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Assessment of Occupational Exposure to N-hexane: A Study in Shoe Making Workshops

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ABSTRACT

The n-hexane is widely used in the production of glues, lacquers, paints, plastic and rubber products. Therefore, a significant potential for exposure to this toxic solvent exists in industrial settings. This study was carried out to assess the individual exposure of workers to n-hexane and their urinary concentration of free 2, 5-hexanedione were determined. Thirty-eight male workers from 6 shoe making workshops were studied. Individual exposure of workers to n-hexane and theiv urinary concentration of free 2, 5-hexanedione were determined. Data were analyzed using the SPSS/PC statistical package version 16.0. The calculated time-weighted average exposure to n-hexane and the average urinary concentration of free 2, 5-hexanedione were estimated to be 76.8 mg m^{-3} and 0.241 mg L^{-1}, respectively. A good correlation ($r = 0.815$) was found between time-weighted average exposure to n-hexane and urinary levels of free 2, 5-hexanedione. Significantly higher urinary concentrations of free 2, 5-hexanedione detected in subjects who did not wear gloves. Our data indicate that workers' average exposure to n-hexane and the levels of free 2, 5-hexanedione in their urine did not exceed the current proposed threshold limit value and biological exposure index for this chemical. Additionally, our findings provide further evidence in favor of the notion that free 2, 5-hexanedione is an appropriate indicator for biological monitoring of workers exposed to n-hexane. Finally, it is implied that dermal exposure also plays an important role in the overall absorption of n-hexane by the human body.

Key words: Shoe making, n-hexane, personal air monitoring, biological monitoring, 2, 5-hexanedione

INTRODUCTION

Hexane is a colorless, clear, highly volatile liquid under standard conditions (ATSDR, 1999). "Hexane" refers to the technical or commercial material that may contain a blend of n-hexane and a variety of other hydrocarbons such as toluene, acetone, Methyl Ethyl Ketone (MEK), dichloromethane, trichloroethylene, pentane isomers and heptane isomers. These formulations may contain anywhere from 20 to 80% n-hexane (ATSDR, 1999). The term "normal" hexane (HEX)

refers to the specific linear unbranched hexane isomer. HEX is widely used in the production of glues, lacquers, paints, plastic and rubber products (Pryor and Rebert, 1992; Jorgensen and Cohr, 1981). Therefore, a significant potential for exposure to this toxic solvent exists in industrial settings. The main route of exposure to HEX is inhalation. However, dermal absorption is also possible (Cardona *et al*., 1993; Prieto *et al*., 2003). Toxicological studies have shown that exposure to HEX can cause sensorimotor peripheral polyneuropathies in occupationally exposed individuals (Chang *et al*., 1993; Pastore *et al*., 2002) and in glue-sniffing addicts (Smith and Albers, 1997; Chang *et al*., 1998).

When entering the body, HEX undergoes a complex metabolism, leading to the formation of 2, 5-hexanedione (2, 5-HD) which has been shown to be the only neurotoxic metabolite of HEX, responsible for the damage of nervous system of exposed individuals. Urinary concentration of this metabolite has been proposed as a useful indicator in biological monitoring of individuals occupationally exposed to HEX (ACGIH, 2010). During 1980s, most of conventional methods used for the determination of urinary concentration of 2, 5-HD involved the acid or enzymatic hydrolysis of the urine samples (Perbellini *et al*., 1981; Ahonen and Schimberg, 1988; Saito *et al*., 1991) and the level of 2, 5-HD in these hydrolysed urine samples (total 2, 5-HD) was used for biological monitoring of workers exposed to HEX. However, later studies showed that treatment of urine with acid hydrolysis converts other HEX metabolite, 4, 5-dihydroxy-2-hexanone, into 2, 5-HD which is not neurotoxic and is conjugated to glucuronic acid and, thus, easily excreted in urine (Fedtke and Bolt, 1986). Moreover, the hydrolysis of urine is not a necessary step in the measurement of 2, 5-HD, since it is excreted unbound in urine (Fedtke and Bolt, 1987). Therefore, in 2001, the American conference of governmental industrial hygienists (ACGIH, 2001) suggested that free urinary 2, 5-HD (without hydrolysis) be measured in biological monitoring of individuals exposed to HEX instead of total 2, 5-HD and proposed a value of 0.4 mg L^{-1} as the reference value for free 2, 5-HD.

