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BIOLOGICAL MONITORING OF EXPOSURE TO BENZENE AND TOLUENE IN TYRE RETREADERS

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ABSTRACT: Workers engaged in tyre retreading face the possibility of occupational exposure to benzene and toluene as these are used as solvents and glue. Exposure to these solvents in other industries has been aptly described in several reports. Considering important from public health point of view, a study on biological monitoring of benzene and toluene exposure in tyre retreaders was performed Urine samples were analyzed for the metabolites of benzene and toluene viz. phenol and hippuric acid respectively. The information like smoking, alcohol intake, age and duration of job was sought after personal interviews. Results showed, that population of smokers excreted less phenol but high hippuric acid in comparison to non smokers. Alcohol intake inhibited the metabolism of benzene and toluene. Excretion of phenol and hippuric acid was lower in alcoholics than non alcoholics. Age also affected the excretion of phenol. It was found to be lowest in the age group of 30-40 years. Excretion of phenol was lowest in workers having the work experience of 16-20 years, whereas, excretion of hippuric acid was lowest in workers having the experience of 6-10 years. These observations confirmed that tyre retreaders are exposed to benzene and toluene through their work environment.

INTRODUCTION: Benzene and toluene have been used in various industries as glues or Solvents all over the world. The main risk to the health of workers is from inhalation of organic solvents in the form of vapour or aerosol. At apparent steady state, 30% to 65% of inhaled benzene enters systemic circulation [1]. Skin absorption of liquid benzene is significant [2]. Since 1981, a preparation containing more than 1% benzene is not allowed to be manufactured, used or dealt with in the workplace, except in laboratories and in those situations benzene must be used in a completely sealed process as specified in Industrial Safety and Health Act (ISHA).

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The carcinogenicity of benzene was well known in human studies, since it caused acute myelocytic leukemia in 1920s [3]. Cases of hematopoietic cancer have been reported in shoe manufacturing factories in Italy and Turkey [4, 5]. Development of suitable biomarkers can be of great help in molecular epidemiology of benzene exposed workers [6].

Toluene is highly soluble in blood and fat. The major route of absorption is through inhalation and skin absorption. The recommended threshold limit value time weighted average (TLV-TWA) of 50ppm of toluene is based on preventing such effects [7]. The most important health concern for human from exposure to toluene is harmful effect on nervous system. Toluene causes an increased tendency to sleep, frequent headache, eye irritation and memory impairment in human [8]; cerebral and hippocampal atrophy as well as a loss in brain volume [9, 10]. The effect of toluene are dependent on both the amount and duration of exposure. Painters,

glue sniffers and shoe makers are also exposed to toluene.

Biological monitoring of human exposure to benzene and toluene has been performed as a component of health surveillance programs in several countries [11-14]. Cases with disabilities attributed to solvent exposure have been intensively studied and solvent induced changes described in previous studies [15-20]. Urinary excretion of unmodified solvents is a highly sensitive and specific exposure index, and can also be applied for the biological monitoring of occupational exposure to solvents or to solvent mixtures [21]. Decharat (2014) determined the hippuric acid level in urine samples of paint workers at steel furniture industry in Thailand [22]. An extensive study to identify workers' exposure to hazardous substances in various tire manufacturing processes was made by Lee et al. [23] in Korea. Present study was undertaken to record exposure to benzene and toluene in a population of tyre retreaders employing specific parameters of biological monitoring. This study will be helpful in developing preventive measures against exposure of organic solvents in industrial workers.

MATERIALS AND METHODS: A large number of Workers were found engaged in tyre retreading industry in Northern India. Tyre retreaders, from different areas of Meerut city were registered and information like age, work experience, smoking habit, alcohol consumption, and overall health conditions were sought after personal interviews. Healthy persons who had never been occupationally exposed to benzene and toluene, and living in clean surroundings were selected as controls. During the survey, urine samples were collected in white sterile bottles from all subjects at the end of work shift. These samples were transported in liquid nitrogen container (Inox India Ltd., India) to the laboratory and stored at -20°C till analyses. At room temperature the samples were processed for the determination of specific gravity, creatinine, phenol and hippuric acid applying following methods.

Specific gravity:
Specific gravity of urine samples was determined by a urinometer (Atago Company Ltd. Tokyo, Japan). Urine samples having specific gravity between 1.010-1.040 were selected for further analysis.

Creatinine:
Creatinine was determined by alkaline picrate method [24] using a commercial kit supplied by Span Diagnostics Ltd., India. The absorbance was recorded at 520 nm by using Systronics visual spectrophotometer (Ahmedabad, India).

