

## Increased aneusomy and long arm deletion of chromosomes 5 and 7 in the lymphocytes of Chinese workers exposed to benzene

Luoping Zhang, Nathaniel Rothman<sup>1</sup>, Yunxia Wang, Richard B. Hayes<sup>1</sup>, Guilan Li<sup>2</sup>, Mustafa Dosemeci<sup>1</sup>, Songnian Yin<sup>2</sup>, Prema Kolachana, Nina Titenko-Holland and Martyn T. Smith<sup>3</sup>

Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA 94720-7360, <sup>1</sup>Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD 20892, USA and <sup>2</sup>Institute of Occupational Medicine, Chinese Academy of Preventive Medicine, Beijing 100051, People's Republic of China

<sup>3</sup>To whom correspondence should be addressed  
Email: martynts@uclink4.berkeley.edu

Two of the most common cytogenetic changes in therapy- and chemical-related leukemia are the loss and long (q) arm deletion of chromosomes 5 and 7. The detection of these aberrations in lymphocytes of individuals exposed to potential leukemogens may serve as useful biomarkers of increased leukemia risk. We have used a novel fluorescence *in situ* hybridization (FISH) procedure to determine if specific aberrations in chromosomes 1, 5 and 7 occur at an elevated rate in the blood cells of workers exposed to benzene. Forty-three healthy workers exposed to a wide range of benzene concentrations (median 31 p.p.m., 8 h time-weighted average) and 44 unexposed controls from Shanghai were studied. Whole blood was cultured and metaphase spreads were harvested at 72 h. Benzene exposure was associated with increases in the rates of monosomy 5 and 7 but not monosomy 1 ( $P < 0.001$ ,  $P < 0.0001$  and  $P = 0.94$ , respectively) and with increases in trisomy and tetrasomy frequencies of all three chromosomes. Long arm deletion of chromosomes 5 and 7 was increased in a dose-dependent fashion ( $P = 0.014$  and  $P < 0.0001$ ) up to 3.5-fold in the exposed workers. These results demonstrate that leukemia-specific changes in chromosomes 5 and 7 can be detected by FISH in the peripheral blood of otherwise healthy benzene-exposed workers. We suggest that aberrations in chromosomes 5 and 7 may be useful biomarkers of early biological effect for benzene exposure.

### Introduction

Benzene is an established human leukemogen which causes aplastic anemia, acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) in exposed workers (1-3). Classical cytogenetics have shown that benzene exposure is also associated with increased levels of chromosomal aberrations in the peripheral blood lymphocytes of exposed workers (4-6). Recent studies have shown that chromosome aberrations are associated with increased cancer risk (7,8), especially for increased mortality from hematological malignancies (8). This finding provides substantial support for the use of chromosome

**Abbreviations:** AML, acute myeloid leukemia; DAPI, 4,6-diamino-2-phenylindole; FISH, fluorescence *in situ* hybridization; MDS, myelodysplastic syndromes; TWA, time-weighted average.

aberrations as biomarkers of early effect for benzene and other potential leukemogens. We reasoned that this approach could be improved in two ways: (i) by detecting disease-specific aberrations; (ii) by using fluorescence *in situ* hybridization (FISH) so that many more metaphases could be analyzed, thereby increasing statistical power.

Specific chromosome aberrations are the hallmark of human leukemia (9-11). The loss or gain of specific chromosomes (such as monosomy 7 and trisomy 8) is commonly observed in AML and MDS, as are specific chromosome translocations, inversions and deletions (10). The loss of chromosomes 5 and 7 (-5 and -7) and their long-arm deletions [del(5q) and del(7q)] are the two most common changes in therapy-related AML and MDS, especially among patients previously treated with alkylating agents (12). These specific chromosome aberrations are also more common among leukemia patients with previous exposure to chemical solvents, including chronic exposure to benzene, insecticides, petroleum, etc. (13,14). For example, one recent study found an association between -7 and del(7q) and previous exposure to paints (odds ratio 7.5) (14). Monosomy 7 was also found in 100% of the bone marrow cells of one benzene-induced MDS case (15). These findings suggest that both therapeutic and occupational chemical exposure may induce the loss of chromosomes 5 and 7 and their long arm deletions, which may lead to leukemia.

To determine whether benzene exposure could potentially induce these specific aberrations in chromosomes 5 and 7, we recently performed an *in vitro* study using a novel FISH procedure which allows for the simultaneous detection of aneusomy and long arm deletions in chromosomes 5 and 7. We observed that the benzene metabolites hydroquinone and 1,2,4-benzenetriol increased the frequencies of these specific chromosome aberrations in cultured human lymphocytes (16). In the current study, we have applied the same FISH methodology to demonstrate that the loss and long arm deletions of chromosomes 5 and 7 occur at an elevated rate in peripheral lymphocytes of healthy benzene-exposed workers. We hypothesize that these specific chromosome aberrations may provide improved markers of early effect and risk of leukemia from benzene exposure.

### Materials and methods

#### Subject enrollment

Identification of factories and enrollment of study subjects has previously been described in detail by Rothman *et al.* (17). Biological samples were collected from 44 healthy workers currently exposed to benzene with minimal exposure to toluene and other aromatic solvents in Shanghai, China, in October, 1992. The same number of healthy controls without current or previous occupational exposure to benzene were enrolled from factories in the same geographic area. Controls were frequency-matched to the exposed subjects by gender and age (5 year intervals). Exclusion criteria for all subjects were history of cancer, therapeutic radiation, chemotherapy or current pregnancy. Factories for exposed subjects were selected so that the study population would have a wide range of exposures to benzene similar to exposure patterns in a larger cohort study (3,18).