Since then, limited studies have been undertaken to measure the free 2, 5-HD. Additionally, important factors that influence the levels of 2, 5-HD in urine such as the treatment of urine samples (acid or enzymatic hydrolysis), simultaneous exposure to other solvents that affect the metabolism of HEX and the time of sampling have been overlooked in many, if not all, earlier studies. Similarly, earlier studies suffer from other limitations such as the lack of accurate exposure estimates, failure to report air concentrations of HEX and therefore failure to report time-weighted average (TWA) exposure of workers. Likewise, some studies did not specify whether the hexane was a commercial formulation or HEX.

Given the above, this study was undertaken with the following objectives:

- To assess the extent to which traditional shoemakers in small workshops were exposed to n-hexane
- To detect the relationship between time-weighted average (TWA) exposure to HEX and urinary concentration of free 2, 5-HD

MATERIALS AND METHODS

Subjects and study design: This cross-sectional study was carried out at local traditional shoemaking workshops in Kermanshah city, west of Iran. Thirty-eight male workers occupationally exposed to glues containing HEX in 6 shoemaking workshops were studied. The number of workers was between 4 and 8 in different workshops who worked in different work shifts. Some of the

workers wore gloves during work, but most of them did not. The concentration of HEX in air samples collected at the breathing zone of workers was measured and workers' TWA exposure was determined. For biological monitoring, urine samples were collected from shoemakers at the end of shift on the last day of a workweek (Thursday).

Chemicals: HEX, carbon disulfide, 2, 5-HD, cyclohexanone and dichloromethane were purchased from Merck, Darmstadt, Germany.

Exposure assessment Air monitoring: A total of 12 subjects were selected (2 workers from each workshop) for air sampling in order to determine the TWA exposure to HEX. For this purpose, 84 air samples were taken (7 air samples for each individual). To determine the individual exposure to HEX, partial-period (%75 of work shift) consecutive air samples were collected from breathing zone of workers, utilizing calibrated battery-powered constant flow pumps (SKC-Universal XR Pump Model PCXR8) and glass tubes (SKC 226-01). The length, external and internal diameter of the tubes was 7, 0.6 and 0.4 cm, respectively. Tubes were flame-sealed ends, containing two sections of activated coconut shell charcoal (front = 100 mg, back = 50 mg) separated by a 2 mM urethane foam plug. The breathing zone air samples were collected at a flow rate of 0.2 L min^{-1} under normal operating conditions. To avoid saturation, the activated charcoal tubes were replaced every 40 min during sampling period. The flow rate was measured before and after each sampling (for each charcoal tube) and the average used for calculation of the volume of air sampled. Absorbed HEX vapors were measured by the method recommended by the NIOSH (2003), desorbing the activated charcoal with 1 mL carbon disulfide. For quantifying the levels of HEX, 1 μL of sample was injected (split 1:50) into a Varian CP-3800 gas chromatograph equipped with a flame ionization detector (GC-FID) and a 30 m long×0.25 mM diameter capillary column, packed with 100% dimethylpolysiloxane. The injection block and detector temperature were 250, 300°C, respectively. Initial oven temperature was 40°C for 6 min, followed by increases of 10°C min^{-1} up to 230°C, maintaining for 1 min. Helium was used as a carrier gas at a flow rate of 1 mL^{-1}. The limit of detection (LOD) was 1.14 μg^{-1} sample.

Biological monitoring: Free urinary 2, 5-HD (without acid hydrolysis) determined by methodology published by Perbellini *et al.* (1981) as modified by Dos Santos *et al.* (2002), because its concentration is directly related to the neurotoxic potential of HEX (unlike other HEX metabolites that are not neurotoxic and are conjugated to glucuronic acid and therefore, easily excreted in urine (Fedtke and Bolt, 1986).

