

## Assessment of DNA Damage in Workers Exposed to Organic Solvents

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### ABSTRACT

*The carcinogenic potential of organic solvents is major issue in defining human health risk in occupationally settings. Hence in the present investigation 108 workers employed in shoe manufacturing factory Nacharam (Ranga Reddy District) and 82 control subjects with similar mean ages, smoking prevalences were selected for analysis of DNA damage in human lymphocytes by comet assay. The present study clearly indicated that chronic exposure to organic solvents in workplace induced significant increase frequencies were observed in smokers thus indicate synergistic effect of occupation exposure and smoking. To ensure maximum occupational safety, biomonitoring is of great value for assessing the risk in shoe-manufacturing workers. Thus DNA damage and cellular death are known to be the important mechanism in chemical carcinogenesis. These results may be relevant in risk assessment for protecting human health and preventing carcinogenesis.*

**Key words:** DNA damage, comet assay, organic solvents

### INTRODUCTION

Organic solvents (OSs) have various effects on human health, whether the exposure is by vapour, mist or liquid form. In fact, about 50% of the synthesized organic solvents are employed for the production of paints and shoes. From the different groups of organic solvents, xylene, toluene, styrene, ethylbenzene, acetone and methyl ethylketone are considered to be some of the most frequently and quantitatively represented solvents in the composition of paints.

Due to the modern industrial and the rapid development in the field of science and technology, man is continuously exposed to the environment pollutants like industrial and

agricultural chemicals, food activities, drugs and cosmetics etc. Further exposure to organic solvents leads to chronic affect on central nervous system has been reported<sup>13</sup>, Occupational exposure to trichloro ethylene an imported volatile organic compound used clock manufacturing factory showed on skin (29.6%) and respiratory symptoms (21.1%) were observed among exposed group<sup>34</sup>, Asthma allergy skin reactions visual disorders were most prevalent in printing factory workers<sup>9</sup>. A significant hearing loss and reproductive end points were observed in chromium alloy factory and tobacco dust exposed population<sup>25,32</sup>.

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People employed in the shoe making industry are at an increased risk of leukemia and nasal cancer<sup>19</sup>, and an excess of mortality due to other types of cancer has also been reported<sup>14,15,40</sup>. Workers in shoe and boot factories are exposed to a mixture of organic solvents, among which toluene and acetone are usually the most common. Neither of these solvents is considered a genotoxin or a carcinogen; the weight of evidence from human *in vivo* studies suggests that exposure to toluene does not cause somatic cell genotoxic damage although this view has been questioned by recent studies of rotogravure printers<sup>23,17,27</sup>.

The glues and gasoline used in shoe manufacture may contain benzene, which could be responsible for some of the cancers found in shoe workers. Benzene is a well-known clastogen that requires metabolic activation to be mutagenic. The genotoxic metabolites are also thought to play an important role in benzene myelotoxicity and leukemogenesis. The quinone metabolites of benzene can break chromosomes by inducing reactive oxygen species but may also act as aneuploidogens, causing microtubule disruption<sup>36,41</sup>. Recent investigations have indicated that structural CA are increased in shoe factory workers exposed to benzene and toluene<sup>4,37</sup>.

Occupational exposure as a organic solvents is associated with DNA damage and development of cancer. Comet assay has been widely adopted as a sensitive and quantitative tool for DNA damage assessment at the individual cell level in populations exposed to genotoxics. The aim of this study was to assess the application of the high-throughput comet assay, to determine the DNA damage in workers employed in shoe manufacturing factory exposed to organic solvents.

## MATERIALS AND METHODS

Chemicals Agarose-normal melting, agarose-low melting, sodium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate, disodium ethylenediaminetetraacetic acid (disodium

EDTA), tris, sodium hydroxide, sodium dodecyl sulphate / sodium lauryl sarcosinate, tritron X 100, trichloro acetic acid, zinc sulphate, glycerol, sodium carbonate, silver nitrate, ammonium nitrate, silicotungstic acid, formaldehyde.

## Subject Recruitment and Sample Collection:

The study was conducted on 108 workers aged 16- 66 years from Telangana. The control groups consisted 80 people, aged 16-66 with no history of exposure to clastogenic and/or aneugenic agents and socio-economic level also similar to that of experimental subjects. At the time of sample collection (3ml/individual) all the tannery workers signed a term of informed consent and replied to Questionnaires elaborated to determine the profile and habits of study population. The protocol has been approved by local ethical committee. The exposed workers to chromium, the duration of service was taken more than five years. Peripheral blood samples (V = 5 ml) were collected under sterile conditions by venipuncture into heparinized tubes for comet assay.

