environ Med 51:50–53 (1994).
11. Sasiadek M. Nonrandom distribution of breakpoints in the karyotypes of workers occupationally exposed to benzene. Environ Health Perspect 97:255–257 (1992).
12. Forni A, Moreo L. Cytogenetic studies in a case of benzene leukaemia. Eur J Cancer 3:251–255 (1967).
13. Forni A. Chromosome changes and benzene exposure. A review. Rev Environ Health 3:5–17 (1979).
14. Erdogan G, Aksoy M. Cytogenetic studies in thirteen patients with pancytopenia and leukaemia associated with long-term exposure to benzene. N Istanbul Contrib Clin Sci 10:230–247 (1973).
15. Aksoy M, Erdem S, Erdogan G, Dincol G. Acute leukaemia in two generations following chronic exposure to benzene. Hum Hered 24:70–74 (1974).
16. Sellyei M, Keleman E. Chromosome study in a case of granulocytic leukaemia with 'Pelgerisation' 7 years after benzene pancytopenia. Eur J Cancer 7:83–85 (1971).
17. Erdogan G, Aksoy M. Cytogenetic studies in 20 patients with pancytopenia and leukaemia with long-term exposure to benzene. In: European and African Division International Society of Hematology, 3rd Meeting, 24–28 August 1975, London.
18. Rowley JD, Blaisdell RK, Jacobson LO. Chromosome studies in preleukemia. I. Aneuploidy of group C chromosomes in three patients. Blood 27(6):782–799 (1966).
19. Winkelstein A, Sparkes RS, Craddock CG. Trisomy of group C in a myeloproliferative disorder. Report of case. Blood 27:722–33 (1966).
20. Sandberg AA, Ishihara T, Crosswhite LH. Group C-trisomy in myeloid metaplasia with possible leukaemia. Blood 24:716–25 (1964).
21. Gray JW, Pinkel D. Molecular cytogenetics in human cancer diagnosis. Cancer 69:1536–1542 (1992).
22. Pinkel D, Landegent J, Collins C, Fuscoe J, Segraves R, Lucas J, Gray J. Fluorescence *in situ* hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. Proc Natl Acad Sci USA 85:9138–9142 (1988).
23. Willard H, Waye J. Hierarchial order in chromosome specific human alpha satellite DNA. Trend Genet 3:192–198 (1987).
24. Zhang L, Venkatesh P, Creek ML, Smith MT. Detection of 1,2,4-benzenetriol induced aneuploidy and microtubule disruption by fluorescence *in situ* hybridization and immunocytochemistry. Mutat Res 320:315–327 (1994).
25. Eastmond DA, Rupa DS, Hasegawa LS. Detection of hyperdiploidy and chromosome breakage in interphase human lymphocytes following exposure to the benzene metabolite hydroquinone using multicolor fluorescence *in situ* hybridization with DNA probes. Mutat Res 322:9–20 (1994).
26. Rothman N, Li G-L, Dosemeci M, Bechtold W, Marti GE, Wang Y-Z, Linet M, Xi L-Q, Lu W, Smith MT, Titenko-Holland N, Zhang L, Blot W, Yin S, Hayes R. Hematotoxicity among Chinese workers heavily exposed to benzene. Am J Ind Med 29:236–246 (1996).
27. Bechtold WE, Willis JK, Sun JD, Griffith WC, Reddy TV. Biological markers of exposure to benzene: S-phenylcysteine in albumin. Carcinogenesis 13:1217–1220 (1992).
28. Eastmond DA, Pinkel D. Detection of aneuploidy and aneuploidy-inducing agents in human lymphocytes using fluorescence *in situ* hydridization with chromosome-specific DNA probes. Mutat Res 234:303–318 (1990).
29. Yardley-Jones A, Anderson D, Parke DV. The toxicity of benzene and its metabolism and molecular pathology in human risk assessment. Br J Ind Med 48:437–444 (1991).
30. Zhang L, Robertson ML, Kolachana P, Davison AJ, Smith MT. Benzene metabolite, 1,2,4-benzenetriol, induces micronuclei and oxidative DNA damage in human lymphocytes and HL60 cells. Environ Mol Mutagen 21:339–348 (1993).
31. Yager JW, Eastmond DA, Robertson ML, Paradisin WM, Smith MT. Characterization of micronuclei induced in human lymphocytes by benzene metabolites. Cancer Res 50:393–399 (1990).
32. Irons RD, Neptune DA. Effects of the prinicipal hydroxy-metabolites of benzene on microtubule polymerization. Arch Toxicol 45:297–305 (1980).
33. Irons RD, Pfeifer RW, Aune TM, Pierce CW. Soluble immune response suppressor (SIRS) inhibits microtubule function *in vivo* and microtubule assembly *in vitro*. J Immunol 133:2032–2036 (1984).
34. Hagmar L, Brogger A, Hansteen IL, Heim S, Hogstedt B, Knudsen L, Lambert B, Linnainmaa K, Mitelman F, Nordenson I, Reuterwall C, Salomaa S, Skerfving S, Sorsa M. Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage. Cancer Res 54:2919–2922 (1994).
35. Bonassi S, Abbondandolo A, Camurri L, Dal Pra L, De Ferrari M, Degrassi F, Forni A, Lamberti L, Lando C, Padovani P, Sbrana I, Vecchio D, Puntoni R. Are chromosome aberrations in circulating lymphocytes predictive of future cancer onset in humans? Preliminary results of an Italian cohort study. Cancer Genet Cytogenet 79:133–135 (1995).