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Cytogenetic Biomonitoring of Workers Occupationally Exposed to Aromatic Solvents

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Abstract

It has long been shown that several organic solvents such as benzene are potent carcinogens. Because of long latency and no radical treatment of cancers, evaluation of the biological effects of potent carcinogens in a population at risk might provide predictive information. Organic solvents are the most widely used chemicals in industry. In the present study, chromosomal damages induced in lymphocytes of workers occupationally exposed to toluene, thinner and benzene are evaluated by standard metaphase analysis method; chromosome and chromatid type aberrations were scored for each subject. Results obtained show that workers exposed to chemicals show a high frequency of chromosomal aberrations compared to controls ($p<0.001$). Of the chemicals, benzene induced a higher frequency of chromosomal aberrations than toluene or thinner ($p<0.01$). Results indicate that aromatic solvents studied in this investigation are potent inducers of chromosome aberrations, therefore cytogenetic methods are reliable techniques for the detection of biological effects and risk assessment of people working with chemical agents.

Keywords: *Chromosomal Aberrations, Lymphocytes, Aromatic Solvents, Occupational Exposure.*

Introduction

Exposure to harmful pollutants in work place can cause various diseases with short or long latent period for manifestation. Therefore

we need a method to predict the potential health effects of chemicals in humans.

It is well documented that the chromosome aberration assay is one of the most sensitive and specific assay for use in the identification of carcinogens and non-carcinogens (Preston *et al*., 1981; Auletta & Ashby, 1988; Tennant *et al*., 1987; Shelby, 1988 and Au *et al*, 1990).

The main conceptual basis for using cytogenetic assays for biological monitoring is that genetic damage in a non-target tissue, most often peripheral blood lymphocytes, reflects similar events in cells involved with carcinogenic process. Therefore, chromosomal damage in human somatic cells may represent events in a process that eventually lead to manifestation of health such as cancer.

The application of cytogenetic end points to a significant cytogenetic cancer risk assessment is still not routine methodology but in combination with other disciplines from molecular biology and epidemiology it is useful and informative (Sorsa *et al*., 1992; Sorsa *et al*., 1990 and Mayer *et al*., 1991). Chromosomal aberration analyses have been used broadly for the detection of mutagenic effects of many chemicals. And in the case of occupational exposure this method was successfully used for detection of genetic damages (Deferrari *et al.*, 1991; Motykiewicz *et al*., 1992; Meng & Zhang, 1990; Kourakis *et al*., 1992 and Lerda & Rizzi, 1992). Therefore, we used this method to monitor workers occupationally exposed to benzene, toluene and thinner. These chemicals are broadly used as solvents in industry and they belong to the chemical class of aromatic compounds with very high solubility. The most important effects of these chemicals are effects on central nervous system and genetic effects.

Occupational exposure to benzene has been associated with aplastic anemia, leukemia and other related blood disorders (Goldstein, 1997 and McMichael, 1988). However, extensive in vitro genotoxicity testing has indicated that benzene is weakly mutagenic or non-mutagenic in standard mutation assays but dose cause chromosomal aberrations (McMichael, 1988; Dean, 1985 and Waters *et al*., 1988). In vivo genotoxicity studies have also demonstrated that benzene causes chromosomal damages that results in dramatic increase in structural chromosomal aberrations and micronucleated erythrocytes (Yager *et*

al., 1990). Cytogenetic studies in benzene exposed workers have reported a similar profile of genotoxicity (Bogadi-Sare *et al*., 1997). Increased frequencies of structural chromosomal aberrations in the lymphocytes of benzene exposed workers have been reported by numerous investigators (e.g. Bogadi-Sare *et al*., 1997; Sorsa & Yager, 1987; Smith *et al*., 1988 and Aksoy, 1988). Because of these effects benzene is replaced by toluene in industry.

Toluene is colorless benzene like aromatic solvent. It is the most toxic aromatic solvent. There are various reports indicating chromosomal aberrations induced by toluene in occupationally exposed workers (Dean, 1978 and 1985). Cytogenetic analysis of these people show a higher frequency of chromatid aberrations compared to unexposed controls (Schmid, 1985 and Sasiade & Jagielski, 1990).

In this study we have included people occupationally exposed to thinner and paint. Thinner is a mixture of various solvents (mainly aromatics) usually used in painting industry. It has been previously shown that workers exposed to paint show a high level of chromosomal aberrations (Haglund, 1980), however, there is not independent reports concerning biological and cytogenetic effects of thinner.

