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DETERMINATION OF URINARY CONCENTRATIONS OF ORGANIC SOLVENT IN URBAN WORKERS

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ABSTRACT

Introduction: Concerns about indoor air quality have drawn researcher's attention in the last years. This becomes more important with knowledge of that 90% of people's daily times are spent inside the home and workplaces. Solvents are an example of prevalent hazard chemical, which are less-studied comparing pesticides or metals. Chlorinated solvents such as carbon tetrachloride, chloroform, and dichloromethane constitute an important class of solvent, which applies for a variety of consumer and industrial cleaning purposes especially in the laboratory. Mentioned components represent various side effects and carcinogenic implication that could adversely affect workers exposed to solvents.

Materials and Methods: In the present study the excretion of urinary carbon tetrachloride, chloroform, and dichloromethane were evaluated as biomarkers of exposure to chlorinated solvents. With this aim, forty chemistry laboratory technicians from several universities in Tehran and forty occupationally non-exposed persons were investigated. Spot urine samples were obtained prior to and at the end of the work shift from each subject. The urinary levels of chlorinated solvents were determined by using headspace gas chromatography and mass spectrometry detection.

Results: The mean concentrations of chloroform and dichloromethane in chemistry laboratory technicians were significantly greater than the control groups. Although the mean levels of carbon tetrachloride before the work shift in technicians were higher than the occupationally non-exposed group, a statistically significant difference could not be observed (P_{value} = 0.324).

Conclusion: The results showed that the laboratory technicians are one of the most exposed groups among occupationally exposed people with the main route of exposure through inhalation.

INTRODUCTION

Recently, there has been wide concern about the effects of indoor air quality on human health. This is frequently, often due to the fact that almost 90% of people's every day time is being spent inside either workplaces and home [1]. Occupational environments have always been a source of chemical exposure to workers from point and non-point sources [2]. However, the indoor air quality has not taken into consideration as it should have been. Furthermore, the places

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which people are in direct contact with chemical compounds, including chemical laboratory could pose human at risk [3]. Therefore, this issue should be paid more attention; otherwise, people may suffer from the poor indoor working place air quality [4]. Situations of human exposure to chemical hazards include during transportation, distribution and application of organic solvents into the immediate environment [5]. Although long considered as possible human disease risk factors, solvents have received somewhat less attention than pesticides or metals despite the prevalence solvent used in many workplaces [6]. Solvents are classified by their chemical characteristics, organic or inorganic, and also by the chemical composition, such as chlorine substitution [7]. Exposure can occur through inhalation due to volatilization of the solvent, or dermal uptake, or ingestion, depending on exposure source and chemical composition. Chlorinated solvents such as carbon tetrachloride (CTC), chloroform, and dichloromethane (DCM) have been used for a variety of consumer and industrial cleaning purposes due to their ability to dissolve organic substances [8]. These three chlorinated solvents are among the most widely used in this solvent group. Most of chlorinated solvents are common solvents in the laboratory because they are relatively unreactive and miscible with most organic liquids, and conveniently volatile [9]. The prevalence use of DCM, CTC and chloroform and on the other hand the resultant potential for exposure to them representing a concern to public health. Many various side effects including toxicity to the liver, kidney, lungs, neurotoxicological effects, and carcinogenic effects have been reported in the literature [8, 10-13]. These chlorinated solvents (DCM, CTC, and chloroform) are classified in the 2B class ("possible" human carcinogen) by the International Agency for Research on Cancer [14].

In recent decades, several studies have demonstrated the ability of urinary chemicals to be used as biomarkers of exposure [15-22]. Recent studies showed that the determination of unmetabolized solvents in urine provides a highly sensitive and specific index of exposure to chlorinated solvent [15, 17-22]**.** Chloroform, CTC and DCM can be eliminated unchanged in exhaled air and urine. For the assessment of occupational exposure to the chlorinated solvents determination of the unmetabolized compounds or their metabolites in urine and blood were suggested [23-28]. Therefore, analysis of the concentration of unmetabolized compounds in urine detected after the work shift seems to give the most reliable estimation of exposure, so in this study the unmetabolized chloroform, CTC and DCM were selected as suitable biomarkers of routine solvents exposure. The aim of this study was to determine the exposure levels of chloroform, CTC and DCM for laboratory technicians during routine work shift, by biological monitoring.