Phenol:
Phenol, metabolite of benzene was estimated following the amino-antipyrine method of Dannis [25]. Pure liquid phenol (Central Drug House Pvt. Ltd., Mumbai) was used as standard. The absorbance was recorded at 510 nm.

Hippuric acid:
Hippuric acid in the urine samples was determined by applying the method of Ogata and Hobara [26], using para-dimethyl amino-benzaldehyde in pyridine as the extraction medium. The absorbance was recorded at 440 nm.

Statistical analyses:
All results were expressed as mean ± SE. Statistical significance (p) was calculated by Student's "t"-test [27].

RESULTS: According to World Health Organization, Occupational Safety and Health Assessment and American Conference of Governmental Industrial Hygienists, the specific gravity of urine samples is the first observation to be made in health risk assessment of human subjects exposed to different occupational agents. In this study specific gravity of urine was higher in smokers compared to non-smoking population of tyre retreaders (**Table 1**). Specific gravity of urine samples of non-alcoholics was higher than alcoholic population (**Table 1**). Tyre retreaders, in age group of 10-20 and 41-50 years showed higher values of specific gravity of urine in comparison to age group of 21-40 years (Table 2). Higher specific gravity values were found in tyre retreaders having work experience of 16-20 and 1-5 years, followed by the subjects having work experience of 11-15 and 6-10 years (**Table 3**). Specific gravity of urine

sample of all tyre retreaders was significantly ($p<0.05$) higher than the control subjects.

For any observation dealing with kidney function or excretion of the toxin, it becomes mandatory to determine creatinine. Creatinine excretion was five times higher in tyre retreaders compared to healthy subjects; It clearly indicated the effect of compounds, used in tyre retreading, on kidney function. All the tyre retreaders showed significantly higher creatinine values than the control subjects. It was higher in non-smoker and non-alcoholic population of tyre retreaders in comparison to smokers and alcoholics (**Fig. 1 and 2**). Urinary excretion of creatinine was lowest in tyre retreaders of age group 31-40 years (**Fig. 3).** Work experience of 1-20 years showed significant differences in creatinine values. It increased initially from 1-5 years, decreased in the subjects having work experience of 6-10 years, again increased in subjects having work experience of 16-20 years (**Fig. 4**).

Phenol is a metabolite of benzene. It is used as a reliable marker of benzene exposure among the industrial workers. It was found Significantly ($p<0.05$) higher in tyre retreaders as compare to control subjects. It was higher in non-smokers and non-alcoholics in comparison to smokers and alcoholics (**Fig. 1 and 2**). Significant differences were observed among different age groups. It was higher in subjects ranging between 10-20 years whereas the values of phenol decreased in age group of 21-50 years (**Fig. 3**). Work experience again was found to be effective, determining the toxicity of benzene in tyre retreaders. Values were found to be higher in workers initially exposed to benzene through tyre retreading (**Fig. 4**).

Hippuric acid is a metabolite of toluene. It is used as a reliable marker of exposure to toluene. Values of hippuric acid in tyre retreaders were significantly increased ($p<0.05$) than the control subjects. It was found to be higher in smokers and non-alcoholics in comparison to non-smokers and alcoholics (**Fig. 1** and **2**). The age also affected the excretion of hippuric acid. It was found to be higher in subjects having age of 41-50 years (**Fig. 3**). Work experience also determined the excretion of hippuric acid. It was found to be higher in tyre retreaders having an work experience of 16-20 years. The minimum values were observed in work group having experience of 6-10 years (**Fig. 4**).

TABLE 1: SPECIFIC GRAVITY OF URINE SAMPLES WITH REFERENCE TO SMOKING AND ALCOHOL CONSUMPTION

Group	Specific gravity
Smokers	1.028 ± 0.002*
Non-smokers	1.027 ± 0.002*
Alcoholics	1.021 ± 0.001*
Non-alcoholics	1.032 ± 0.0009*
Controls	1.012 ± 0.0006

Results are expressed as mean ± SE (n=5). *values are significantly different from controls. $p<0.05$