The protocol was explained to all potential participants and informed

Table I. Demographic characteristics by exposure category

	Age	Cigarettes/day		Alcoholic drinks/week
		Males (23)	Females (21)	
Controls <sup>a</sup> (n = 44)	35.4 ± 7.3 <sup>b</sup>	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

<sup>a</sup>Controls were frequency matched to exposed subjects on age and gender.

<sup>b</sup>Mean ± SD.

consent was obtained using Institutional Review Board approved procedures. Each subject was administered a questionnaire by a trained interviewer. Data collected included age, gender, current and lifelong tobacco use, current alcohol consumption, medical history and work history. Height and weight of each subject were measured. The demographic characteristics of the study subjects are described in Table I. Peripheral blood was obtained by phlebotomy from study subjects; blood from one person could not be successfully cultured and results are reported here for 87 subjects.

#### Exposure assessment

Individual exposure was monitored by organic vapor passive dosimetry badges (3M no. 3500; 3M, St Paul, MN), which were worn by each worker for a full work shift on five separate days during the 1–2 week period prior to phlebotomy. Badges were analyzed by gas chromatography with flame ionization detection. An 8 h time-weighted average (TWA) exposure was calculated for benzene as the geometric mean of the five air measurements. Current exposures to benzene were confirmed by the analysis of phenol and other metabolites in urine (17). A detailed assessment of factory records and operations showed that no other known marrow-toxic chemicals or physical agents were present in these workplaces. Historical benzene exposure during subjects' employment at the study factories was estimated by trained field personnel using work histories obtained by interview, company employment records, benzene area measurements obtained from factory records, information on benzene use in the factory, ventilation practices and current individual exposure levels. Cumulative exposure to benzene was calculated by multiplying the historical time-specific exposure estimates and the duration worked. All exposure assessment was performed blind with respect to chromosomal analysis.

#### Blood cell cultures and slide preparation

Whole blood collected with the anticoagulant heparin was cultured in RPMI-1640 medium supplemented with 15% fetal bovine serum, 1% penicillin/streptomycin, 1% L-glutamine (Gibco BRL, Grand Island, NY) and 1% phytohemagglutinin-P (Pharmacia, Piscataway, NJ). Blood cells were incubated at 37°C in a 5% CO<sub>2</sub> moist atmosphere and harvested 72 h after culture initiation. In order to obtain a sufficient number of metaphase spreads, colcemid (0.1 µg/ml; Gibco BRL) was added to each culture 4 h before harvesting. 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 pre-labeled glass slides, allowed to air dry and stored at -20°C under a nitrogen atmosphere. Prepared slides were later shipped on dry ice to the USA.

#### Fluorescence in situ hybridization

The FISH methodology used to simultaneously measure numerical and structural changes in chromosomes 1, 5 and 7 in metaphase spreads has recently been described in detail (16). Briefly, four different types of DNA probes were used for this triple color FISH analysis: a biotinylated human centromeric probe (α-satellite) for chromosomes 1 (D1Z7), 5 (D5Z2) and 19 (D19Z3); a digoxigenin-labeled centromeric probe specific for chromosome 1 (α-satellite, D1Z5); a unique sequence biotinylated DNA probe specific for chromosome 5q31; a digoxigenin-labeled chromosome 7 probe which targets both its telomere (7q36-qter) and centromere (α-satellite, D7Z1). All probes were purchased from Oncor (Gaithersburg, MD). Since several different types of probes were used, a sequential hybridization procedure was performed to obtain an optimal view of each target. After detection and amplification (where necessary) of the hybridization signals, the biotin-labeled probe was stained green (FITC) and the digoxigenin-labeled probe red (rhodamine). The mixture of both was observed as yellow. Thus, the centromeres of chromosomes 1, 5 and 7 were stained yellow, green and red, respectively. The long arms of chromosomes 5 (5q31) and 7 (7q-telomere) were also detected in the same color as their centromeres. All chromosomes were counterstained with a blue fluorescent dye, 4,6-diamino-2-phenylindole (DAPI) (0.1 µg/ml; Sigma, St

Louis, MO), prepared in mounting medium (Vector, Burlingame, CA). The hybridization signals were viewed under a Nikon fluorescence microscope equipped with epifluorescent illumination and a 100× oil immersion lens. A triple bandpass filter for DAPI/FITC/Texas red (excitation at 405, 490 and 570 nm, emission at 460, 525 and 635 nm) was used for multicolor staining of metaphase FISH probes. The specific aberrations of chromosomes 5 and 7, such as aneusomy, q arm deletions, breaks and translocations, were detected as described in Zhang et al. (16). The total number of scored metaphase cells in this study was 30 200, and the average number per subject was 347, but at least 200 cells were scored per subject. Metaphase cells were considered scorable if they met specific criteria (16).

