RESEARCH COMMUNICATION

# Cytogenetic Methods for Assessing Human Exposure to Toluene in Coimbatore, South India

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## Abstract

**The aim of the present study was to identify genetic alterations occurring in rotograving workers chronically exposed to toluene. A total of 60 samples from 30 exposed subjects and 30 age-matched controls, selected based on the toluene level present in their urine, were recruited. Exposed subjects were categorized based on their duration of exposure and smoking habitats. Controls were normal and healthy and categorized based on their smoking habits. Cell cultures were established from blood samples collected from the control and experimental subjects after obtaining informed consent. G-banding and comet assays were used to identify genetic alterations. A higher degree of total chromosome aberration was identified in exposed subjects compared to controls. As expected, controls exhibited minimal number of alterations. The overall CA frequency due to toluene exposure was significantly different from that of the controls for both chromatid and chromosome type aberrations (P\0.05 by ANOVA). . The habit of cigarette smoking among the workers had a synergistic effect on inducing DNA damage. In conclusion, this work shows a clear genotoxic effect associated with toluene exposure, our results also reinforcing the conclusion of higher sensitivity of cytogenetic assays for the biomonitoring of occupationally exposed populations. There is a strong need to educate those who work with potentially hazardous materials about adverse effects and highlight the importance of using protective measures.**

**Keywords:** Human lymphocytes - toluene - chromosome aberrations - comet assay - occupational exposure

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## Introduction

The International Agency for Research on Cancer reported that as an occupation that increases certain risk factor on cancers (IARC, 1987). Furtherly numerous reports are indicated an excess risk for cancers (Walker et al., 1993; Fu et al., 1996) from occupational exposed chemicals. Workers in rotograving industry are exposed to a mixture of organic solvents, particularly to toluene. Toluene is apparently not a carcinogen or genotoxic in vitro, and has not given genotoxic in vivo (McGregor, 1994). But recent investigations have indicated that structural chromosome aberrations are increased in workers exposed to toluene (Tunca and Egeli., 1996; Bogadi-Sare et al., 1997).

The toluene metabolites also show mutagenic and carcinogenic properties more than toluene itself and these compounds (Ohashi et al., 2006) are widely used as organic solvents in rotogravure machine (Saijo et al., 2004). The exposure to high concentration of toluene causes adverse health effects in nervous system (Harkonen et al., 1978), mucous and dermal irritations (Uchida et al., 1993), and chromosome dislocation (Bilban, 2004; Celik and Akbas, 2005). Toluene is metabolized in liver microsomes (Kim et al., 1997) by the hepatic cytochrome to hippuric acid, which is then excreted by urine (Siqueira and Paiva, 2002). This metabolic pathway is mediated by cytochrome-P450 and may initially form arene oxides, which rearrange spontaneously to *o*- and *p*-cresol (Pelclova et al., 2000) and these compounds mutagenicity have been reviewed and show mixed results. In view of the predominantly in vivo reactivity which has been described (McGregor, 1994).

Toluene exposure has also been found to induce chromosomal aberrations, while results concerning exposure to toluene are controversial (Forni et al., 1971; Funes-Cravioto et al., 1977). Furthermore, there is concern about their potential carcinogenicity and adverse effects at low levels of exposure will give negative effects (Adgate et al., 2004; Son et al., 2004; Petronella et al., 2005; Sapkota et al., 2005; Lee et al., 2006; Sunesson et al., 2006). Cytogenetic studies on Toluene are mostly related to unintentional occupational exposure to this and other organic solvents in rotogravure printing workers (Forni et al., 1971; Popp et al., 1992; Pelclova

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et al., 2000). Recently, chromosomal aberrations (CA) in human peripheral lymphocytes (PBLC) are recognized as a valuable biomarker of genotoxic effect, probably the only one which has been internationally standardized and validated (Albertini et al., 2000) and it have been used as biomarkers for a reasonable evaluation of cancer predictivity (Hagmar et al., 1998). Similar DNA damage as measured by the Comet assay (Adgate et al., 2004), and DNA repair capacity as measured by the cytogenetic challenge assay (Lee et al., 2006), provide information on possible early biological effects of toxic chemical exposure and may be indicative of health risks.

Therefore, the present study was carried out to evaluate possible chromosome aberration and DNA damage in rotogravure workers intentionally exposed to toluene and to determine the hippuric acid excretion.

