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# Association between occupational exposure to benzene and chromosomal alterations in lymphocytes of Brazilian petrochemical workers removed from exposure

**Rozana Oliveira Gonçalves · Neli de Almeida Melo · Marco Antônio Vasconcelos Rêgo**

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**Abstract** We aimed to investigate the association between chronic exposure to benzene and genotoxicity in the lymphocytes of workers removed from exposure. The study included 20 workers with hematological disorders who had previously worked in the petrochemical industry of Salvador, Bahia, Brazil; 16 workers without occupational exposure to benzene served as the control group. Chromosomal analysis was performed on lymphocytes from peripheral blood, to assess chromosomal breaks and gaps and to identify aneuploidy. The Kruskal-Wallis test was used to compare the mean values between two groups, and Student's *t* test for comparison of two independent means. The frequency of gaps was statistically higher in and the exposed group than in the controls ($2.13 \pm 2.86$ vs. $0.97 \pm 1.27$, $p = 0.001$). The frequency of chromosomal breaks was significantly higher among cases ($0.21 \pm 0.58$) than among controls ($0.12 \pm 0.4$) ($p = 0.0002$). An association was observed between chromosomal gaps and breaks and occupational exposure to benzene. Our study showed that even when removed from exposure for several years, workers still demonstrated genotoxic damage. Studies are still needed to clarify the long-term genotoxic potential of benzene after removal from exposure.

**Keywords** Occupational exposure · Benzene · Chromosomal aberrations · Aneuploidy

## Introduction

Workers in the petrochemical industry are exposed to a mixture of pollutants from different sources, such as lead (Pb), gasoline, benzene, toluene, and xylene (Martínez et al. 2014). In many parts of the world, some industrial processes still employ the controlled use of benzene (Chanvaivit et al. 2007; Ji et al. 2012). Benzene is considered a ubiquitous environmental contaminant, as it is a component of cigarette smoke, gasoline, and automobile emissions (Borgie 2014; Fracasso et al. 2010).

Benzene toxicity, caused by chronic exposure to benzene, is characterized by a specific set of symptoms (malaise, myalgia, drowsiness, dizziness, and recurrent infections). However, effects on bone marrow are the most significant symptoms, initially manifesting as anemia, leukopenia, thrombocytopenia, or a combination of the three (West et al. 2000; Schnatter et al. 2005; Maffei et al. 2005).

Benzene exposure is associated with genetic damage such as aneuploidy, sister chromatid exchange (SCE),

R. O. Gonçalves (✉)
Postgraduate Program in Biotechnology in Health and Investigative Medicine, Gonçalo Moniz Research Center, Oswaldo Cruz Foundation (FIOCRUZ), 121 Waldemar Falcão Street, 40296710 Salvador, Bahia, Brazil
e-mail: rozana26oliveira@hotmail.com

N. de Almeida Melo
Federal University of Bahia, Salvador, Brazil

M. A. V. Rêgo
Department of Preventive and Social Medicine, Federal University of Bahia, Salvador, Brazil

micronucleus, and chromosomal aberrations (CA), along with an increased risk for leukemia (Tung et al. 2012; Carrieri et al. 2012). Specific chromosomal aneuploidies and aberrations have been detected in the blood cells of benzene-related leukemia patients, as well as in healthy benzene-exposed workers. Studies have shown an increase in the rates of monosomy (in chromosomes 5, 6, 7, 10, 16, and 19) and trisomy (in chromosomes 5, 6, 7, 8, 10, 14, 16, 21, and 22) associated with benzene exposure in a dose-dependent manner (Pedersen et al. 2006; Zhang et al. 2011; Zhang et al. 2005a). Celi and Akbaş (2005) conducted a study of gasoline station attendants, and found significant differences in SCE values in the exposed workers compared with controls ($p < 0.01$).

It is not known whether benzene causes DNA damage and mutation; controversial results have been reported in the literature. Chemical mutagenicity is a complex process that depends on individual genetic susceptibility, as well as on the duration and severity of exposure, and can be influenced by other modifying factors (Mrdjanović et al. 2014).

The aim of our study was to investigate the long-term association between occupational exposure to benzene and genotoxicity, by studying the lymphocytes of workers removed from exposure to benzene between the preceding 3 and 13 years.