In the present study chromosomal aberrations are analyzed in lymphocyte culture of workers chronically exposed to aromatic solvents.

Materials and Methods

Subjects: Cytogenetic analysis of peripheral blood lymphocytes was performed as a biological test system. The subjects were 22 male shoe industry workers with an age range of 25-50 years exposed to toluene. Toluene concentration was varied from 135-230 ppm in this workplace as measured using a passive gas detector (Gastec universal tester, functioning at a constant air flow rate of 200 ml/min); 16 male workers with age range of 20-50 years in the paint industry using thinner as a solvent. Because thinner is a mixed solvent, air concentration measurement was not possible. In this study three male workers exposed to benzene have also participated. Benzene concentration varied irregularly in this work place. The range varies from 15 ppm –

135 ppm at the time of investigation as measured by gas detector (Gastec universal tester), workers were working in rotation in areas with low or high benzene concentration, so they were presumably exposed similarly to benzene. Twenty male subjects (age range 25-55 years) belong to the office staff of the same factories who were not exposed to chemicals, were chosen as control group. All subjects were interviewed about recent viral infections, vaccinations, previous occupational exposure to chemicals, drug intake, X-ray exposure and smoking. Those with recent X-ray exposure and drug treatment were excluded from the study.

***Cell Culture**:* Venous blood was drawn into heparinized tubes and cultures were established the same day. 0.4 ml whole blood was cultivated for 52 h in 4.5 ml RPMI-1640 (Sigma) supplemented with 15% fetal calf serum (FCS), 1% phytohaemaglutinin (PHA) and antibiotics (100 iu/ml Penicillin and 100 ?g/ml Streptomycin) (Sigma). Two hours prior to harvesting 0.2 ?g/ml colchicine (Sigma) was added to the cultures. After hypotonic treatment with 0.075 M KCl for 10 mins, lymphocytes were fixed in Carnoy's fixative and dropped onto glass slides (IAEA, 1986). Preparations were stained with Giemsa stain and 100 mitoses were screened for chromosomal aberrations classified following International System of Cytogenetic Nomenclature for acquired chromosome aberration (ISCN, 1985). Chromosomal aberrations were divided into chromosome and chromatid types, including gaps (acromatic lesions), breaks and exchanges.

Results were analyzed with student's t-test, analysis of variance and regression analysis was performed using logit model.

Results

Analysis of chromosome aberrations was performed for all samples and results are summarized in Tables 1 and 2 and shown in Figure 1.

Of the 20 subjects in control group, a total of 21 break and 24 gaps were observed mainly of chromatid type. Some of workers in this group had smoking habit, however, regression analysis showed no influence of age and smoking habit on the frequency of chromosomal aberrations ($p > 0.05$).

Table 1 - Frequency and types of chromosomal aberration in workers occupationally exposed to chemicals. Values in parenthesis indicate the number of aberrations per 100 cells.

Subjects	Years of exposure	No. of Cases	No. of cells analyzed	Chromatid Breaks	Chromosome Breaks	Chromosomal Exchanges	Breaks / 100 cells	Gaps	Percent Aberrant Cells
Control		20	2000	12 (0.6)	3 (0.15)	5(0.25)	1	25(1.25)	2.25
Benzene Exposed	4 13 16	1 1 1	100 100 100	3 3 4	2 2 3	0 2 2	5 7 9	7 7 11	12 13 15
***Toluene* Exposed**	<1 1 – 4 5 – 9 >10	5 8 3 7	500 800 300 700	3 (0.6) 9 (1.3) 7 (2.33) 24 (3.43)	0 6 (0.75) 2 (0.67) 17 (2.43)	0 1 (0.13) 0 5 (0.71)	0.6 2 3 6.57	4 (0.8) 15 (1.88) 7 (2.33) 32 (4.37)	1.4 3.38 4.67 8.86
Thinner And Paint Exposed	>10 10 – 14 15 – 19 >20	3 2 8 3	300 200 800 300	4 (1.33) 3 (1.50) 9 (1.13) 6 (2)	2 (0.87) 2 (1) 15 (1.88) 5 (1.67)	--- --- 2 (025) 1 (033)	2 2.5 3.25 3.3	7 (2.33) 6 (3) 25 (3.13) 16 (5.33)	4.0 5.5 5.75 6.67

Those workers exposed to benzene and were studied as positive control showed a high frequency of chromosomal aberrations. A total of 33 chromatid and 13 chromosome type aberrations were observed which is statistically significant with unexposed control group ($p < 0.001$).