## Materials and Methods

The study subjects contained 30 rotogravure workers and 30 healthy individuals as controls (Table 1). The subjects were selected from various cities of southern India. A questionnaire was used to collect the information on sex, age, duration of exposure, use of protective masks, general health status, smoking habits and exposure to drugs for each exposed and control subject. All the experimental and control subjects analyzed for urinary hippuric acid levels before the sample compilation. Informed written consent was obtained from all the individuals, and the study was conducted in accordance with the guidelines of the Declaration of Helsinki.

*Estimation of hippuric acid o- and p-cresol in urine*

Urine samples were diluted 100 times with a distilled water and methanol (1:1) solution. After being centrifuged for 5 min at 300 rpm, hippuric acid in the supernatant was measured with a high performance liquid chromatography (HPLC, Hitachi L6210) equipped with an UV detector.

*Chromosome aberration assays*

About 1.5 mL of blood was collected by vein puncture and was inserted in to heparinized vacutainer for cytogenetic analysis. All chemical reagents were purchased from Sigma Chemical (St. Louis, MO, USA), except for colcemid that was obtained from Gibco Laboratory (Grand Island, NY, USA). Upon arrival in the laboratory, blood samples were set up to establish cell cultures following standard procedures of our laboratory (Moorhead et al., 1960). Briefly, 0.5 ml whole blood was added to 4.5 ml RPMI 1640 medium, supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1% streptomycin–penicillin antibiotics, 0.2 ml reagent grade phytohemagglutinin, and incubated at 37°C. At 71 h, cultures were treated with 0.1 lg/ml colcemid to block cells in mitosis. Lymphocytes were harvested at 72 h by centrifuging cells to remove culture medium (800-1,000 rpm/7 min), adding hypotonic solution (KCl 0.075 M) at 37°C for 20 min to swollen the cells, following treatment with fixative (3:1 ratio of methanol:acetic acid) twice. Cytological preparations were made by dropping 2-3 drops of the concentrated cell suspension onto slides wet with ice cold acetic acid (60%). Slides were carefully dried on a hot plate (56°C, 2 min). For CA analysis, 100 complete metaphase cells of the first cell cycle were evaluated per subject under a microscope (1009) to identify numerical and structural CA. The collected data were registered on master tables Figure 1 Shows the study area map and later transferred to a computer file.

*Comet assay in exfoliated buccal cells*

Buccal cells were collected three times from each subject at 3 alternate days after the work shift. Workers were asked to rinse the mouth with distilled water. The buccal cells were collected using a toothbrush by scrapping the inside cheek of the mouth. The toothbrush was agitated in 30 ml cold PBS buffer and the suspension was centrifuged at 2500rpm at 4°C for 10 min. The cell pellet was resuspended in 100$\mu$l PBS buffer.

Comet assay was performed as described by Szeto et al., (2005). 10$\mu$l of the buccal cell suspension was mixed with 85$\mu$l of pre-warmed (40°C) 1% (w/v) low melting point agarose in phosphate buffered saline (PBS: 0.137MNaCl, 2.68mMKCl, 6.4mM Na2HPO4·7 H2O, 1.47mM KH2PO4, pH 7.4), and immediately applied on a clean microscopic slide precoated with 85$\mu$l of 1% (w/v) normal melting agarose in PBS buffer. The slideswere allowedto solidify in roomtemperature.A50$\mu$l of trypsin solution was layered onto the gel and left for 30 min at 37°C followed by washing with PBS buffer, proteinase K (1mg/ml) treatment for 1 h at 37°C and immersion in lysis solution (2.5M NaCl, 0.1M EDTA, 10mM Tris, 1% Triton X-100, pH 10) for 1 h at 4°C. The slides were kept in electrophoresis tank filled with electrophoresis buffer (0.01M NaOH and 1mM EDTA, pH 9.1) for 20min and electrophoresed at 12V for 18 min. After electrophoresis the slides were removed and neutralized three times with 0.4M Tris at pH 7.5. The slides were then stained with ethidium bromide (50$\mu$l of 20$\mu$g/ml). The stained slides were examined under Nikon fluorescent microscope with a 580nm emission filter. The comets were analyzed by visual classification and the damage was assigned to 5 classes (Jaloszynski et al., 1997). Comets with a bright head and no tail were classified as class 0 (cells with no DNA migration) and comets with a small heads and a long diffuse tails were classified as class 4 (severely damaged cells). Comets with intermediate appearance were classified into classes 1, 2 and 3. The percentage of tail DNA was calculated using the formula [(2.5×cells at class 0 + 12.5×cells at class 1 + 30×cells at class 2+60×cells at class 3 + 90×cells at class 4)/total number of cells scored)] (Zhao et al., 2006).