## Materials and methods

### Study population

The exposed group (cases) included 20 male workers from the petrochemical industry of Salvador, Bahia, who had ceased occupational exposure to benzene in the preceding 3 to 13 years. The study also included 16 controls without a history of occupational exposure to benzene. The cases were recruited through the Workers Health Study Center (CESAT); all had confirmed hematological abnormalities, such as a leukocyte count lower than $4 \times 10^9$/L or a neutrophil count lower than $2 \times 10^9$/L.

All subjects were interviewed about their work, lifestyle habits (such as smoking and alcohol consumption), medication use, and disease status. The cases were matched with controls for age and gender. Signed informed consent forms were obtained from all patients and subjects prior to the study. The study was approved by the Research Ethics Committee of the Maternity Climério of Oliveira, under resolution 010/2010. The protocol and procedures were in accordance with the ethical standards of the committee on human subjects and the Helsinki Declaration of 1964 (as revised in 2008).

### Peripheral blood lymphocyte cultures

Peripheral blood was collected by venous puncture, and 0.5 mL of whole blood was added to 5 mL in RPMI-1640 cell culture medium supplemented with 20 % fetal calf serum and 2 % phytohemagglutinin. Peripheral blood lymphocyte cultures were incubated at 37 °C from 60 to 72 h. Three cultures were prepared for each individual (cases and controls). The procedure for staining chromosomes to obtain G banding followed the method of Seabright (1971), with some modifications. For analysis of breaks and gaps, the slides were treated with Giemsa stain. Fifty metaphases per individual were analyzed.

### Statistical analysis

Epi Info (Centers for Disease Control and Prevention, Atlanta, GA, USA) was employed to calculate frequencies and comparisons of means of gaps, chromosomal breaks, and aneuploidy in the two groups using the Kruskal-Wallis one-way analysis of variance, with alpha set at 0.05, and Student's *t* test for comparison of two independent means.

**Table 1** Epidemiological characteristics of individuals exposed to benzene poisoning and controls

| Characteristic | Cases | Controls | *p* value |
|---|---|---|---|
| Mean age | 52.4 | 51.4 | >0.05 |
| Smoker | 5 % | 18.75 % | 0.303 |
| Coffee drinker | 90 % | 93.75 % | 1.000 |
| Disease | 10 % | 31.25 % | 0.024 |
| Use of medication | 40 % | 56.25 % | 0.526 |
| Mean leukocytes | 3244 | 6668 | >0.05 |
| Mean neutrophils | 1400 | 3892 | 0.001 |
| Mean monocytes | 234 | 344 | 0.001 |

**Table 2** Means of chromosomal gaps, breaks, and aneuploidy from cultures of lymphocytes of cases and controls

| | Cases | Controls | *p* value* |
|---|---|---|---|
| Gaps | 2.13 ± 2.86 | 0.97 ± 1.27 | 0.001 |
| Breaks | 0.21 ± 0.58 | 0.12 ± 0.4 | 0.0002 |
| Aneuploidies | 0.031 ± 0.18 | 0.027 ± 0.17 | 0.614 |

*Kruskal-Wallis test

## Results

The mean age among the cases was 52.4 years, ranging from 38 to 67 years; mean age among controls was 51.4 years, ranging from 37 to 72 years. Coffee consumption was observed in 90.0 % of cases and 93.75 % of controls. The consumption of alcohol was more frequent among cases (81.25 %) than among controls (50.0 %); smoking was more frequent among controls (18.75 %) than among cases (5.0 %). Hypertension was the most-reported disease in both groups. However, there were no significant differences in general characteristics and lifestyle habits between cases and controls (Table 1).

The main hematological difference in the cases recruited for this study was the reduced number of leukocytes and neutrophils. Neoplastic diseases were not reported by cases.

Table 2 shows that the frequency of gaps was more prevalent in cases (2.13 ± 2.86) than in controls (0.97 ± 1.27) ($p = 0.001$). The frequency of chromosome breaks was also higher among cases (0.21 ± 0.58) than among controls (0.12 ± 0.4) ($p = 0.0002$). The frequency of aneuploidy was not statistically different between cases and controls ($p = 0.614$) (Table 2). An increased frequency of aneuploidy in specific chromosomes reported in the literature was not observed.

Figure 1 shows a chromosomal break found in one of the cases. Unidentified chromosomes were observed in eight cases and five controls (Fig. 2); a quadriradial chromosome was found in one case (Fig. 3).