MATERIALS AND METHODS

Study population

Eighty healthy men from the city of Tehran, Iran, were enrolled in this study. The study population included 40 chemistry laboratory technicians from several universities in Tehran as an occupationally exposed group and for the measurement of any background levels originating from other sources such as ambient air, urine samples were collected from 40 occupationally non-exposed persons in the same organization acted as referents. All of the subjects were men between 27 and 43 (mean) 29years old and none of them were smokers. In order to dermal protection, it was requested from laboratory technicians to wear plastic gloves during work shift.

Sampling

The exposure measurements were carried out in September 2012. Urine samples were collected at the beginning and the end of the shift. Urine samples were stored in a cooling box until transferred to the laboratory, where they were divided into several fractions and frozen at -20°C until analysis.

Analysis of urine samples

Head space solid-phase microextraction (HS-SPME) appears to be a solvent- free extraction technique as an attractive alternative to most of the conventional sampling techniques [29]. This technique is based on the distribution of the analytes to a fused-silica fiber coated with a stationary phase. For the HS- SPME determinations, a manual SPME holder and fibers were used. The fibers were conditioned at 20°C higher than the desorption temperature (270°C). Two blank injections were performed before the actual analysis. Between uses, fibers were kept sealed from ambient air by piercing the tip of the SPME needle into a small piece of septum to prevent accidental contamination. The HS-SPME parameters were determined by experiments in which some parameters kept constant and the remaining one was modified to find an optimum condition [30]. Urine samples were collected in polyethylene bottles. An amount of 2 ml of urine was immediately transferred into the 10-ml headspace vials containing 1g NaCl to saturate the aqueous solution. Then 0.2 ml of internal standard (hexane in water, 160 ug/l) was added. The vials were sealed using the caps with a Teflon membrane. The vial was placed in a water bath maintained at 60±0.1 °C for 15 min to establish phase equilibrium. The vial and SPME holder were clamped into a stand that allowed the vial to be immersed in the water bath only up to the level of the liquid in the vial. Next, the SPME needle was exposed to headspace so as to locate the tip of the exposed fiber approximately 0.5 cm from the top of the liquid. Headspace adsorption time was 35 min. The fiber was then retracted, removed from the vial, and placed immediately into the injector of the GC system. After that, the fiber was collected and inserted in the chromatograph injector. Desorption time in the injector was 4.5 min, and the splitter was opened after 3 min. Determinations were performed by means of gas chromatography (Agilent GC/MS 6890/5973 detector; HP-5, 30 m × 0.25 mm × 1 μm). The temperatures were as follows: injector temperature170°C, initial oven temperature 45 °C (held for 5 min), increased to 90°C at a rate of 5°C/min and held for 2 min then, increased to the final temperature 280°C at a rate of 30°C/min where it was held for 1 min .Helium was used as carrier gas, at flow rate of 1 mL/min. The limits of quantification (LOQ) values for all compounds were 0.005 ng/mL.

Statistical analysis

Mean urinary concentration of Chloroform, Dichloromethane and carbon tetrachloride between two groups (laboratory technicians and Control group) were analyzed and because the distribution of data was not normal, the analysis was carried out by means of two statistical procedures: analysis of variance (one- way ANOVA) followed by Scheff's post hoc test and Kruskal-Wallis test. Results were expressed as mean ± S.D. and 95% confidence intervals. The level of significance was set to 0.05 and P_{values} >0.05 were assumed to be non-significant.

RESULTS AND DISCUSSION

Tables 1 and 2 show the results of the mean urinary concentration of chloroform, dichloromethane and carbon tetrachloride determinations (ng/mL) in the two groups of workers in two different samples collected: before starting work and at the end of the shift.

Table 1. Mean urinary concentration (S.D.) of Chloroform, Dichloromethane and carbon tetrachloride in two groups (n = 40) before starting work.

Groups	Chloroform (ng/ml)	Dichloromethane (ng/ml)	Carbon tetrachloride (ng/ml)
Laboratory technicians	0.62 (0.43)	1.15 (1.25)	0.09 (0.08)
Control group members	0.12 (0.21)	0.79 (0.09)	0.03 (0.08)
P_{value} *	0.024	0.048	0.325
P_{value} **	0.000	0.000	0.000

*One-way ANOVA, **Kruskal–Wallis test. SD: Standard deviation

Table 2. Mean urinary concentration (S.D.) of Chloroform, Dichloromethane and carbon Tetrachloride in two groups (n = 40) at the end of work shift.

