Biological monitoring of *n*-hexane exposure in shoe workers

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To analyse working conditions and to provide information about the degree to which shoe workers are exposed to *n*-hexane, the urinary excretion of total 2,5-hexanedione (2,5-HD) was determined in 81 employees in 12 shoe factories. Twenty-five individuals who had experienced no exposure to solvents were used as controls. 2,5-HD was measured in spot urine samples collected from workers at the end of shift. In the urine of shoe workers, the 2,5-HD presented a mean value of 2.33 mg g^{-1} creatinine, a median of 1.96 mg g^{-1} creatinine. The mean 2,5-HD concentration in the urine samples from non-exposed subjects was 0.28 mg g^{-1} creatinine, the median value was 0.18 mg g^{-1} creatinine. The mean time-weighted average (TWA) concentration of *n*-hexane in 12 shoe workshops was 126.1 ppm, ranging from 23 to 215 ppm. We found a significant, but low, correlation ($r = 0.40$; $p < 0.001$) between TWA intensity of environmental exposure to *n*-hexane and the concentration of 2,5-HD in urine. The probable effect of toluene on the concentration of 2,5-HD was also discussed in the present study.

Keywords: *n-hexane, 2,5-hexanedione, urine, biological monitoring.*

Abbreviations: *ACGIH, American Conference of Governmental Industrial Hygienists; BEI, biological exposure index; GC, gas chromatography; 2,5-HD, 2,5-hexanedione; NIOSH, National Institute of Occupational Safety and Health; SD, standard deviation; TLV, threshold limit value; TWA, time-weighted average.*

Introduction

n-Hexane is one of the most popular constituents of solvent-containing industrial products, despite its potency as a neurotoxin. *n*-hexane is believed to exert its neurotoxic effect via the major metabolite, 2,5-hexanedione (2,5-HD) (Krasavage *et al.* 1980, Perbellini *et al.* 1981).

Analysis of urinary 2,5-HD has been suggested for the biological monitoring of *n*-hexane exposures by several authors (Perbellini *et al.* 1981, Ahonen and Schimberg 1988, Kawai *et al.* 1991, Periago *et al.* 1993), although some results on the correlation between environment concentration of *n*-hexane and urinary excretion of 2,5-HD show some degree of variability (Iwata *et al.* 1983, Kawai *et al.* 1991, Saito *et al.* 1991). The importance of *n*-hexane exposure monitoring has also been emphasized by the observation of testicular toxicity and of modulation of the immune system in experimental animals and humans (Nylen *et al.* 1983, Karakaya *et al.* 1996).

The numbers of polyneuropathy cases caused by occupational exposure to *n*-hexane are still of concern for our country. However, to our knowledge, the urinary 2,5-HD levels of Turkish shoe-makers potentially exposed to *n*-hexane have not been previously reported. The aim of the study was to analyse working conditions and to provide information about the degree to which shoe workers were exposed to *n*-hexane by measuring urinary 2,5-HD. Efforts were also undertaken to evaluate whether there was a correlation between the urinary 2,5-HD and *n*-hexane concentration in the workplace air and to interpret the usefulness of 2,5-HD as a biological marker of *n*-hexane exposure.

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MATERIALS AND METHODS

Subjects studied

We studied a population of 81 workers (all men) employed in 12 shoe workshops where the jobs include use of glues and adhesives containing *n*-hexane. Concurrent control subjects consisted of 25 non-exposed male employees in the office department. Informed consent was obtained after the nature of the study was fully explained. Each person was interviewed and a questionnaire was filled in. The questions covered a brief occupational and medical history.

Environmental monitoring

The concentration of solvents in breathing-zone air was measured at each workshop throughout the shift. *n*-Hexane concentrations in the air were determined by means of a single beam infrared spectrophotometer (Miran IBX-Portable Ambient Air Analyzer). For each workshop, vapour concentrations in the workers' breathing area were continuously monitored for 4 h in order to calculate the time-weighted average (TWA) *n*-hexane concentrations. The ceiling concentrations of toluene at each workstation were determined over a sampling period of 15 min by infrared analyser. The detection limits for *n*-hexane and toluene were 1 and 7 ppm respectively. Breathing-zone benzene was measured with Casella London SP15 passive samplers (flow rate 0.2 l min^{-1}) based on diffusion and adsorption by activated NIOSH-approved charcoal filters. Filters were desorbed with CS_2 and the resulting sample was injected into a gas chromatograph (Shimadzu-GC-AM).