*Statistical analysis*

Results are expressed as mean±standard deviation. Student's t-test was performed to compare the chromosome aberration and DNA damage levels between the rotogravure workers and controls. Statistically significant levels were considered at $p < 0.05$.

## Results

The subjects were selected from the rotogravure

workers, exposed to toluene. There were 10 smokers and 20 non-smokers in each of the exposed and control groups. The average cigarette consumption of smokers in both groups was nearly 10.2±2.93 (mean±standard deviation) cigarettes per day.

Table 1 depicts that the chromosome aberration in both control and experimental subjects. Significant aberrations were notitified in experimental subjects. Experimental subjects contained more chromosome aberrations (6.5±3.39, Chromosome type was 2.46±1.61, Chromatide type was 4±2.5) than controls (1.43±1.67 Chromosome type was 0.6±0.96, Chromatide type was 1±0.83). Among the experimental subjects, smokers (9.7±3.3) had more number of chromosome alterations then non smoking experimental subjects (4.9±2.07) and controls (Smokers/3.09±1.7; Non-Smokers/0.47±0.51). To determine the effect of duration of exposure to toluene on chromosome aberrations, the workers were divided into 3 groups as less than 10 years of exposure, 10-20 years of exposure and more than 20 years of exposure (Table 4). No statistically significant results were identified with duration of exposure and toluene exposure in chromosome aberrations.

The classes of comets observed in the buccal cells of exposed and controls are given in Table 3. In the control subjects, a decrease in the comet class 0 and an increase in the comet classes 3, 4 and percentage of DNA damage were observed among smokers then non smokers, but there was no statically significant data observed except the 5 subjects with 31-40 and 51-60 years of age. An age related increase in DNA damage was observed in both control and exposed groups. Exposed subjects with age above 51 years showed maximum DNA damage. Significant increase ($p < 0.05$) in the comet classes 2-4, decrease in comet class 0, 1 and increase in the percentage of tail DNA was identified in most of the exposed groups when compared to control which shows that exposed subjects carry more DNA damage than control subjects. An increased level of DNA damage was observed in the rotogravure workers with smoking habits when compared with smoking controls and nonsmoking rotogravure workers. To determine the effect of duration of exposure to toluene on DNA damage, no statistically significant increase in DNA damage was found.

Table 4. depicts the levels of excreted urinary toluene metabolites were also significantly elevated. Hippuric acid , *p*-cresol, and for *o*-cresol, which was increased the based on duration of exposure. But the correlation between chromosome and DNA was not significantly.

## Discussion

Chromosome aberrations in human lymphocytes has been established as a sensitive method for the detection of genotoxicity caused by environmental mutagens in