#### Statistical analyses

Study subjects (n = 87) were divided into three groups: controls (n = 44), workers exposed to ≤31 p.p.m. benzene (n = 21), the median exposure level, and workers exposed to >31 p.p.m. (n = 22). Workers in the lower exposure group (≤31 p.p.m.) had a median benzene exposure level of 13.6 p.p.m. and range of 1.6–30.6 p.p.m. and those in the higher exposure group (>31 p.p.m.) had a median exposure level of 91.9 p.p.m. and range of 31.5–328.5 p.p.m. We have previously shown that these exposure categories are strongly correlated with urinary benzene metabolite levels and various measures of hematotoxicity (19). Summary data for relatively frequently occurring aberrations of chromosomes 1, 5 and 7 within these exposure groups (i.e. aneusomies, long arm deletions and total structural changes of chromosomes 5 and 7 and centromere breakage of chromosome 1) are presented as the mean of the number of aberrations per 100 metaphases scored for each subject. Differences between controls and all exposed workers combined were tested by the Wilcoxon rank sum test. Linear regression was used to compare aberration frequencies between controls and lower exposed workers and controls and higher exposed workers and to calculate a test for trend. For these analyses, a natural log transformation was used to normalize each outcome and the original matching variables, age and gender, were included in each model. There was no evidence of confounding by current or previous tobacco use, alcohol use or body mass index and these were excluded from the final models. Because several structural aberrations of chromosomes 5 and 7 (i.e. long arm breaks and translocations) occurred one to two orders of magnitude less frequently than other aberrations evaluated, data were pooled for all subjects within each exposure category. Summary data are presented as the total number of events detected over the total number of metaphases scored within each group and a test for trend was performed using exact methods.

## Results

### Monosomy of chromosomes 1, 5 and 7

Aneusomies of chromosomes 1, 5 and 7 were detected using the FISH assay by examining the target signals on each centromere. The frequencies of monosomy 5 and 7, but not of 1, were increased in benzene-exposed workers. The monosomy rates in total exposed workers were compared with controls using the Wilcoxon rank sum test. In comparison with controls (n = 44), the mean frequency (%) of monosomy 5 in the total exposed group (n = 43) was increased from 1.63 to 2.74 (P < 0.001) and of monosomy 7 from 2.72 to 4.97 (P < 0.01). There was no difference in monosomy 1 between controls (mean 3.66) and exposed subjects (mean 3.53). In order to determine whether the increased frequencies of monosomy 5 and 7 were correlated with the increased exposure levels, we divided the exposed category into two groups at the median exposure level (31 p.p.m., 8 h TWA). In the lower exposure group (≤31 p.p.m.), the rate of monosomy 5 was significantly increased (P < 0.01) over the controls but the monosomy 7 rate was not (Figure 1). In the higher exposure group (>31 p.p.m.), monosomy 5 and 7 were both significantly increased (P < 0.001). Again, there was no difference in monosomy 1 between either the lower or higher exposure groups and controls (Figure 1). A test for trend was performed by linear regression on natural log transformed data which was adjusted for age and gender. Significant trends were observed for both monosomy 5 (P < 0.001) and 7 (P < 0.0001) but not for 1 (Figure 1).

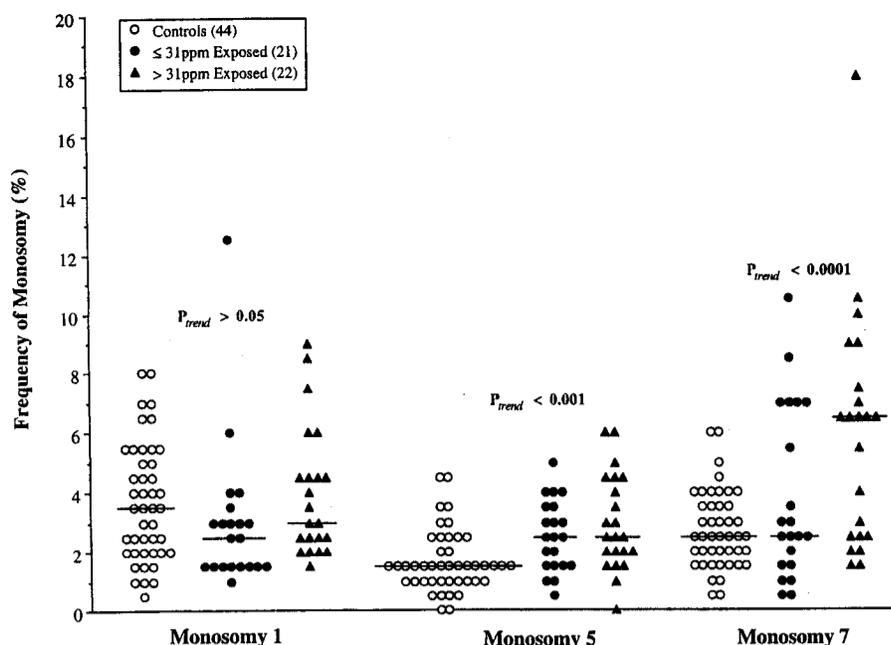


Fig. 1. Frequencies of monosomy 1, 5 and 7 in the peripheral blood lymphocytes of workers exposed to benzene and unexposed controls. Individual values for each study subject are presented and the median is shown as a horizontal bar. The three exposure categories are: controls (○), workers exposed to  $\leq 31$  p.p.m. (●) and to  $> 31$  p.p.m. (▲) benzene. A natural log transformation was used to normalize each outcome and a test for trend was performed by linear regression, controlling for age and gender, the original matching variables. A statistically significant increasing trend was observed for monosomy 5 ( $P_{\text{trend}} < 0.001$ ) and 7 ( $P_{\text{trend}} < 0.0001$ ) but not for monosomy 1 ( $P_{\text{trend}} > 0.05$ ).