Groups	Chloroform (ng/ml)	Dichloromethane (ng/ml)	Carbon tetrachloride (ng/ml)
Laboratory technicians	2.55 (2.10)	1.89 (2.91)	0.09 (0.07)
Control group members	0.23 (0.38)	0.097 (0.11)	0.03 (0.08)
P_{value} *	0.010	0.031	0.431
P_{value} **	0.000	0.000	0.000

*One-way ANOVA, **Kruskal–Wallis test. SD: Standard deviation

The urinary concentrations of all analysts of subjects with job associated with exposure to solvents (chemistry laboratory technicians) before starting the work compared with the no exposure group. The mean concentrations of chloroform and DCM in chemistry laboratory technicians were significantly greater than the control groups. Although the mean levels of CTC before the work shift in technicians were higher than the occupationally non-exposed group, a statistically significant difference could not be observed (P_{value}= 0.324). The total chloroform and DCM uptake during the work shift in laboratory technicians and occupationally non-exposed group were calculated to be on average 1.93±2.1, 0.35±0.6 and 0.69±1.87, 0.02±0.04 ng/mL, respectively. The concentration of all the analyets increased during the day for both groups. The increase is more marked among laboratory technicians. As can be seen in Table 2, excretion of chloroform, DCM in laboratory technicians after the work shift were significantly higher than the control group (P<0.001). The concentration of chloroform, DCM in laboratory technicians increased during the day and reached 2.54±2.1 and 1.84±1.9 respectively. The urine concentration of chloroform and dichloromethane in both work shifts followed the order: technicians> control group members.

The aim of this study was to monitor the degree of chronic and acute exposure to chlorinated solvents (chloroform, dichloromethane and carbon tetrachloride) of two groups of laboratory technicians and control group members, through the evaluation of urinary concentrations.

The chemistry laboratory technicians were chosen for the study because their exposure was expected to be relatively high and there were few reports in literature on the biological monitoring of coincident chlorinated solvents in urine samples from this group. Regarding the choice of biomarkers (chloroform, carbon tetrachloride, dichloromethane) some previous studies [24, 27, 31, 32] showed a good correlation between their airborne exposure concentrations and the biological results. In general, the advantage of using unmetabolized compounds as biomarkers is that the urinary concentration of the unmetabolized substance is less influenced by inter individual metabolic differences than the urinary disposition of corresponding metabolites [33]**.** For the assessment of exposure to chlorinated solvents the urinary concentrations of analytes (chloroform, CTC and DCM) after the work shift can be used as an indicator of a day's (acute) exposure, whereas the urinary concentration of solvents before the one day shift allows the monitoring of repeated (chronic) exposure. A significant difference was observed in chloroform and DCM concentrations in both pre and post-shift samples among job categories (P<0.05 by ANOVA and Kruskal-Wallis test). The mean chloroform and DCM concentrations in exposed workers were significantly greater than unexposed group. Since chloroform and DCM are widely consumed in the chemistry laboratories, usually occur at higher concentrations than CTC (EPA's Toxic Release Inventory (TRI) database, 2008).

Since there might be a significant risk of dermal exposure, the laboratory technicians were requested to wear plastic gloves during the work shift, so no significant skin exposure occurred during the study. The CTC level detected in this study was much lower than levels which are reported to photocopy centers workers [31]. In

another study urinary concentration of DCM in the unexposed general population (120 subjects; mean age, 38.6±6.6 years) conducted; their results showed the uptake of small amounts of DCM [34]. The result of this study showed that the laboratory technicians are one of the most exposed groups among occupationally exposed people. Inhalation is presumably the main route of exposure in chemistry laboratory technicians.

CONCLUSIONS

The result of this study showed that the laboratory technicians are one of the most exposed groups among occupationally exposed people. Inhalation is presumably the main route of exposure in chemistry laboratory technicians.

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COMPETING INTERESTS

The authors declare that they have no conflicts of interest.

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ETHICAL CONSIDERATIONS

The authors state that they have no ethical considerations.

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