**Table 1. Chromosome Alteration of Toluene Exposed Workers and Controls**

| S.No | Age | | Smoking Habitats | | DE | Chromosomal aberrations | | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Chromatid | | Chromosomal | | | |
| | Cont | Exp | Cont | Exp | | Cont | Exp | Cont | Exp | Con | Exp |
| 1 | 44 | 34 | NS | NS | 15 | 0 | 6 | 0 | 2 | 0 | 8 |
| 2 | 31 | 47 | NS | S | 13 | 0 | 5 | 0 | 3 | 0 | 8 |
| 3 | 42 | 51 | NS | S | 23 | 1 | 2 | 0 | 4 | 1 | 6 |
| 4 | 47 | 26 | S | NS | 7 | 0 | 2 | 4 | 1 | 4 | 3 |
| 5 | 48 | 38 | NS | S | 7.5 | 1 | 4 | 0 | 6 | 1 | 10 |
| 6 | 27 | 41 | S | S | 12 | 2 | 6 | 1 | 4 | 3 | 10 |
| 7 | 54 | 54 | NS | NS | 21.5 | 1 | 3 | 0 | 3 | 1 | 6 |
| 8 | 28 | 29 | NS | NS | 7.5 | 0 | 5 | 1 | 2 | 1 | 7 |
| 9 | 26 | 28 | NS | S | 9 | 1 | 6 | 0 | 4 | 1 | 10 |
| 10 | 53 | 32 | NS | NS | 9 | 1 | 3 | 0 | 4 | 1 | 7 |
| 11 | 37 | 45 | NS | NS | 15.5 | 0 | 3 | 0 | 2 | 0 | 5 |
| 12 | 46 | 45 | S | NS | 16 | 4 | 2 | 3 | 1 | 7 | 3 |
| 13 | 32 | 57 | S | S | 23 | 1 | 7 | 1 | 1 | 2 | 8 |
| 14 | 37 | 29 | S | NS | 8 | 1 | 2 | 2 | 0 | 3 | 2 |
| 15 | 39 | 50 | NS | NS | 24 | 0 | 0 | 0 | 3 | 0 | 3 |
| 16 | 46 | 27 | NS | S | 6 | 0 | 4 | 0 | 3 | 0 | 7 |
| 17 | 29 | 31 | NS | NS | 17 | 0 | 3 | 0 | 1 | 0 | 4 |
| 18 | 56 | 53 | NS | NS | 23.5 | 0 | 1 | 1 | 2 | 1 | 3 |
| 19 | 50 | 29 | NS | NS | 4 | 0 | 3 | 0 | 2 | 0 | 5 |
| 20 | 27 | 48 | NS | NS | 23 | 1 | 1 | 0 | 4 | 1 | 5 |
| 21 | 45 | 37 | S | S | 11.5 | 2 | 11 | 1 | 6 | 3 | 18 |
| 22 | 53 | 30 | S | NS | 6 | 1 | 3 | 1 | 2 | 2 | 5 |
| 23 | 36 | 53 | NS | NS | 24 | 0 | 4 | 0 | 5 | 0 | 9 |
| 24 | 28 | 25 | S | S | 9.5 | 0 | 8 | 0 | 3 | 0 | 11 |
| 25 | 30 | 43 | S | NS | 26 | 2 | 6 | 1 | 0 | 3 | 6 |
| 26 | 43 | 55 | NS | S | 28 | 0 | 8 | 0 | 1 | 0 | 9 |
| 27 | 54 | 46 | S | NS | 25.5 | 3 | 5 | 1 | 2 | 4 | 7 |
| 28 | 28 | 29 | NS | NS | 7 | 0 | 0 | 0 | 1 | 0 | 1 |
| 29 | 33 | 39 | S | NS | 14 | 2 | 4 | 1 | 1 | 3 | 5 |
| 30 | 24 | 39 | NS | NS | 19 | 1 | 3 | 0 | 1 | 1 | 4 |

DE-Duration of exposure (Years); Cont-Controls; Exp-Experimentals; S-Smokers; NS-Non-Smokers

**Table 2. Classes of Comets and Percentage of DNA Damage Among the Control Subjects and Rotogravure Workers**

| | N | Classes of comet | | | | | % of DNA damage |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | |
| Control Smokers | | | | | | | |
| <30 Years | 2 | 71.6 ± 0.9 | 14.2 ± 0.6 | 2.9 ± 0.3 | 0.5 ± 0. 5 | 1.0 ± 0.3 | 4.9 ± 0.5 |
| 31-40 Years | 3 | 68.1 ± 0.5* | 14.5 ± 0.3* | 4.3 ± 0.6* | 2.3 ± 0. 2* | 1.2 ± 0.6* | 7.6 ± 0.6* |
| 41-50 Years | 3 | 65.4 ± 02 | 7.2± 0.5 | 6.4 ± 0.4 | 5.5 ± 1. 7 | 2.9 ± 0.9 | 11.5 ± 1.0 |
| 51-60 Years | 2 | 63.2± 06* | 6.3 ± 0.7* | 5.5 ± 0.2* | 8.2 ± 0. 6 | 4.1 ± 1.1* | 13.0 ± 0.6* |
| Control Non Smokers | | | | | | | |
| <30 Years | 7 | 71.0 ± 05 | 15.7 ± 1.6 | 2.6 ± 0.4 | 0.4 ± 0.3 | 0.3 ± 0.6 | 5.5 ± 1.1 |
| 31-40 Years | 4 | 68.3 ± 0.6 | 11.3 ± 0. | 4.2 ± 0.8 | 2.3 ± 0. 5 | 1.0 ± 0.2 | 7.4 ± 0.3 |
| 41-50 Years | 6 | 66.1± 0.3 | 7.4± 0.9 | 6.8 ± 0.4 | 6.2 ± 0. 7 | 2.6 ± 1.1 | 10.1 ± 0.4 |
| 51-60 Years | 3 | 64.2 ± 1.1 | 6.0 ± 1.5 | 7.2 ± 0.6 | 7.3 ± 0.6 | 4.1 ± 0.2 | 13.8 ± 0.7 |
| Experimental smokers | | | | | | | |
| <30 Years | 3 | 71.1± 0.7 | 2.7 ± 0.3# | 7.5 ± 0.3 | 7.9 ± 0. 5 | 1.8 ± 0.8 | 9.3 ± 0.6 |
| 31-40 Years | 2 | 63.6 ± 0.6 | 2.6 ± 1.0# | 9.1 ± 0.5# | 8.5 ± 0.4# | 4.2 ± 0.3# | 11.3 ± 0.3 |
| 41-50 Years | 2 | 55.2 ± 0.2# | 5.3 ± 09# | 11.2 ± 1.0# | 9.7 ± 07# | 6.5 ± 0.7# | 15.0 ± 1.2 |
| 51-60 Years | 3 | 48.0 ± 0.1# | 5.9 ± 0.3 | 11.1 ± 0.8 | 13.4 ± 0.9# | 9.2 ± 0.4 | 19.2 ± 1.2 |
| Experimental Non Smokers | | | | | | | |
| <30 Years | 6 | 71.2 ± 0.9 | 2.3 ± 1.2* | 8..1 ± 0.3* | 6.4 ± 1.0* | 1.3 ± 0.3* | 10.1 ± 0.4 |
| 31-40 Years | 5 | 65.5 ± 0.4* | 3.2 ± 0.3* | 8.7 ± 0.4* | 7.6 ± 0.3* | 3.2 ± 0.7* | 13.1 ± 1.4 |
| 41-50 Years | 6 | 57.3 ± 3.3* | 4.1 ± 1.2* | 10.7 ± 0.8* | 9.0 ± 0.9* | 6.1 ± 0.6* | 18.1 ± 0.5 |
| 51-60 Years | 3 | 49.5 ± 0.5* | 6.0 ± 0.6 | 11.3 ± 1.0* | 12.0 ± 2.1* | 8.0 ± 0.8* | 21.4 ± 0.3 |