#### Trisomy of chromosomes 1, 5 and 7

Unlike monosomy, the frequencies of trisomy 1 as well as 5 and 7 were all significantly increased in benzene-exposed workers. Compared with controls ( $n = 44$ ), the mean frequency of trisomy 1 in all exposed workers ( $n = 43$ ) was increased from 0.27 to 0.53 ( $P < 0.01$ ), of trisomy 5 from 0.51 to 0.88 ( $P < 0.05$ ) and of trisomy 7 from 0.87 to 1.66 ( $P < 0.001$ ). These increases in trisomy were detected especially in the  $> 31$  p.p.m. group (Figure 2). In the  $\leq 31$  p.p.m. group, frequencies of trisomy 1 and 5 were not significantly increased, whereas trisomy 7 showed an increase of borderline significance ( $P = 0.05$ ). Significant trends were also observed for chromosomes 1 ( $P < 0.001$ ), 5 ( $P < 0.01$ ) and 7 ( $P < 0.0001$ ).

#### Tetrasomy of chromosomes 1, 5 and 7

As with trisomy, the frequencies of tetrasomy 1, 5 and 7 were all significantly increased in benzene-exposed workers. In comparison with controls, the mean frequency of tetrasomy 1 was increased from 0.47 to 0.74 ( $P < 0.01$ ), of tetrasomy 5 from 0.49 to 0.72 ( $P < 0.05$ ) and of tetrasomy 7 from 0.51 to 0.79 ( $P < 0.05$ ). The frequency of tetrasomy in the  $\leq 31$  p.p.m. group was not significantly increased for any of the three chromosomes (Table II). Tetrasomy was increased in the  $> 31$  p.p.m. group for chromosomes 1 ( $P < 0.01$ ), 5 ( $P < 0.01$ ) and 7 ( $P < 0.05$ ) (Table II). Significant trends were observed for chromosomes 1, 5 and 7 ( $P < 0.05$ ). Interestingly, all three chromosomes have very similar tetrasomy frequency levels in each category (controls, lower exposure and higher exposure) (Table II), whereas trisomy rates are quite different from one chromosome to another (Figure 2). In fact, most of the tetrasomies we detected ( $> 93\%$ ) were tetraploidy rather than real tetrasomies, which means that four copies of each chromosome were present in the same cell. The tetraploidy rates were estimated from tetrasomy data in each category (Table II). Thus, increased tetraploidy was

the primary mechanism of tetrasomy induction in the benzene-exposed workers, especially in the  $> 31$  p.p.m. group.

#### Long arm deletions of chromosomes 5 and 7

Long arm deletions were detected as the loss of target signals at 5q31 and 7q36-pter. Table III shows the long arm deletions in chromosomes 5 and 7 in benzene-exposed workers and their matched controls. Although the frequency of del(5q) was more than doubled in the total exposed workers ( $n = 43$ ) (0.73) above the control level (0.30), the increase was not significant. The del(7q) rate, on the other hand, was significantly increased from 1.41 to 3.85 ( $P < 0.0001$ ). Similarly, the  $\leq 31$  p.p.m. group showed an increase in del(7q) ( $P < 0.05$ ) but not in del(5q) (Table III). In the  $> 31$  p.p.m. group, both rates of del(5q) and del(7q) were increased ( $P < 0.01$  and 0.0001, respectively). Significant trends were observed for both del(5q) and del(7q) ( $P < 0.001$  and 0.0001, respectively), with the latter showing a slightly stronger trend. The overall increase in both del(5q) and del(7q) was 3.5-fold in the  $> 31$  p.p.m. category (Table III).

#### Other structural changes on chromosomes 1, 5 and 7

In addition to the deletions, long arm breaks and translocations between chromosomes 5 and 7 and other chromosomes, breaks in the centromere of chromosome 1 could also be detected by the FISH assay employed here. The sums of all structural chromosome alterations for chromosomes 5 and 7 are also shown in Table III. In chromosome 5, the only significant increase in total structural aberrations was in the  $> 31$  p.p.m. group and a significant trend ( $P < 0.001$ ) was observed (Table III). In chromosome 7, however, there were significant increases over controls in both the  $\leq 31$  p.p.m. ( $P = 0.05$ ) and  $> 31$  p.p.m. ( $P > 0.0001$ ) exposure groups. Again, the test for trend showed a significant dose-response effect ( $P < 0.0001$ ) (Table III). Together, the total structural aberration rate of