## Discussion

In the present study, we found significant differences for gaps and chromosomal breaks when comparing cases and controls ($p = 0.001$ and 0.0002, respectively). This finding concurs with other studies that report the clastogenic and mutagenic potential of benzene (Bindhya et al. 2010; Mrdjanovic et al. 2014). Discordant findings were obtained by Lovreglio et al. (2014), Gonçalves et al. (2005) and Trevisan et al. (2014), who found no difference in the frequency of CA between individuals exposed to benzene and matched controls. However, as gaps and chromosomal breaks are not specific to benzene, we cannot exclude the possibility that the observed genotoxic effects could also depend on other pollutants present in the complex toxic mixture of chemicals encountered in the petrochemical industry (Schettgen et al. 2009; Mansi et al. 2012). Nonetheless, the findings are in accord with a diagnosis of benzene toxicity (Ministério da Saúde 2004).

Few studies have examined the duration of genetic alterations caused by benzene. Augusto et al. (1993) observed that 48 % of patients 5 years removed from

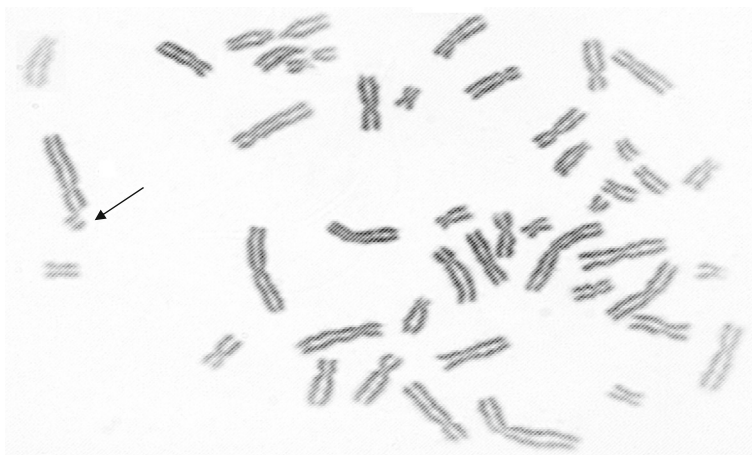

**Fig 1** Metaphase showing a chromosomal break found in one of the cases

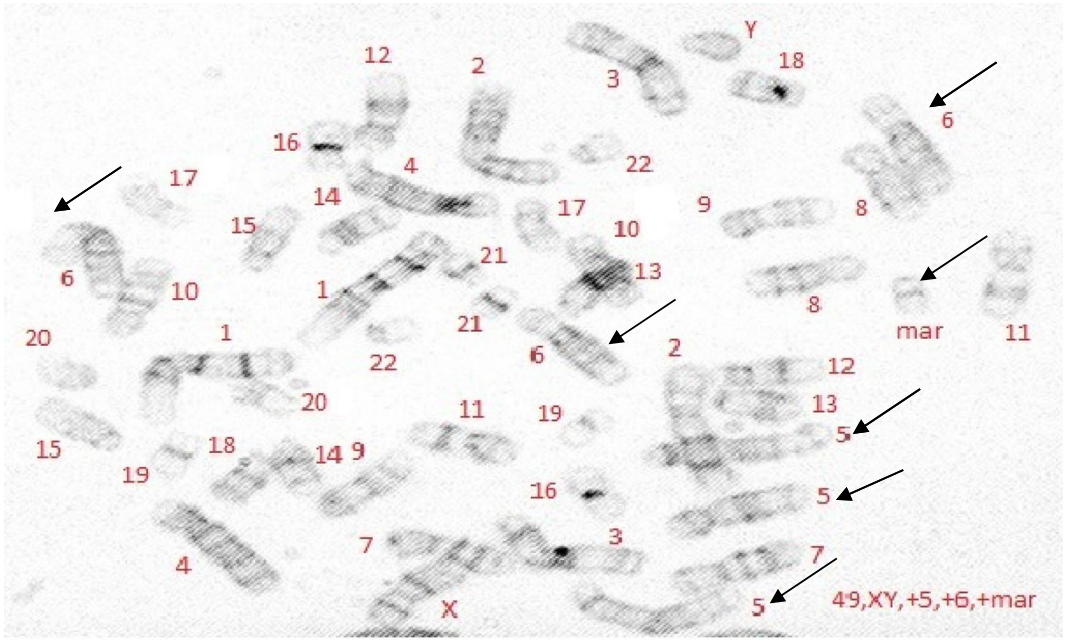


**Fig 2** Metaphase showing trisomy of chromosomes 5 and 6 and a marker chromosome, found in one of the cases

exposure to benzene did not exhibit normalized blood cells. According to Forni (1996), the levels of CA remained elevated for 30 years after exposure to benzene. Giver et al. (2001), in experiments with mice that received oral doses of benzene, observed the persistence of 14 % of aneuploid cells up to 8 months after exposure. In the present study, we observed differences in the frequency of gaps and chromosomal breaks between cases and controls.