*n*: number of subjects studied; *$p < 0.05$ compared with non-smoking control subjects; #$p < 0.05$ compared with smoking control subjects

**Table 4. Combined Results of Toluene Exposed Workers**

| Exposure period | n | % of Chromosome aberrations | % of DNA | Hippuric acid (g/g creatinine) | o-Cresol (mg/g creatinin) | p-Cresol (mg/g creatinin) |
|---|---|---|---|---|---|---|
| <10 Years of exposure | 11 | 6.18±3.34 | 12.27 ±2.4 | 1.61±1.12 | 1.22±1.63 | 149±49.2 |
| 10-20 Years of years | 09 | 7.22±4.65 | 13.95 ± 3.3 | 2.31±0.92 | 1.72±0.32 | 152±82.0 |
| > 20 Years exposure | 10 | 6.20±2.14 | 12.38 ± 3.6 | 2.87±1.46 | 3.41±0.70 | 229±73.7 |

n-Number of subjects

populations exposed to genotoxicants, through direct contact with ingested or inhaled compounds (Salama et al., 1999). Once the hazardous workplace or exposures are identified, the major objectives are to establish the relationship between exposure levels and genetic risk and to define safe levels of exposure on a sound toxicological basis. In this context, biomonitoring exposed individuals are extremely important, especially in evaluating genotoxic effects of toluene, as in this study. At present, large follow-up studies suggest that increases in chromosome alterations may predict an increased cancer risk (Bonassi et al., 1995; Hagmer et al., 1998).

Pelclova et al., (2000) studied the toxicity of toluene exposed to workers and found significantly higher number of structural chromosome aberrations than normal subjects. Some of these studies found evidence of a genotoxic effect in workers in a rotogravure factory (Nise et al., 1991), whereas others did not (Forni and Pacifico, 1971). These differing results can be attributed to the variability of populations analyzed with various factors such as age, smoking habits and duration of exposure. On the other hand negative results also find out the genotoxicity of toluene exposure (Nakamura et al., 1997; Zarani et al., 1999; SCGOC, 2002).