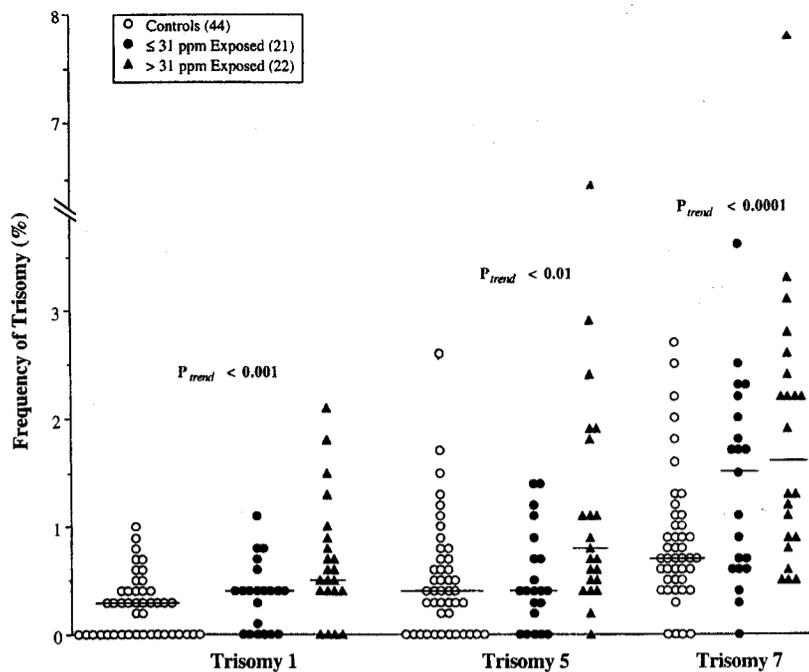


Fig. 2. Frequencies of trisomy 1, 5 and 7 in the peripheral blood lymphocytes of workers exposed to benzene, and unexposed controls. Individual values for each study subject are presented and the median is shown as a horizontal bar. The three exposure categories are: controls (○), workers exposed to  $\leq 31$  p.p.m. (●) and to  $> 31$  p.p.m. (▲) benzene. A natural log transformation was used to normalize each outcome and a test for trend was performed by linear regression, controlling for age and gender, the original matching variables. A statistically significant increasing trend was seen for trisomy of all three chromosomes (1,  $P_{trend} < 0.001$ ; 5,  $P_{trend} < 0.01$ ; 7,  $P_{trend} < 0.0001$ ).

Table II. Frequency of tetrasomy and tetraploidy in benzene-exposed workers

Benzene exposure (n)	Chromosome 1	Chromosome 5	Chromosome 7	Estimated tetraploidy <sup>a</sup>
Controls (44)	0.47 $\pm$ 0.06 <sup>b</sup>	0.49 $\pm$ 0.07	0.51 $\pm$ 0.06	0.45
$\leq 31$ p.p.m. (21)	0.69 $\pm$ 0.15	0.66 $\pm$ 0.14	0.71 $\pm$ 0.16	0.65
$> 31$ p.p.m. (22)	0.79 $\pm$ 0.11 <sup>c</sup>	0.77 $\pm$ 0.11 <sup>c</sup>	0.87 $\pm$ 0.13 <sup>c</sup>	0.75

<sup>a</sup>Tetraploidy was estimated from tetrasomy data because  $>93\%$  observed tetrasomies were tetraploidy.

<sup>b</sup>Frequency (%) as mean  $\pm$  SE.

<sup>c</sup>Trend test by linear regression on natural log transformed data, adjusted for age and sex:  $P < 0.05$ .

Table III. Structural alterations of chromosomes 5 and 7 in benzene-exposed workers

Benzene exposure (n)	Long arm deletion	Total structural chromosomal aberrations <sup>a</sup>
Chromosome 5		
Controls (44)	0.30 $\pm$ 0.05 <sup>b</sup>	0.31 $\pm$ 0.05
$\leq 31$ p.p.m. (21)	0.40 $\pm$ 0.21	0.40 $\pm$ 0.21
$> 31$ p.p.m. (22)	1.05 $\pm$ 0.23 <sup>c</sup>	1.20 $\pm$ 0.27 <sup>c</sup>
Chromosome 7		
Controls (44)	1.41 $\pm$ 0.15	1.54 $\pm$ 0.16
$\leq 31$ p.p.m. (21)	2.79 $\pm$ 0.50	2.94 $\pm$ 0.54
$> 31$ p.p.m. (22)	4.86 $\pm$ 0.78 <sup>d</sup>	5.52 $\pm$ 0.98 <sup>d</sup>

<sup>a</sup>Deletions, breaks and translocations.

<sup>b</sup>Frequency (%) as mean  $\pm$  SE.

<sup>c,d</sup>Trend test by linear regression on natural log transformed data, adjusted for age and sex: <sup>c</sup> $P < 0.001$ ; <sup>d</sup> $P < 0.0001$ .

chromosome 7 was increased from 1.54 in controls to 4.26 ( $P < 0.0001$ ) in the exposed workers. These results are similar to those for long arm deletions shown in Table III, because long arm deletions are the major form ( $>85\%$ ) of structural chromosome aberrations detected in this study.

Although rare events, long arm translocations between chromosomes 5 and 7 and other chromosomes were increased only in the  $>31$  p.p.m. group, from 0 to 0.04 in chromosome 5 ( $P_{trend} < 0.05$ ) and from 0.07 to 0.25 in chromosome 7 ( $P_{trend} < 0.01$ ). The only significant increase in long arm breaks was in chromosome 5 among the  $>31$  p.p.m. group (from 0.01 to 0.07,  $P_{trend} < 0.05$ ). In chromosome 7, long arm breaks were elevated from 0.02 in controls to 0.04 in the  $>31$  p.p.m. group, but not significantly.

In the FISH assay used here breakage at the centromere of chromosome 1 could also be detected because the centromere of this chromosome was targeted with two different color probes at adjacent loci (20). Our results showed that there was no difference in frequencies of centromere breaks on chromosome 1 between controls (0.10  $\pm$  0.03, mean  $\pm$  SE) and exposed workers (0.16  $\pm$  0.05). Similarly, there was no significant increase between either the  $\leq 31$  p.p.m. (0.12  $\pm$  0.05) or  $>31$  p.p.m. (0.19  $\pm$  0.09) exposure groups and controls, although a modest trend and almost 2-fold increase was observed.

## Discussion

The measurement of chromosome aberrations in peripheral blood lymphocytes has been used for many years to monitor human populations exposed to carcinogens or potential carcinogens. For example, there are numerous studies in the literature which show that human exposure to the leukemogen benzene increases the level of chromosome aberrations in lymphocytes

detected by conventional methods (4,5,21,22). Recent reports have confirmed the validity of this approach by demonstrating that elevated levels of chromosome aberrations in peripheral blood lymphocytes are associated with subsequent increased cancer risk, especially for increased mortality from hematological malignancies, including AML (7,8).