Single Cell Gel Electrophoresis (SCGE) The comet assay was conducted under alkali conditions according to Singh et al. <sup>35</sup>. All chemicals were obtained by Sigma. Two microlitre of whole blood were suspended in 0.5% low melting agarose and sandwiched between a layer, of 0.6% normal melting agarose and a top layer of 0.5% low melting agarose on fully frosted slides. The slides were kept on ice during the polymerization of each gel-layer. After the solidification of 0.6% agarose layer, the slides were immersed in lysis solution (1% sodium sarcosinate, 2.5 M NaCl, 100 mM Na<sub>2</sub>EDTA, 10 mM Tris-HCl, 1% Triton X-100 and DMSO 10%) at 4 °C. After 1hr, the slides were placed in the electrophoresis buffer (0.3 M NaOH, 1 mM Na<sub>2</sub>EDTA, pH 10) for 20 min at room temperature to allow for DNA to unwind. The buffers were then chilled and the electrophoresis was performed at 300 mA and 19V in a horizontal electrophoresis platform for 20 min. The slides were neutralized with

Tris–HCl buffer (pH 7.5) and stained with 10% ethidium–bromide for 10 min. Each slide was analyzed by using Leitz Orthoplan epifluorescence microscope. For each subject 50 cells were analyzed by automatic digital analysis system Comet assay II (Perceptive Instruments Ltd., Suffolk, Halstead, UK),

determining tail length and tail moment (tail length×tail % DNA/100). DNA damage was further quantified by visual classification of cells into categories of ‘comets’ corresponding to the amount of DNA in the tail according to Anderson et al<sup>1</sup>.

## RESULTS

**Table 1: Demographic characteristics of study subjects**

Group	No. of subjects	Age in years	Duration of exposure
Control	82	43.33±7.3	19.5±2.8
Exposed	108	43.52±6.8	19.8±2.91
Non smokers	68	42.11±8.01	19.5±2.27
Smokers	40	42.83±7.01	19.3±2.64

**Table 2: Frequency of comet tail length in study subjects**

Group	No. of subjects	Frequency of comet tail length
Control	82	3.12±1.10
Exposed	108	
Non smokers	68	12.60±2.10*
Smokers	40	18.12±3.60*
Years of exposure (<10)	58	10.10±1.60*
Years of exposure (>10)	40	16.20±1.20*

\*P<0.01

A total of 190 subjects in shoe manufacturing unit were studied for basal DNA damage using comet assay. The results of DNA damage was given in Table 2. In exposed subjects a significant increase (P<0.05) in DNA mean tail length was observed when compared with control values. In exposed group, the frequency of comet tail length is 12.60±2.10 in non smokers and 18.12±1.20 frequency of DNA damage was observed in smokers. Thus the data indicate significant differences between smokers and non smoker groups. The frequency of comet tail length were found to be significantly higher in subjects with longer duration of exposure (P<0.05 Table 2).

## DISCUSSION

Several investigations reported that increased mortality from cancer in foot wear workers

because of exposure to organic solvents such as toluene h-hexane , acetone, dust particles, additives in shoe manufacturing workers are continuously exposed to these organic mixtures and elevated risk for cancer and respiratory diseases<sup>38</sup>.

The present results clearly indicate genotoxic nature of occupational exposure and can be comparable with that Kista et al.,<sup>21</sup> who reported increased frequency of comet tail length in solvent based adhesive group (n=16) and controls. Similarly increased frequency of micronuclei in exfoliated buccal cells human lymphocytes of workers exposed to organic solvents.<sup>33</sup>.

Mutagenesis is involved in the pathogenesis of many neoplasias. Occupational exposure may contribute to the development of pernicious illnesses, many

times through mechanisms that involve genotoxic changes. Continuous efforts have been made to identify genotoxic agents, to determine conditions of harmful exposure and to monitor populations that are excessively exposed<sup>8,39</sup>.

The present study was designed to assess the DNA damage among viscose plant workers who are occupationally exposed to CS2. Comet assay is a valuable method for detection of occupational and environmental exposures to genotoxicants, and it can be used as a tool in risk assessment for hazard characterization<sup>11,24</sup>, air pollution<sup>39</sup>, cigarette smoking<sup>31</sup> and various in vitro and in vivo studies<sup>12</sup>.

A possible indication of the heavy organic solvent exposure of the workers was the low level of blood haemoglobin when compared with control values. Similar observations were observed in shoe factory workers of Bulgenia<sup>28</sup> Anemia and other hemotological abnormalities have been associated with organic solvent exposure in petroleum workers<sup>18</sup>.

The glues and gasoline used in shoe manufacturing factory contain benzene which is well known clastogen, quinine metabolites of benzene can break chromosomes by inducing reactive oxygen species. The results of the present study also indicate significant increase in the frequency of comet tail length in workers exposed to organic solvents. The harmful effect of dust in various forms of human health have already been demonstrated<sup>10,16,29</sup> in petrol station employees<sup>2,3</sup> Cigarette smokers and tobacco users<sup>30</sup> workers exposed to pesticides<sup>26</sup> Polycyclic Hydrocarbons<sup>20</sup> timber dust<sup>6</sup> Ozone and Cancer patients<sup>3,7</sup>.

The results of present investigation clearly indicate combined effect of cigarette smoke and occupational exposure enhances the frequency of basal DNA damage in lymphocytes of human subjects exposed to organic solvents. Similar results were obtained in our previous studies occupational exposed to lead based painters<sup>22</sup> population exposed to organic solvents<sup>33</sup>.

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