Other results were obtained in a multitude of studies with biological monitoring of various genotoxic effects in peripheral blood lymphocytes from workers exposed to toluene in the occupational environment (Bauchinger et al., 1982; Pelclova et al., 1990; Nise et al., 1991; Popp et al., 1992; Hammer et al., 1998; Pelclova et al., 2000; Ok et al., 2004). Some positive results have been obtained in toluene exposed workers, but these might be benzene contamination (Tunka and Egeli, 1996; Bogadi et al., 1997). Pitarque et al., (1999) obtained negative results by comet test in exposed to toluene, but found increased values for MN in peripheral lymphocytes in the same group. Numerous reports are found, possibility of cytogenetic damage in various occupationally exposed to toluene (Pitarque et al., 1999; Pelclova et al., 2000). Unfortunately, most of these studies contain some deficiencies, particularly a lack of information about cumulative past exposures, and the overall studies do not provide conclusive evidence of an association between toluene exposure and increased incidence of cancer. While the neurotoxicity of toluene is an accepted fact (Spencer and Schaumburg, 1985; Greenberg, 1997), its genotoxicity in complex mixtures is still under discussion; perhaps its effects are increasing the host susceptibility to carcinogens and other genotoxic products (Wang et al., 1993; Nakajima et al., 1994; Pitarque et al., 1999; Hammmer, 2002). This high degree of variability in the available data represents possible exposure and makes the explanation of biomonitoring results. But, the present study showed toluene has a synergistic effect on peripheral lymphocytes. In view of the current inconclusive evidence of a direct relationship between in vivo toluene exposure and induction of genetic damage, in that the positive findings presented here are satisfactory evidence of a

genetic risk associated with toluene exposure. So we may assume that as early biological effects in carcinogenesis, and lymphocytes are valid surrogate cells for the changes taking place in tissues where neoplasms may eventually develop.

Exfoliated cells hold strong potential as a tool for biomonitoring human populations exposed to genotoxic agents because they can be easily collected from the tissues by noninvasive procedures. In addition, more than 90% of cancers arise in epithelial tissues; in many cases, these tissues are the actual targets of carcinogens, as indicated by the sites of cancers related to the exposures (Fairbairn et al., 1995; Fenech et al., 1999; Grover et al., 2003). The increasing use and diversity of solvents raises concern about possible risks toluene exposure and in vitro and in vivo cytogenetic findings are reported for occupationally exposed to toluene (McGregor, 1994). Accordingly Pitarque et al., (1999) used the comet assay to evaluate DNA damage in female shoe workers exposed to organic solvents and observed that organic solvents did not affect the comet values. On the other hand toluene exposure in rotogravure printing workshops has been reported (Pelclova et al., 2000). The range of toluene concentrations to which the studied workers exposed may have been relatively small (McGregor, 1994). Popp et al., (1992) observed significant DNA damage in workers employed in a shoemaking factory as measured by the alkaline elution techniques. Although this study provides suggestive evidence that the DNA damage in buccal cells of rotogravure workers is caused by toluene, further in vitro experiments with toluene may be useful to provide support for this assumption and also play an important role.

Several markers have been proposed including benzyl alcohol in urine (BeOH-U) (Kawai et al., 2008), *o*-cresol in urine (o-CR-U) (Truchon et al., 1999), and mercapturates (Angerer et al., 1998) such as benzylmercapturic acid in urine (BMA-U) (Inoue et al., 2004), and un-metabolized toluene in blood and urine (Tol-B and Tol- U, respectively) (Kawai et al., 1992 a,b). Hippuric acid is still the most commonly used solvent in various workplaces (Yasugi et al., 1998; Moon et al., 2001; Samoto et al., 2006; Ukai et al., 2007). The use of biological monitoring, typically through urinalysis, makes it possible to estimate levels of exposure of individual workers to this solvent. For this purpose of biological monitoring, hippuric acid in urine (HA-U) has been the marker of Toluene exposure (Ikeda, 1996) in rotogravure workers. In addition, possibility has been pointed out that the marker of choice may vary depending on the intensity of toluene exposure (Kawai et al., 2008). Similarly the present findings showed that these metabolic compounds are effective biomarker for toluene exposure. Hippuric acid is the main metabolite resulting from toluene exposure and has been suggested as a marker for estimating exposure to both high and low concentrations of toluene (De Rosa et al., 1985), even in solvent mixtures (Burgaz et al., 2002; Ok et al., 2003).

In the present study described rotogravure industry workers exposed to toluene using the Comet assay and the chromosome aberration test as sensitive cytogenetic endpoints for the detection of genotoxic effects and compared the data produced to the traditional parameters for assessing exposure to toluene with correlation of urinary hippuric concentration. In our study, will make the large sample size and the wide variability found in the urine toluene levels made it possible to obtain a good relationship between the exposure and the biomarkers. In summary, this study shows a clear genotoxic effect associated with the occupational exposure to toluene. These data are relevant and permit an estimate of the genetic risk of toluene exposure by using biomarkers of exposure.

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