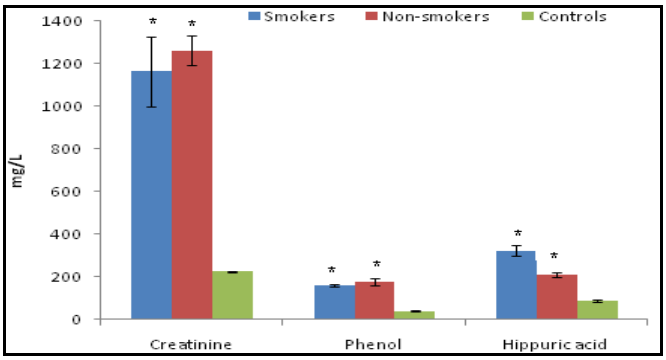

FIG. 1: CREATININE, PHENOL AND HIPPURIC ACID IN URINE SAMPLES OF TYRE RETREADERS WITH REFERENCE TO SMOKING. ASTERISK (*) REPRESENTS SIGNIFICANT DIFFERENCES FROM CONTROL. (p<0.05)

TABLE 2: SPECIFIC GRAVITY OF URINE SAMPLES WITH REFERENCE TO AGE OF TYRE RETREADERS

Age group	Specific gravity
10-20 years	1.026 ± 0.002*
21-30 years	1.020 ± 0.003*
31-40 years	1.021 ± 0.003*
41-50 years	1.026 ± 0.001*
Controls	1.012 ± 0.0006

Results are expressed as mean ± SE (n=5). *values are significantly different from controls. $p<0.05$

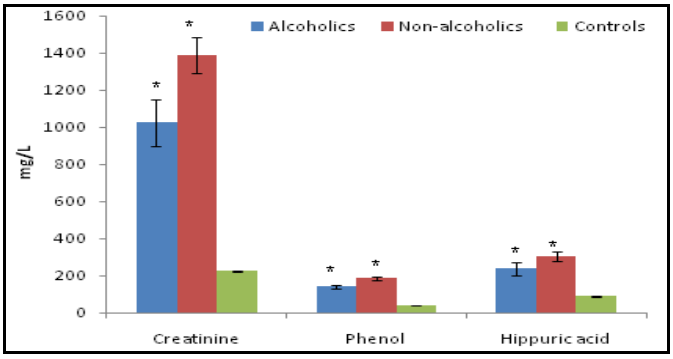

FIG. 2: CREATININE, PHENOL AND HIPPURIC ACID IN URINE SAMPLES OF TYRE RETREADERS WITH REFERENCE TO ALCOHOL CONSUMPTION. ASTERISK (*) REPRESENTS SIGNIFICANT DIFFERENCES FROM CONTROL. (p<0.05)

TABLE 3: SPECIFIC GRAVITY OF URINE SAMPLES WITH REFERENCE TO WORK EXPERIENCE OF TYRE RETREADERS

Work experience	Specific gravity
1-5 years	1.029 ± 0.001*
6-10 years	1.022 ± 0.002*
11-15 years	1.018 ± 0.001*
16-20 years	1.031 ± 0.002*
Controls	1.012 ± 0.0006

Results are expressed as mean ± SE (n=5). *values are significantly different from controls. p<0.05

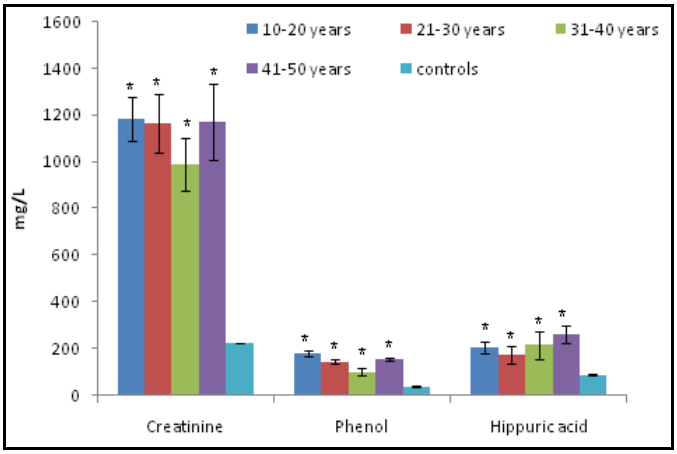

FIG. 3: CREATININE, PHENOL AND HIPPURIC ACID IN URINE SAMPLES OF TYRE RETREADERS WITH REFERENCE TO AGE. ASTERISK (*) REPRESENTS SIGNIFICANT DIFFERENCES FROM CONTROL. (p<0.05)