Workers exposed to thinner and paint showed relatively high frequency of chromosomal aberrations compared to control group (Table 1). Of the 1600 metaphases analyzed, 47 breaks and 55 gaps were scored. Seven workers in this group were smokers but analysis of variance performed did not show a significant difference for smoking habit and chromosomal aberration frequency ($p > 0.05$). Because workers had different working experience, regression analysis performed by the logit model show a significant relationship for years of exposure to thinner and aberration induction ($p = 0.01$). Effect of

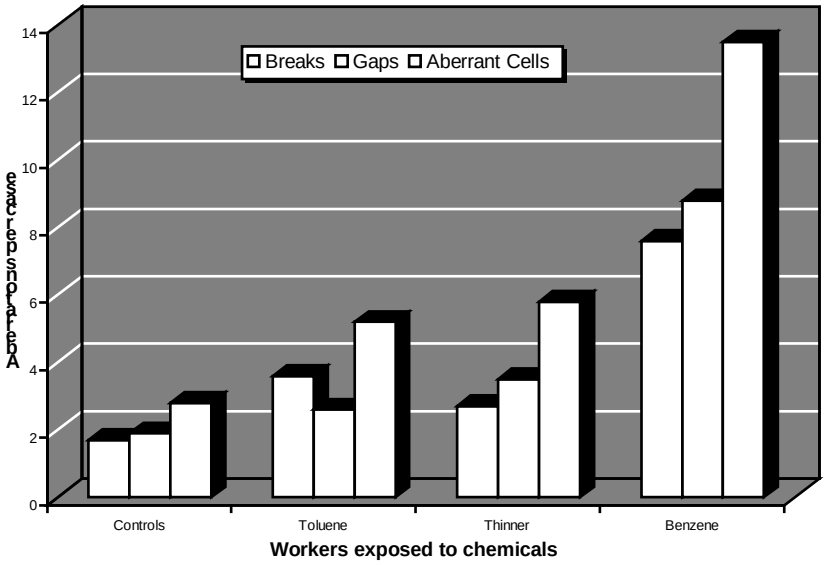
this pollutant was found for all types of aberrations (breaks, gaps, and aberrant cells) statistically significant different with control ($p < 0.001$).

Table 2 - Mean frequency of total chromosomal aberrations in occupationally exposed workers to different chemical agents.

Subjects	Number of cases	Years of exposure	Breaks ± SD	Gaps ± SD	Aberrant Cells ± SD
Control	20	-----	1.05 ± 0.8	1.2 ± 0.65	2.25 ± 1.1
Benzene	3	11.5 ± 1	7 ± 1.63	8.3 ± 1.9	12.3 ± 2.25
Toluene	4 8 3 7	0.38 ± 0.1 2.06 ± 0.88 8 ± 0.82 13.75 ± 2.44	1 2.17 ± 0.81 2.67 ± 0.47 6.14 ± 1.88	1.25 ± 0.43 2.21 ± 0.67 2.33 ± 0.47 4.57 ± 2.77	2 3.38 ± 1.1 4.67 ± 0.47 8.16 ± 2.47
Thinner And Paint	3 2 8 3	4 ± 1.6 12 ± 1 17.88 ± 0.88 20.3 ± 0.47	2 ± 0.82 2.5 ± 1.5 3.25 ± 1.2 3.3 ± 1.44	2.3 ± 0.47 3 3.25 ± 1.09 5.33 ± 0.94	4 ± 0.8 2.5 ± 1.5 5.75 ± 1.39 6.67 ± 1.25

Of 22 workers exposed to toluene, 2200 cells were scored and a total of 103 cells with lesions observed. In this group, 43 chromatid type breaks, 25 chromosome type breaks, 6 exchanges and 54 chromosomal gaps were observed (Tables 1 & 2). Frequency of chromosomal breaks was more than gaps in toluene exposed group which indicate this solvent is highly mutagenic and clastogenic. Results show a significant difference for chromatid breaks (4 times) and chromosome breaks ($p < 0.01$).

Figure 1 compares the frequency of chromosomal aberrations induced by various chemicals used in this study. As it is seen aberrations induced by benzene are significantly different from those of toluene and thinner ($p < 0.01$). Also, workers exposed to toluene show



a higher frequency of chromosomal breaks compared to those workers exposed to thinner ($p < 0.001$).

Figure 1 - Comparison of chromosomal aberrations induced by various chemical solvents in workers occupationally exposed.

Discussion

On the basis of the cytogenetic analysis of lymphocytes, it can be concluded that occupationally exposed workers to chemicals used in this study represent a group with increased risk of health. Human peripheral blood lymphocytes are suitable for use in surveillance studies because they are easily accessible and can integrate exposures over a relatively long life span.