Most workshops lacked appropriate local ventilation, and none of the workers wore gloves while using adhesives and solvents.

Gas chromatographic analysis of the glues and fluids revealed that they contained mainly *n*-hexane (25–98%), toluene (25–40%) and, at low concentrations, heptane, acetone and benzene. Benzene concentrations were always less than 1% in the samples analysed.

Biological monitoring

Urine samples were obtained at the end of shift and stored at –20 °C. The total 2,5-HD in urine samples was measured by the method of Perbellini *et al.* (1990). Five millitres of urine were put into glass tubes stoppered with screw caps and the pH adjusted to lower than 0.1 by adding 1 ml of concentrated hydrochloric acid. Samples were heated at 100 °C for 45 min in an oven and then allowed to cool to room temperature. The extraction of 2,5-HD was carried out by using C18 cartridges (Extract Clean ™RC-500 mg) prewashed by 3 ml of methanol and 5 ml of acid water (pH < 1), after which the urine samples were added: 2,5-HD was eluted with 3 ml of 5% acetonitrile/water solution. This solution was extracted

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using dichloromethane (2 ml) containing cyclohexane as an internal standard by shaking for 5 min and then by centrifuging for 5 min. The recovered solvent was evaporated to 0.3 ml under nitrogen flow and used for the GC analysis.

A Packard 438 GC with a flame ionization detector (FID) and an HP-5 capillary column (25 m × 0.2 mm, i.d. × 0.11 μm film thickness) was used for analysis of the total 2,5-HD in the urine of subjects. Running conditions were as follows: injector block temperature 200 °C; detector temperature 220 °C; initial oven temperature 65 °C for 3 min, followed by increments of 25 °C per min. Nitrogen was used as the carrier gas at a flow rate of 1 ml min^{-1}, split 1/40. A spiked quality control sample was used. The recovery rate of 2,5-HD was estimated by using three different concentrations of the metabolite: 0.9, 2.9 and 6.8 mg l^{-1}. Three different samples of each concentration was used. The average extraction efficiency of 2,5-HD was 88.9% (SD 12.9%). The variation coefficient intra-assay was 1.3%. The detection limit for 2,5-HD under the conditions employed was 0.05 mg l^{-1} urine. The 2,5-HD concentration was expressed either as observed (the observed value), or after correction for creatinine concentration (Donald and Zimmer 1967).

Statistical analysis

The statistical differences were calculated using the Mann–Whitney test. Correlations were evaluated using simple regression analysis.

Results

The general characteristics of the study population are summarized in Table 1. Breathing-zone concentrations of solvents at the 12 workplaces are indicated in Table 2 together with the maximum concentrations observed. All workers were exposed to a mixture of *n*-hexane and toluene and they were exposed to greater concentrations of *n*-hexane than toluene.

Table 3 shows the mean and median values of 2,5-HD excretion in urine samples of shoe workers as well as control subjects. Before and after correction using the creatinine, mean values of urinary 2,5-HD levels in workers were about 8–10 times higher than those of control subjects.

The urinary 2,5-HD concentrations in shoe workers were significantly higher than those in controls, both on observed values and after correction for creatinine ($z = -5.85$, $z = -6.66$; $p < 0.001$ respectively). Table 4 illustrates the correlation between TWA intensity of environmental exposure to *n*-hexane and the concentration of 2,5-HD in urine. 2,5-HD concentrations correlated significantly ($r = 0.4486$; $p < 0.001$) and positively with the *n*-hexane concentration in air. However, the correlation coefficient was still lower ($r = 0.3979$; $p < 0.001$) after correction for creatinine. The correlation coefficient between atmospheric toluene concentration (ceiling values) and the *n*-hexane/2.5-HD ratio ($r = 0.3435$; $p < 0.01$) was statistically significant, even after correction for creatinine ($r = 0.2856$; $p < 0.01$).