Urine samples were collected from shoemakers at the end of shift on the last day of a workweek (Thursday) and stored at -4°C until analysis. Urine samples with a specific gravity below 1.01 g cm^{-3} or higher than 1.03 g cm^{-3} were discarded. To determine free 2, 5-HD, 1 g of NaCl, 60 μL of cyclohexanone (as internal standard) and 2 mL of dichloromethane were added to 5 mL of a urine sample and shaken for 90 s, then the mixture was centrifuged (~2300 g) for 15 min. The resulting extracts were evaporated by nitrogen flow up to a volume of 0.3 mL and 1 μL injected (split 1:20) into a Varian CP-3800 gas chromatograph equipped with a flame ionization detector (GC-FID) and a 30 m long×0.25 mM diameter capillary column, packed with 100% dimethylpolysiloxane using the following oven temperature: initial temperature of 60°C for 2 min followed by increasing the temperature at a pace of $10°C^{-1}$ min up to 120°C maintained for 4 min and subsequent increment

of 50°C min up to 180°C maintained for 2 min. The temperature of injector and detector were 200 and 250°C, respectively. Helium was used as carrier gas at a flow rate of 2 mL min^{-1}. The detection limit was 0.02 mg L^{-1}.

Statistical analysis: Data were analyzed using SPSS, V 16.0 on a personal computer. Descriptive statistical parameters for values of the quantitative variables obtained by biological and personal air monitoring were calculated. Relationship between the urinary concentration of free 2, 5-HD and TWA exposure to HEX was studied by linear regression analysis. The comparison of the mean of urinary levels of free 2, 5-HD in workers wearing and not wearing gloves was conducted using the Mann-Whitney non-parametric U-test.

RESULTS AND DISCUSSION

The average age (year) and duration of employment (year) for workers exposed to HEX were 25.9±6 (17-44 year) and 3±1.9 (0.5-12 year), respectively. The duration of the shifts of the workers studied was 9.2 h daily and they were working 6 days a week. Only 2 workers were smoker and the remaining 36 workers were not smoker. Qualitative analysis of air samples revealed HEX, cyclohexane, pentane, toluene and ethyl acetate were present in the workshops. The results of measurement of personal exposure to HEX and biological monitoring of workers are shown in Table 1. As seen, TWA exposure to HEX was 76.8 mg m^{-3} which is lower than the respective TLV-TWA proposed by the ACGIH (2010). Urinary concentration of free 2, 5-HD in workers either individually or as an average (0.241 mg L^{-1}) was lower than the proposed BEI (0.4 mg L^{-1}, ACGIH, 2010). Only in 1 worker with TWA exposure of 43 mg m^{-3}, 2,5-HD was not detected.

Table 2 depicts the linear correlation between 9.2-h TWA exposure to HEX and urinary concentration of free 2, 5-HD in urine collected at the end of the working shift. As seen, there was a very good correlation between 9.2 h TWA exposure to HEX and urinary concentration of free 2, 5-HD ($r = 0.815$). Applying this regression equation, the urinary concentration of free 2, 5-HD following exposure to recommended TLV-TWA for HEX (176 mg m^{-3}, ACGIH, 2010) was calculated to be 0.6 mg L^{-1}.

The levels of free 2, 5-HD in urine of workers in association with the use of gloves are shown in Table 3. The arithmetic average 9.2-h TWA exposure for workers wearing gloves and those who did not were 70.6 mg m^{-3} and 80.2 mg m^{-3}, respectively. At equal exposure to HEX the concentration of free 2, 5-HD in urine was significantly higher ($p<0.001$) in workers not using gloves (0.281 mg L^{-1}) than in those who did (0.184 mg L^{-1}).