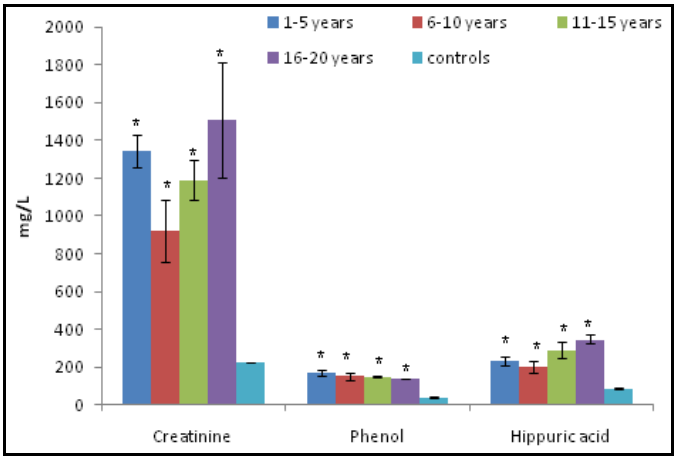

FIG. 4: CREATININE, PHENOL AND HIPPURIC ACID IN URINE SAMPLES OF TYRE RETREADERS WITH REFERENCE TO WORK EXPERIENCE. ASTERISK (*) REPRESENTS SIGNIFICANT DIFFERENCES FROM CONTROL. (p<0.05)

DISCUSSION: Investigations were made to find out the exposure to benzene, if any, in tyre retreaders. High values of phenol in urine samples indicate that these workers are facing the problem of occupational exposure to benzene. Exposure to benzene in workplaces can occur mainly via inhalation and dermal absorption. Almost one-third of retained benzene is excreted rapidly in urine as conjugate phenol and dihydroxy phenol [28, 29].

Oral exposure can occur from accidental or intentional consumption of benzene containing products. Benzene can be irritating to mucous membrane; acute exposure to high concentration can produce pulmonary irritation and edema, dermatitis and gastro-intestinal irritation in tyre retreaders. Exposures to benzene can cause symptoms typical to organic solvent toxicity. Symptoms may progress from exposure euphoria, headache, vertigo, depression, confusion, seizures and the respiratory failure in severe exposures. There are chances that chronic exposure to benzene and toluene in tyre workers may lead to serious health problems. Several factors may affect the toxicity of benzene and toluene. These include exposure to other xenobiotics, diet, age, sex and genetics, etc.

Toluene is used as a precursor in the production of polyurethane polyamide and simple polymer of isocyanates including foams. It is highly volatile compound with poor olfactory warning properties. Exposure to toluene may cause skin irritation [30], pulmonary irritation, bronchitis and pulmonary edema. Both acute and chronic exposures to toluene are related with central nervous system (CNS) alterations such as fatigue, confusion and in-coordination. At 200-400ppm concentration of toluene, headache, nausea, eye irritation, loss of aptitude, bad taste, lassitude and in-coordination are reported with long term exposure with long term exposure. Blood abnormalities, cytochrome disorders, change in lens of eye, immune system, and renal problems have also been observed in industrial workers. No such report is available in tyre workers in India. Our results showed that workers in this industry are facing problems related to toluene exposure.

No gender-specific study was conducted as no female worker was found to be engaged in tyre retreading business. However, among the male workers, effects of smoking, alcohol consumption, age and work experience was observed. Smokers excreted less phenol but high hippuric acid compared to non-smokers. Similarly, consumption of alcohol inhibited the metabolism of benzene and

toluene. Excretion of both phenol and hippuric acid were lower in alcoholics than non-alcoholics. Although the activities of liver drug metabolizing enzymes is increased by chronic alcohol administration [31].

Phenol excretion was lowest in the age group of 30-40 years whereas, excretion of hippuric acid in age group of 20-30 years. Work experience or total exposure duration to benzene and toluene also affected the excretion of phenol and hippuric acid. Excretion of phenol was lowest in workers having experience of 16-20 years whereas, lowest hippuric acid was found in 6-10 year group. This suggested that the relation of exposure duration to performance is also remarkable. Although, results do not suggested any widespread subclinical change resulting from occupational exposure to either benzene or toluene used in tyre retreading process. All these observations showed that in addition to styrene, ethyl butadiene etc., the tyre retreaders also suffered the problem of exposure to benzene and toluene.

CONCLUSION: In this study, results indicated that smoking, alcohol consumption, age and period of exposure to chemicals used in tyre retreading determine the toxicity of benzene and toluene. Present result confirmed the exposure to benzene and toluene in the tyre retreaders. Tyre retreaders are directly exposed to these solvents through their work environment, thus it is mandatory to aware them about the safety measures and protective devices.

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