	Exposed workers ($n = 81$)	Controls ($n = 25$)
Age (years)	29.4 (± 13.1)	36.0 (± 8.7)
Range	12–73	25–58
Duration of exposure (years)	13.6 (± 11.2)	–
Range	0.12–40	
Smoking habits (%)		
Non-smokers	45.7	48.0
Smokers	54.3	52.0
Alcohol intake (%)		
Yes	30	28
No	70	72

Table 1. General characteristics of study population.

Values are mean (± standard deviation).

Concentration in breathing-zone air (ppm)		Workplace
n-Hexane		
TLV—TWA	126.1 (± 47.5)	1–12
Range	23–215	
Ceiling value	485.5 (± 263.1)	
Range	97–1109	
Toluene		
Ceiling value	171.9 (± 115.2)	1–12
Range	36–413	
Benzene		
TLV–TWA	0.65 (± 0.65)	1–12
Range	0–17	

Table 2. Results of air monitoring studies in workplaces.

Values are mean (± standard deviation).

	2,5-HD in urine	
Group	(mg l^{-1})	(mg g^{-1} creatinine)
Exposed workers ($n = 81$)	2.36 ± 1.97[a] 1.74[b] (0.08–8.48)[c]	2.33 ± 1.78[a] 1.96[b] (0.11–7.69)[c]
Controls ($n = 25$)	0.43 ± 0.52[a] 0.23[b] (ND–1.76)[c]	0.28 ± 0.35[a] 0.18[b] (ND–1.55)[c]

Table 3. Concentrations of 2,5-HD in urine specimens from exposed workers and controls.

[a] Values are arithmetic mean ± standard deviation.

[b] Median.

[c] (Range).

ND, Not detected.

n	Observed values				Corrected for creatinine			
	α	β	*r*	*p*	α	β	*r*	*p*
81	0.019	0.013	0.4486	< 0.001	0.015	0.446	0.3979	< 0.001

Table 4. Correlation between time-weighted average *n*-hexane vapour concentrations in breathing zone air and 2,5-HD in urine.

The parameters α and β represent the slope and the intercept, respectively on the vertical axis such that $y = \alpha x + \beta$, where *y* is the amount of the compound in the urine and *x* is the time-weighted average *n*-hexane concentration (in ppm) in the breathing-zone air. The units for *y* are mg l^{-1} for observed values and mg g^{-1} creatinine for values corrected for creatinine concentration. *r*, correlation coefficient.

Discussion

Our results show that *n*-hexane continues to be a major contaminant, along with toluene, in the shoe workshops studied. Breathing-zone concentrations of *n*-hexane at the

workshops we investigated ranged from 23 to 215 ppm, levels that may involve health risks to individuals exposed. The concentration of 2,5-HD in the urine of workers was elevated, suggesting that occupational exposure to *n*-hexane was evident. In our study, small amounts of 2,5-HD are detectable in the urine of persons not occupationally exposed to *n*-hexane. Fedtke and Bolt (1986a) observed that strong acid hydrolysis results in elevated background levels in the urine of non-exposed subjects, in agreement with the finding by Kawai *et al.* (1990). Fedtke and Bolt (1986b) clearly showed that most of the urinary 2,5-HD detected in humans exposed to *n*-hexane was formed by 4,5-dihydroxy 2-hexanone, which gave rise to 2,5-HD during strong acid hydrolysis (pH 0.1) of urine. In our study, for the measurement of 2,5-HD in urine, as also carried out by Perbellini *et al.* (1990), we used strong acid hydrolysis (pH < 0.1). Thus, our results confirm previous studies regarding the presence of 2,5-HD in urine of control subjects (Fedtke and Bolt 1986a, Ogata *et al.* 1991, Mutti *et al.* 1993, Perbellini *et al.* 1993). According to Kawai *et al.* (1991), 2-acetylfuran may confound 2,5-HD analysis in acid-hydrolysed urine samples, giving rise to values overlapping those measured in subjects, especially when a polar capillary column is used for GC analysis. The same authors concluded that acid hydrolysis should be carried out at a pH of $\geq$0.5 and recommended that a non-polar capillary column is used for better separation of 2,5-HD from 2-acetylfuran so as to exclude the confounding effect of co-formed 2-acetylfuran. In the present study, acid hydrolysis is carried out at a pH of lower than 0.1 and a non-polar capillary column, HP-5, is used.