We reasoned that conventional chromosome analysis could be improved upon in two ways: (i) by detecting disease-specific aberrations; and (ii) by using FISH so that many more metaphases could be analyzed, thereby increasing statistical power. The data presented here clearly show that exposure to benzene is associated with a marked increase in the loss and long arm deletions of chromosomes 5 and 7 [-5, -7, del(5q) and del(7q)] in workers' lymphocytes. These specific chromosome changes are known to be associated with the development of AML, especially subsequent to chemical exposure (12,14,23). However, they will not confer the same selective growth advantage on lymphoid cells as they do on myeloid cells. The presence of AML-specific chromosomal changes in lymphocytes in non-diseased individuals therefore reflects the rate at which these events are being produced by genotoxic insults. Further, since lymphocytes have a much longer lifespan than granulocytes, the production of aberrations in the former is cumulative over a period of weeks to months. Quantitation of AML-specific aberrations in peripheral blood lymphocytes may therefore provide a measure of damage and leukemia risk incurred by exposure to genotoxic agents such as benzene, radiation and the environment in general.

The present study utilized FISH to analyze metaphase spreads with a novel combination of probes. Approximately 500 cell equivalents were scored, which allowed us to detect several AML-specific aberrations at an increased rate in a relatively small, but highly exposed, population of 43 exposed workers and 44 matched controls. An even larger number of cells could have been scored by interphase cytogenetics, but this method is difficult to use to detect monosomy or loss of specific chromosome regions because of artifacts associated with probe overlap and hybridization. Interphase cytogenetics can and has been used, however, to measure chromosome gain, such as trisomy 9 in human cells treated with benzene metabolites (20,24). Indeed, in this same population we have previously reported that benzene exposure was associated with increased levels of trisomy of chromosome 9 in workers' lymphocytes (25).

Deletions in 5q and 7q were the major structural aberrations observed in chromosomes 5 and 7 both in controls and benzene-exposed workers. While very few translocations and breaks were detected, the FISH method employed was not designed to detect these structural changes readily. The effect of benzene exposure in elevating del(5q) and del(7q) appeared to be approximately equal at ~3-fold. However, the effects of benzene exposure on del(7q) might actually be greater than on del(5q), because the FISH method employed here may underestimate AML-related damage to 7q. This is because the majority of del(5q) and del(7q) in myeloid leukemias contain deletions of either band 5q31 or band 7q22 (26,27). Small interstitial deletions would be detected in 5q using the 5q31 probe employed here, but would not be detected at the 7q22 or 7q34 bands by the 7q36-qter probe. On the other hand, it is possible that 7q22 deletions are overestimated because terminal breakage could occur between 7q23 and 7qter that would appear as a probe deletion. Further studies are planned

using a new YAC probe specific for 7q22 to examine effects at this location in greater detail.

In addition to long arm deletions of chromosomes 5 and 7, increased monosomy of these two chromosomes was also clearly associated with benzene exposure, with monosomy 7 being increased more than that of chromosome 5. We also measured monosomy of chromosome 1 and breakage at the chromosome 1 centromere in metaphase spreads from the exposed workers and matched controls. Breakage at the centromere of chromosome 1 has been proposed as a potential marker of chromosome damage by benzene and other agents (20,28). In the present study, the increase in centromere breaks observed in chromosome 1 was not statistically significant, although there was a trend to higher values in the exposed groups. These results suggest that centromere 1 breakage is not likely to be a sensitive biomarker of early effect for benzene. Further, although the background rate of monosomy 1 was higher than that of chromosomes 5 and 7, no significant difference in monosomy of chromosome 1 was observed in benzene-exposed workers compared with controls. Conversely, high levels of benzene exposure were associated with approximately equal 2.5-fold increases in trisomy of all three chromosomes 1, 5 and 7, even though the background rates in unexposed controls were different ( $7 > 5 > 1$ ). The selective effects of benzene, therefore, apparently apply only to monosomy induction and perhaps deletion and translocation, but the reasons for this apparent selectivity are unclear. One might speculate, however, that benzene causes telomere elimination to a greater extent on some chromosomes than on others and that this selective telomere elimination then leads to selective chromosome loss (29). Alternatively, the spatial positioning of the chromosomes during prometaphase may be disrupted by benzene exposure, leading to selective chromosome loss (30).

Using conventional methods, Sasiadek previously reported that the effects of benzene on human lymphocyte chromosomes are not random, with selective changes being induced in chromosomes 2, 4 and 7 (6). She reported that the long arm of chromosome 7 was especially sensitive to deletions induced by benzene. Further, Mamuris *et al.* examined lymphocyte metaphase spreads from a series of patients treated with melphalan, an alkylating agent known to cause secondary AML (31-33). They found that some chromosomes were far more affected than others, including chromosomes 5, 7, 11 and 17 (31). They also reported that melphalan produced selective effects on chromosome regions rearranged in secondary leukemia, including those on 5 and 7, in cultured cells *in vitro* (32). We recently demonstrated that the benzene metabolites hydroquinone and 1,2,4-benzenetriol are able to produce AML-specific aberrations in chromosomes 5 and 7 in human lymphocytes cultured *in vitro* as whole blood (16). Significantly more hydroquinone than 1,2,4-benzenetriol is formed from benzene metabolism and, thus, there is a need to test additional benzene metabolites for their ability to cause specific aberrations. Our findings to date especially implicate hydroquinone and its subsequent oxidation to benzoquinone as the potential cause of the aberrations observed in the benzene-exposed workers. The fact that benzene hematotoxicity is associated with lack of functional NAD(P)H:quinone oxidoreductase activity, which reduces benzoquinone back to hydroquinone, further suggests that hydroquinone/benzoquinone play a central role in benzene-induced leukemogenesis (34,35). The development of benzene-induced AML may

therefore progress in a similar fashion to that induced by chemotherapeutic drugs, with changes on chromosomes 5 or 7 playing a key early role. The results described here and our *in vitro* findings (16) suggest that this is likely to be at least one pathway in benzene-induced leukemogenesis.