Of the 20 subjects in control group a total of 21 breaks and 24 gaps were observed mainly of chromatid types. Some of these workers in this group were smoker but regression analysis indicated that age and

smoking habits did not influence frequency of chromosomal aberrations ($p > 0.05$). Other independent studies also showed no confounding effect of smoking on cytogenetic variables such as micronuclei and sister chromatid exchanges in human lymphocytes (Holz *et al*., 1995). Andreoli *et al*. (1997) showed no correlation between the extent of DNA damage and the ages or smoking habits of the subjects in lymphocytes by alkaline single cell gel electrophoresis after exposure to benzene or benzene metabolites. Those workers exposed to benzene who were as positive control, show a high frequency of chromosomal aberrations. A total of 33 chromatid and 13 chromosome type aberrations were observed which is statistically significant with unexposed control group ($p < 0.001$) (Table 1). Although we could have access to only three benzene exposed samples, considering a high frequency of gap formation in the cells of these individuals, a relatively high frequency of aberrant cells were scored for all subjects, i.e. about 6 times more than spontaneous aberrant cells observed for controls. In vivo genotoxicity studies have demonstrated that benzene causes chromosomal damages (Fujie *et al*., 1992 and Mozdarani & Kamali, 1998), even at doses lower than the current limit for occupational exposure (Farris *et al*., 1996). Cytogenetic studies of exposed workers have reported a similar profile of genotoxicity and increased frequency of chromosomal aberrations (Bogadi-Sare *et al*., 1997 and Aksoy, 1988). Therefore, our results are in agreement with other reports and validate our observation on other study groups.

Frequency of chromosomal breaks was more than gaps in toluene exposed group which indicate that this solvent is highly mutagenic and clastogenic (Tables 1 & 2). Results obtained for this group show a significant different for chromatid breaks (4 times) and chromosome breaks (8 times), compared to controls ($p<0.01$). Observation of high frequency of chromatid type breaks rather than gaps in toluene exposed workers for more than five years, might be due to enzyme activity after toluene exposure converting gaps into breaks (Preston, 1980 and Mozdarani & Bryant, 1987).

Workers exposed to thinner and paint showed relatively high frequency of chromosomal aberrations compared to control group (Tables 1 & 2). Seven workers in this group were smokers but analysis

of variance performed did not show a significant difference for smoking habit and chromosomal aberration frequency ($p > 0.05$) (Holz *et al*., 1995 and Andreoli *et al*., 1997). Because workers had different working experience, regression analysis performed by the use of logit model show a significant relationship for years of exposure to thinner and aberration induction ($p = 0.01$). Effect of this solvent was found to be statistically different with controls for all types of aberrations (breaks, gaps and aberrant cells) ($p < 0.001$). This observation is consistent with a report previously published by Haglund (1980). The mechanism of chromosomal aberration induction by thinner can not be uniform, because it is a mixture of various solvents and also the molecular nature of thinners are different. However, it is obvious that those people exposed to this chemical are at high risk of cytogenetic damages.

Conclusion

In conclusion, results obtained show that all chemicals investigated in this study are genotoxic agents and produce morphological changes in chromosomes which are consistent with other reports (Dean, 1978, 1985; Yager *et al*., 1990; Sasiade & Jagielski, 1990; Haglund, 1980 and Farris *et al*., 1996). The frequencies of chromosomal aberrations induced by various chemicals are compared in figure 1. As seen, aberrations induced by benzene are significantly different from those of toluene and thinner ($p < 0.01$). Also, frequency of aberrations in toluene exposed workers was higher than those exposed to thinner ($p < 0.001$). Chromosomal aberrations are induced directly or indirectly by a wide variety of physical and chemical agents which can be classified into either S-dependent or S-independent agents. S-independent agents are capable of inducing chromatid and chromosome type aberrations at any cell cycle stage but S-dependent agents induce aberrations only after an intervening S-period (Evans, 1977).

An increased frequency of chromosomal aberrations in a population may be considered to indicate an increased risk of cancer. Numerous reports indicate that most neoplasms are associated with chromosomal rearrangements (Mitelman, 1988, Trent *et al*., 1989 and Heim &

Mitelman, 1996) which can lead to the activation of proto-oncogenes and elimination of tumor suppressor genes; therefore represent an important mechanism of tumorogenesis. Thus, chemicals capable of chromosomal aberration induction might be involved in carcinogenic process.

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