Table 1: Statistical analysis for HEX present in the shoemaker's breathing zone air samples (mg m^{-3}), urinary concentration of free 2, 5-HD (mg L^{-1}) and reference values proposed by ACGIH (2010)

Chemicals	n	Average	S.D	Median	GM	TLV-TWA[b]	TLV-TWA[c]	TLV-TWA[d]	BEI
HEX	84	108.2[a] (8-298)	56	89	90.1	76.8 (43-119)	176	141	NA
Free 2,5-HD	38	0.241 (0.02-0.36)	0.06	0.204	0.23	NA	NA	NA	0.4

[a]Average of HEX concentrations in workers' breathing zone air samples. [b]Average of shoemakers' 9.2-h TWA exposure measurement . [c]The 8-h TWA Proposed by ACGIH, 2010. [d]The 9.2-h TLV-TWA calculated from Brief and Scala's equation

Table 2: Correlation between time-weighted average exposure to HEX (mg m^{-3}) and the urinary concentration of free 2, 5-HD (mg L^{-1}) in workers (n = 38)

Metabolite	Equation	r	p-value	Value for 176 mg m^{-3*} of HEX
Free 2, 5-HD	Y = 0.0037 X-0.05	0.815	<0.001	0.6

*The proposed TLV-TWA by ACGIH (2010)

Table 3: Average urinary concentration of free 2, 5-HD mg L^{-1} in the workers in relation with the use of gloves (n = 38)

Metabolite	With gloves (n = 15)	Without gloves (n = 23)	Z	p-value
Free 2,5-HD	0.184	0.281	-3.54	0.001

Table 4: Correlation between TWA exposure to HEX (mg m^{-3}) and the urinary concentration of free 2, 5-HD (mg L^{-1}) in relation with the use of gloves in workers (n = 38)

The use of gloves	n	Equation	Value for 176 mg m^{-3*} of HEX	r	p-value
Yes	15	Y = 0.0031 X – 0.033	0.51	0.842	<0.001
No	23	Y = 0.004X – 0.039	0.66	0.797	<0.001

*The proposed TLV-TWA by ACGIH (2010)

The linear correlation between TWA exposure and urinary concentration of free 2, 5-HD in association with use of gloves is shown in the Table 4. As seen, this correlation was weaker in workers not wearing gloves than in those who did (r = 0.797). To date, in a number of studies the correlations between environmental or personal air concentrations of HEX and free and total 2, 5-HD have been evaluated (Perbellini *et al*., 1981; Ahonen and Schimberg, 1988; Saito *et al*., 1991; Prieto *et al*., 2003) . The levels of atmospheric HEX has generally had a better correlation with the urinary concentration of total 2, 5-HD than that of free 2, 5-HD. However, as discussed earlier in this paper, levels of total 2, 5-HD reflect both neurotoxic and nonneurotoxic metabolites of HEX. Therefore, it is generally believed that free 2, 5-HD is a better indicator for biological monitoring of workers exposed to HEX (Kawai *et al*., 1990; Van-Engelen *et al*., 1995; Dos Santos *et al*., 2002; Prieto *et al*., 2003; Hamelin *et al*., 2004). In the present study, a good correlation was found between TWA exposure and the urinary concentration of free 2, 5-HD. Based on obtained regression equation in the present study, the average level of free 2, 5-HD in workers following exposure to 176 mg m^{-3} HEX was calculated to be 0.6 mg L^{-1} (Table 2) which is higher than the proposed BEI for this chemical. The cause of this discrepancy in the amount of free 2, 5-HD can be sought in the duration of exposure in a day and the number of working days in a week. The average working hours for the subjects in this study was 9.2 per day, 6 days a week. However, the TLV-TWA value for HEX of 176 mg m^{-3} corresponds with 8 h exposure a day and 40 h per week. Therefore, the urinary concentration of free 2, 5-HD in our study may not necessarily be comparable with that of the ACGIH and those obtained by other authors.