Benzene genotoxicity can be influenced by individual genetic susceptibility and effect-modifying factors. Environmental exposures and lifestyle factors are known to modulate metabolite proportions and contribute to DNA damage. In the general population, non-occupational exposure to benzene varies from 1 to 10 ppb, and is derived mainly from motor vehicle exhaust fumes and cigarette smoke. However, some benzene metabolites are also present in coffee (hydroquinone and catechol) and many foods (phenol). Age, smoking status, and length of occupational exposure must also be carefully considered as additional factors in the evaluation of DNA damage (Tunsaringkarn et al. 2011; Mrdjanović et al. 2014; Mansi et al. 2012; Lovreglio et al. 2014). Bukvic et al. (1998) observed that the occurrence of SCE was significantly associated with both age and smoking.

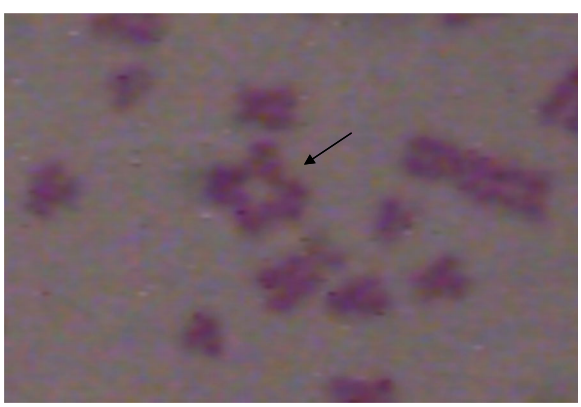

**Fig 3** Metaphase showing a quadriradial chromosome found in one case

Zhang et al. (2011) found an increase in the frequency of specific aneuploidies, such as monosomy of chromosomes 5 and 7 or trisomy of chromosomes 8 and 21, in workers exposed to benzene. In the present study, the principal kind of aneuploidy observed was monosomy. However, this occurrence was quite varied, and did not involve chromosomes specifically mentioned in the literature. The frequency of aneuploidy was higher in the cases, but there was no statistically significant difference when compared with controls. The small

sample size may have been insufficient to detect differences.

The presence of structural rearrangement, such as dicentric chromosomes, unidentified chromosomes, and quadriradials, is reported in some studies as a possible marker of the clastogenic effect of benzene (Fracasso et al. 2010; Santiago et al. 2014). In this study, unidentified chromosomes were found in eight cases and five controls, and a quadriradial was found in one case.

As clinical signs of chronic poisoning by benzene only develop some time after exposure, CA present in peripheral blood lymphocytes have been shown to be an early and sensitive biomarker of exposure. These CA, when persistent, are associated with an increased risk of developing cancer and therefore should be investigated further. In some cases, deaths from acute erythroleukemia, brain tumors, lung cancer, and paranasal sinus cancer were attributed to exposure to high concentrations of benzene (Forni 1996; Zhang et al. 2005b).

## Conclusion

The present study found that chronic exposure to benzene was associated with genotoxic effects in peripheral lymphocytes of 20 males with leukopenia who had been removed from exposure in the preceding 3 to 13 years. These data are supported by other studies that have shown that individuals exposed to benzene for long periods presented with persistent hematological and genetic damage. In addition, studies of individuals exposed to benzene reported gaps and chromosomal breaks as the chromosomal abnormalities most often detected (Santiago et al. 2014; Marchetti et al. 2012). However, because of the small sample size and possible confounding factors, studies are still needed to clarify the genotoxic effects of benzene even after cessation of occupational exposure.

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**Compliance with ethical standards** Signed informed consent forms were obtained from all patients and subjects prior to the study. The study was approved by the Research Ethics Committee of the Maternity Climério of Oliveira, under resolution 010/2010. The protocol and procedures were in accordance with the ethical standards of the committee on human subjects and the Helsinki Declaration of 1964 (as revised in 2008).