Tetrasomy of chromosomes 1, 5 and 7 was also increased significantly in the benzene-exposed workers. The effect was smaller than that on trisomy, being only 1.5-fold, but was again approximately equal on all three chromosomes. We calculated that the majority (>93%) of this increased tetrasomy could be accounted for as polyploidy. The link between benzene exposure and tetraploidy in previous studies has been controversial (36–39), but the data presented here suggest that moderate polyploidy induction is an effect associated with benzene exposure.

In conclusion, we have shown that exposure to benzene is associated with a marked increase in the loss and long arm deletions of chromosomes 5 and 7 [–5, –7, del(5q) and del(7q)] in workers' lymphocytes. This implicates these specific changes in at least one pathway in benzene-induced leukemogenesis. Further, it suggests that detection of these specific changes in chromosomes 5 and 7 by metaphase FISH analysis may provide an innovative approach to determining the risk posed by exposure to benzene and other potential leukemogens.

### Acknowledgements

The authors are indebted to the study subjects and the staff of the Chinese Academy of Preventive Medicine, Beijing and the Shanghai Hygiene and Anti-Epidemic Center, Shanghai, China, for their assistance and participation in this study. We gratefully acknowledge the technical assistance of Pravina Venkatesh and Weihong Guo and thank Dr Robert E.Tarone (NCI) for expert statistical advice. This study was supported by funds from the National Cancer Institute and by grants RO1 ES06721, P42 ES04705 and P30 ES01896 from the National Institute of Environmental Health Sciences and the California Environmental Protection Agency (to M.T.S.).