For estimating the exposure of workers in different shifts, the ACGIH recommends the application of Brief and Scala's equation (ACGIH, 2010). This equation corrects the effects of differences in work and rest periods on workers' exposure. Applying this equation, in the present study the TLV-TWA exposure to HEX for 9.2 h shift was calculated to be 141 mg m^{-3} that based on linear regression equation (Table 2) this, corresponds with a free 2, 5-HD concentration of 0.47 mg L^{-1}, which is very close to the BEI of 0.4 mg L^{-1} proposed by ACGIH. Furthermore, when with Brief and Scala's equation, TWA exposure of workers who wore gloves was corrected for the number of working hours per day (Table 4), the urinary concentration of free 2, 5-HD was calculated to be 0.404 mg L^{-1} which is practically equal to the proposed BEI. It is worth noting that in our study both, daily working hours (9.2 h) and weekly working days (6 days) were higher than the bases on which TLV-TWA was set by ACGIH (8 h per day and 5 days per week). Therefore, it is not surprising that the urinary free 2, 5-HD concentration predicted by our regression equation for 176 mg m^{-3} of HEX was higher than the proposed BEI by ACGIH.

The subjects of this study were exposed to cyclohexane, pentane, toluene and ethyl acetate in addition to HEX, of which, toluene suppresses the metabolism of HEX (Iwata *et al*., 1983; Cardona *et al*., 1993). Experimental study on Wistar rats exposed to 3520 mg m^{-3} HEX plus 3770 mg m^{-3} toluene (1000 ppm) revealed that the total concentrations of metabolites of HEX decreased by approximately one-sixth following co-exposure to HEX and toluene (Iwata *et al*., 1983). Althogh, in our study the effect of extended workshifts seems to be greater than the suppressing effect of toluene on urinary excvetion of 2,5-HD.

Because of the nature of work, shoe makers in addition to inhalation, are exposed to HEX via skin absorption. Cardona *et al*. (1993) reported that the urinary levels of 2, 5-HD in workers who did not wear gloves were higher than those who did. These authors concluded that among working conditions considered, wearing gloves had the most significant effect on urinary excretion of 2, 5-HD. Similar findings have been reported by other authors (Prieto *et al*., 2003; Nolasco *et al*., 2007).

In line with these observations, in our study, the urinary concentration of free 2,5-HD was found to be significantly higher ($p<0.001$) in workers not wearing gloves (0.281 mg L^{-1}) than in those who did (0.184 mg L^{-1}), indicating that dermal exposure also plays an important role in the overall absorption of HEX. Prieto *et al*. (2003) in biological monitoring of 132 workers exposed to HEX (4-709 mg m^{-3}) reported significant differences between the levels of 2, 5-HD in urine of workers who wore gloves and those who did not. These authors concluded that in biological monitoring of workers exposed to HEX special consideration to their working conditions must be taken into account. However, in the study of Prieto *et al*. (2003) the workers in addition to HEX were exposed to toluene, heptane, acetone, ethyl acetate and other hexane isomers of which, toluene and acetone suppresses and increases the urinary concentration of 2, 5-HD, respectively.

The correlation between TWA exposure and the levels of free 2,5-HD in urine of workers not wearing gloves was weaker than in those who did (Table 4). This could be explained, at least in part, due to the differences in the severity of skin absorption among workers and individual differences that exist in the metabolism of any chemical in different individuals.

CONCLUSION

The findings of this study provide further evidence in favor of the notion that free 2, 5-HD is an appropriate indicator for biological monitoring of workers exposed to HEX. we suggest that in the biological monitoring of workers exposed to HEX, their working conditions such as the working hours per day, the number of working days per week and the use of suitable and efficient protective gloves could be taken into account as important factors that influence the urinary concentration of 2, 5-HD. Therefore, decrement in the number of working hours per day and the use of personal protective devices such as appropriate gloves could decrease the overall absorption of HEX and the levels of 2, 5-HD in urine and thus protect the workers against the neurotoxic effects of 2,5-HD due to excessive exposure to HEX.

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that the investigations undertaken and described in this article have been derived from the materials embodied in the thesis of our MSc student of Occupational Health, Mr. Soleimani, the second author of this paper."

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