**Competing interests** The authors declare that they have no competing interests.

## References

Augusto, L. G. S., Vigorito, A. C., & Souza, C. A. (1993). Alterações histológicas da medula óssea secundárias à exposição ao benzeno e a evolução hematológica do sangue periférico em pacientes acometido. *Revista Brasileira de Saúde Ocupacional, 21*(78), 85–89.

Bindhya, S., Balachandar, V., Sudha, S., Mohana Devi, S., Varsha, P., Kandasamy, K., et al. (2010). Assessment of occupational cytogenetic risk, among petrol station workers. *Bulletin of Environmental Contamination and Toxicology, 85*, 121–124.

Borgie, M. (2014). Traffic-related air pollution. A pilot exposure assessment in Beirut, Lebanon. *Chemosphere, 96*, 122–128.

Bukvic, N., Bavaro, P., Elia, G., Cassano, F., Fanelli, M., & Guanti, G. (1998). Sister chromatid exchange (SCE) and micronuclei (MN) frequencies in lymphocytes of gasoline station attendants. *Mutatation Research, 415*, 25–33.

Carrieri, M., Bartolucci, G. B., Scapellato, M. L., Spatari, G., Sapienza, D., Soleo, L., et al. (2012). Influence of glutathione S-transferases polymorphisms on biological monitoring of exposure to low doses of benzene. *Toxicology Letters, 213*(1), 63–68.

Celi, K. A., & Akbaş, E. (2005). Evaluation of sister chromatid exchange and chromosomal aberration frequencies in peripheral blood lymphocytes of gasoline station attendants. *Ecotoxicology and Environmental Safety, 60*(1), 106–112.

Chanvaivit, S., Navasumrit, P., Hunsonti, P., Autrup, H., & Ruchirawat, M. (2007). Exposure assessment of benzene in Thai workers, DNA-repair capacity and influence of genetic polymorphisms. *Mutation Research, 626*, 79–87.

Forni, A. (1996). Benzene-induced chromosome aberrations: a follow-up study. *Environmental Health Perspectives, 104*(6), 1309–1312.

Fracasso, M. E., Doria, D., Bartolucci, G. B., Carrieri, M., Lovreglio, P., Ballini, A., et al. (2010). Low air levels of benzene: correlation between biomarkers of exposure and genotoxic effects. *Toxicology Letters, 192*(1), 22–28.

Giver, R. C., Wong, R., Moore, D. H., & Pallavicini, M. G. (2001). Persistence of aneuploid immature primitive hemopoietic sub-populations in mice 8 months after benzene exposure in vivo. *Mutation Research, 491*, 127–138.

Gonçalves, R. O., Melo, N. A., Carvalho, F., et al. (2005). Efeitos genotóxicos e alterações de enzimas hepáticas em trabalhadores do refino de petróleo. *Jornal Brasileiro de Patologia e Medicina Laboratorial, 41*(5), 297–299.

Ji, Z., Weldon, R. H., Marchetti, F., Chen, H., Li, G., Xing, C., et al. (2012). Comparison of aneuploidies of chromosomes 21, X, and Y in the blood lymphocytes and sperm of workers

exposed to benzene. *Environmental and Molecular Mutagenesis, 53*(3), 218–226.

Lovreglio, P., Maffei, F., Carrieri, M., D'Errico, M. N., Drago, I., Hrelia, P., et al. (2014). Evaluation of chromosome aberration and micronucleus frequencies in blood lymphocytes of workers exposed to low concentrations of benzene. *Mutation Research Genetic Toxicoogyl and Environmental Mutagenesis, 770*, 55–60.

Maffei, F., Hrelia, P., Angelini, S., Carbone, F., Cantelli, F. G., Barbieri, A., et al. (2005). Effects of environmental benzene: micronucleus frequencies and haematological values in traffic police working in an urban area. *Mutation Research, 583*, 1–11.

Mansi, A., Bruni, R., Capone, P., Paci, E., Pigini, D., Simeoni, C., et al. (2012). Low occupational exposure to benzene in a petrochemical plant: modulating effect of genetic polymorphisms and smoking habit on the urinary t,t-MA/SPMA ratio. *Toxicology Letters, 213*(1):57–62.