### References

- Aksoy, M. (1988) *Benzene Carcinogenicity*. CRC Press, Boca Raton, FL.
- Yin, S.N., Hayes, R.B., Linet, M.S. et al. (1996) An expanded cohort study of cancer among benzene-exposed workers in China. Benzene Study Group. *Environ. Health Perspect.*, **104** (suppl. 6), 1339–1341.
- Hayes, R.B., Yin, S.N., Dosimeci, M., Li, G.L., Wacholder, S., Travis, L.B., Li, C.Y., Rothman, N., Hoover, R.N. and Linet, M.S. (1997) Benzene and the dose-related incidence of hematologic neoplasms in China. Chinese Academy of Preventive Medicine–National Cancer Institute Benzene Study Group. *J. Natl Cancer Inst.*, **89**, 1065–1071.
- Tough, I.M. and Court-Brown, W.M. (1965) Chromosome aberrations and exposure to ambient benzene. *Lancet*, **7387**, 684–685.
- Forni, A. (1979) Chromosome changes and benzene exposure. A review. *Rev. Environ. Health*, **3**, 5–17.
- Sasiadek, M. (1992) Nonrandom distribution of breakpoints in the karyotypes of workers occupationally exposed to benzene. *Environ. Health Perspect.*, **97**, 255–257.
- Hagmar, L., Brogger, A., Hansteen, I.L. et al. (1994) 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.
- Bonassi, S., Abbondandolo, A., Camurri, L. et al. (1995) 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.
- Le Beau, M.M. (1990) Chromosomal abnormalities in hematologic malignant diseases. In Mendelsohn, M.L. and Albertini, R.J. (eds) *Progress in Clinical and Biological Research: Mutation and the Environment*. Wiley-Liss, New York, NY, Vol. 340, pp. 325–335.
- Hagemeyer, A. and Grosveld, G. (1996) Molecular cytogenetics of leukemia. In Henderson, E., Lister, T. and Greaves, M. (eds) *Leukemia*, 6th edn. Saunders, Philadelphia, PA.
- Kagan, J. (1993) Molecular biology of chromosomal aberrations in leukemia/lymphoma. *Hematol. Pathol.*, **7**, 159–201.
- Pedersen-Bjergaard, J., Pedersen, M., Roulston, D. and Philip, P. (1995) Different genetic pathways in leukemogenesis for patients presenting with therapy-related myelodysplasia and therapy-related acute myeloid leukemia. *Blood*, **86**, 3542–3552.
- Mitelman, F., Nilsson, P.G., Brandt, L., Alimena, G., Gastaldi, R. and Dallapiccola, B. (1981) Chromosome pattern, occupation and clinical features in patients with acute nonlymphocytic leukemia. *Cancer Genet. Cytogenet.*, **4**, 197–214.
- Crane, M., Strom, S., Halabi, S., Berman, E., Fueger, J., Spitz, M. and Keating, M. (1996) Correlation between selected environmental exposures and karyotype in acute myelocytic leukemia. *Cancer Epidemiol. Biomarkers Prev.*, **5**, 639–644.
- Van den Berghe, H., Louwagie, A., Broeckert-Van Orshoven, A., David, G. and Verwilghen, R. (1979) Chromosome analysis in two unusual malignant blood disorders presumably induced by benzene. *Blood*, **53**, 558–566.
- Zhang, L., Wang, Y., Shang, N. and Smith, M.T. (1998) Benzene metabolites induce the loss and long arm deletion of chromosomes 5 and 7 in human lymphocytes. *Leukemia Res.*, **22**, 105–113.
- Rothman, N., Haas, R., Hayes, R.B. et al. (1995) Benzene induces gene-duplicating but not gene-inactivating mutations at the glycophorin-A locus. *Proc. Natl Acad. Sci. USA*, **92**, 4069–4073.
- Yin, S.N., Linet, M.S., Hayes, R.B. et al. (1994) Cohort study among workers exposed to benzene in China: I. General methods and resources. *Am. J. Ind. Med.*, **26**, 383–400.
- Rothman, N., Li, G.-L., Dosimeci, M. et al. (1996) Hematotoxicity among Chinese workers heavily exposed to benzene. *Am. J. Ind. Med.*, **29**, 236–246.
- Eastmond, D.A., Rupa, D.S. and Hasegawa, L.S. (1994) 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.
- Dean, B.J. (1985) Recent findings on the genetic toxicology of benzene, toluene, xylenes and phenols. *Mutat. Res.*, **154**, 153–181.
- Tompa, A., Major, J. and Jakab, M.G. (1994) 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.
- Irons, R.D. and Stillman, W.S. (1996) The process of leukemogenesis. *Environ. Health Perspect.*, **104** (suppl. 6), 1239–1246.
- Zhang, L., Venkatesh, P., Creek, M.L. and Smith, M.T. (1994) Detection of 1,2,4-benzenetriol induced aneuploidy and microtubule disruption by fluorescence *in situ* hybridization and immunocytochemistry. *Mutat. Res.*, **320**, 315–327.
- Zhang, L., Rothman, N., Wang, Y. et al. (1996) Interphase cytogenetics of workers exposed to benzene. *Environ. Health Perspect.*, **104** (suppl. 6), 1325–1329.
- Le Beau, M.M., Espinosa, R.D., Neuman, W.L., Stock, W., Roulston, D., Larson, R.A., Keinänen, M. and Westbrook, C.A. (1993) Cytogenetic and molecular delineation of the smallest commonly deleted region of chromosome 5 in malignant myeloid diseases. *Proc. Natl Acad. Sci. USA*, **90**, 5484–5488.
- Le Beau, M.M., Espinosa, R.D., Davis, E.M., Eisenbart, J.D., Larson, R.A. and Green, E.D. (1996) Cytogenetic and molecular delineation of a region of chromosome 7 commonly deleted in malignant myeloid diseases. *Blood*, **88**, 1930–1935.
- Rupa, D.S., Hasegawa, L. and Eastmond, D.A. (1995) Detection of chromosomal breakage in the 1cen–1q12 region of interphase human lymphocytes using multicolor fluorescence *in situ* hybridization with tandem DNA probes. *Cancer Res.*, **55**, 640–645.
- Sandell, L.L. and Zakian, V.A. (1993) Loss of a yeast telomere: arrest, recovery and chromosome loss. *Cell*, **75**, 729–739.
- Nagele, R., Freeman, T., McMorrow, L. and Lee, H.Y. (1995) Precise spatial positioning of chromosomes during prometaphase: evidence for chromosomal order. *Science*, **270**, 1831–1835.
- Mamuris, Z., Prieur, M., Dutrillaux, B. and Aurias, A. (1989) The chemotherapeutic drug melphalan induces breakage of chromosomes regions rearranged in secondary leukemia. *Cancer Genet. Cytogenet.*, **37**, 65–77.
- Mamuris, Z., Prieur, M., Dutrillaux, B. and Aurias, A. (1989) Specificity of melphalan-induced rearrangements and their transmission through cell divisions. *Mutagenesis*, **4**, 133–139.
- Mamuris, Z., Gerbault-Seureau, M., Prieur, M., Pouillart, P., Dutrillaux, B. and Aurias, A. (1989) Chromosomal aberrations in lymphocytes of patients treated with melphalan. *Int. J. Cancer*, **43**, 80–86.

34. Rothman, N., Smith, M.T., Hayes, R.B. *et al.* (1997) Benzene poisoning, a risk factor for hematological malignancy, is associated with the NQO1 609C→T mutation and rapid fractional excretion of chlorzoxazone. *Cancer Res.*, **57**, 2839–2842.
35. Smith, M.T. (1996) The mechanism of benzene-induced leukemia: a hypothesis and speculations on the causes of leukemia. *Environ. Health Perspect.*, **104** (suppl. 6), 1219–1225.
36. Forni, A. (1971) Chromosome studies in workers exposed to benzene or toluene or both. *Arch. Environ. Health*, **22**, 373–378.
37. Khan, H. and Khan, M.H. (1973) Cytogenetic studies following chronic exposure to benzene (author's translation). *Arch. Toxicol.*, **31**, 39–49.
38. Ding, X.J., Li, Y., Ding, Y. and Yang, H.Z. (1983) Chromosome changes in patients with chronic benzene poisoning. *Chin. Med. J.*, **96**, 681–685.
39. Turkel, B. and Egeci, U. (1994) Analysis of chromosomal aberrations in shoe workers exposed long term to benzene. *Occup. Environ. Med.*, **51**, 50–53.

Received on March 12, 1998; revised on July 2, 1998; accepted on July 17, 1998