Marchetti, F., Eskenazi, B., Weldon, R. H., & Li, G. (2012). Occupational exposure to benzene and chromosomal structural aberrations in the sperm of Chinese men. *Environmental Health Perspectives, 120*(2), 229–234.

Martínez, P. N. A., Batres-Esquivel, L., Carrizales-Yáñez, L., & Díaz-Barriga, F. M. (2014). Genotoxic and hematological effects in children exposed to a chemical mixture in a petrochemical area in Mexico. *Archives of Environmental Contamination Toxicology., 67*(1), 1–8.

Ministério da Saúde. (2004). *Portaria no. 776/GM de 28 de Abril de 2004*. Brasília: Ministério da Saúde.

Mrdjanović, J., Šolajić, S., Dimitrijević, S., Đan, I., Nikolić, I., Jurišić, V. (2014). Assessment of micronuclei and sister chromatid exchange frequency in the petroleum industry workers in province of Vojvodina, Republic of Serbia. *Food and Chemical Toxicology, 69*, 63–8.

Pedersen, B. J., Christiansen, D. H., Desta, F., & Andersen, M. K. (2006). Alternative genetic pathways and cooperating genetic abnormalities in the pathogenesis of therapy-related myelodysplasia and acute myeloid leukemia. *Leukemia, 20*, 1943–1949.

Santiago, F., Alves, G., Otero, U. B., Tabalipa, M. M., Scherrer, L. R., Kosyakova, N., et al. (2014). Monitoring of gas station attendants exposure to benzene, toluene, xylene (BTX) using three-color chromosome painting. *Molecular Cytogenetics, 7*(1), 15.

Schettgen, T., Ochsmann, E., Alt, A., Kraus, T. (2009). A biomarker approach to estimate the daily intake of benzene in non-smoking and smoking individuals in Germany. *Journal of Exposure Science & Environmental Epidemiology, 20*, 427–433.

Schnatter, A. R., Rosamilia, K., & Wojcik, N. C. (2005). Review of the literature on benzene exposure and leukemia subtypes. *Chemico-biological Interaction, 153*(154), 9–21.

Seabright, M. (1971). A rapid banding technique for human chromosomes. *Lancet, 2*, 971–2.

Trevisan, P., Silva, J. N., Silva, A. P., Rosa, R. F., Paskulin, G. A., Thiesen, F. V., et al. (2014). Evaluation of genotoxic effects of benzene and its derivatives in workers of gas stations. *Environmental Monitoring and Assessment, 186*(4), 2195–2204.

Tung, E. W., Philbrook, N. A., Macdonald, K. D., & Winn, L. M. (2012). DNA double-strand breaks and DNA recombination in benzene metabolite-induced genotoxicity. *Toxicological Sciences, 126*(2), 569–577.

Tunsaringkarn, T., Ketkaew, P., Suwansaksri, J., Siriwong, W., Rungsiyothin, A., Zapuang, K., et al. (2011). Chromosomal damage risk assessment to benzene exposure among gasoline station workers in Bangkok metropolitan, Thailand. *Asian Pacific Journal of Cancer Prevention, 12*(1):223–7.

West, R. R., Stafford, D. A., White, A. D., et al. (2000). Cytogenetic abnormalities in the myelodysplastic syndromes and occupational or environmental exposure. *Blood, 95*(6), 2093–2097.

Zhang, L., Lan, Q., Guo, W., Li, G., Yang, W., Hubbard, A.E., Vermeulen, R., Rappaport, S. M., Yin, S., Rothman, N. (2005a). Use of OctoChrome fluorescence in situ hybridization to detect specific aneuploidy among all 24 chromosomes in benzene-exposed workers. *Chemico-biological Interaction, 153-154*, 117-122.

Zhang, L., Yang, W., Hubbard, A. E., and Smith, M. T. (2005b). Nonrandom aneuploidy of chromosomes 1, 5, 6, 7, 8, 9, 11, 12, and 21 induced by benzene metabolites hydroquinone and benzenetriol. Environmental and Molecular Mutagenesis. *45*(4): 388-396.

Zhang, L., Lan, Q., Guo, W., Hubbard, A. E., Li, G., Rappaport, S. M., et al. (2011). Chromosome-wide aneuploidy study (CWAS) in workers exposed to an established leukemogen, benzene. *Carcinogenesis, 32*(4), 605–612.