Our findings revealed a significant, but low, linear exposure–excretion relationship between the TWA *n*-hexane concentration and urinary 2,5-HD excretion. The reason why a relatively small correlation coefficient (i.e. 0.3–0.4), in contrast to 0.6–0.9 in previous studies (Perbellini *et al.* 1981, Iwata *et al.* 1983, Mutti *et al.* 1984, Saito *et al.* 1991, Cardona *et al.* 1993), was found in the present study may need some discussion. Perbellini *et al.* (1981) analysed urine samples from workers for 2,5-HD by acid hydrolysis at pH 2, and obtained a correlation coefficient of 0.67 between *n*-hexane exposure and urinary 2,5-HD and Iwata *et al.* (1983) determined 2,5-HD after enzymatic and then acid hydrolysis at pH 2 and found a significant correlation between *n*-hexane exposure and urinary 2,5-HD with a correlation coefficient of 0.896. However, Kawai *et al.* (1990) obtained a poor correlation coefficient of 0.359 after acid hydrolysis at pH < 0.5 regardless of the significant relationship between the *n*-hexane concentration in air and the urinary 2,5-HD. The same authors also concluded that the correlation coefficient was always higher in the absence of acid hydrolysis and was smallest with creatinine correction. Mutti *et al.* (1993) also analysed urine samples from workers for 2,5-HD by acid hydrolysis at pH < 0.1 and obtained a correlation coefficient of 0.44 between TWA *n*-hexane exposure and end of shift 2,5-HD. All the findings in the literature, including this study on the relationship between TWA *n*-hexane exposure and excretion of 2,5-HD (after acid hydrolysis) in urine samples indicated that the correlation was statistically significant, although the coefficient and the slopes of the regression lines varied greatly (Perbellini *et al.* 1981, Mutti *et al.* 1984, Ahonen and Schimberg 1988, Kawai *et al.* 1991).

We also agree that the possible causes of this variation may be explained by the differences in the methodology for air sampling (Ahonen and Schimberg 1988, Saito *et al.* 1991, Periago *et al.* 1993), in the methods of urinanalysis for 2,5-HD (Fedtke and Bolt 1986b, Kawai *et al.* 1990, Perbellini *et al.* 1990, Saito *et al.* 1991), in the urine sampling strategy (Cardona *et al.* 1993, Mutti *et al.* 1993) and in the number of workers studied (Iwata *et al.* 1983, Mutti *et al.* 1984, Kawai *et al.* 1991).

Another reason may be the variable composition of commercial mixtures which may or may not contain solvents interfering with hexane metabolism. In animal experiments and *in vitro* studies, metabolic and toxicological interference has been demonstrated between *n*-hexane and toluene (Perbellini *et al.* 1982, Iwata *et al.* 1984). Although a definite effect of other solvents on the concentration of 2,5-HD was not suggested in the present study we investigated the correlation between the atmospheric *n*-hexane/urinary 2,5-HD ratio and environmental levels of toluene at the workplaces to get a rough idea of the possible influence of toluene on the biotransformation of 2,5-HD. The correlation coefficient between atmospheric toluene concentrations and the *n*-hexane/2,5-HD ratio we obtained was statistically significant ($r = 0.3435$; $p < 0.01$). Cardona *et al.* (1993) investigated the possible effect of toluene on the biotransformation of 2,5-HD in shoe factory workers and they found that the correlation coefficient between atmospheric toluene concentration and the *n*-hexane/2,5-HD ratio was statistically significant ($r = 0.4619$; $p < 0.001$). Takeuchi *et al.* (1993) also demonstrated that the slope of the regression line and correlation coefficient decreased in parallel with an increase of toluene exposure level and that co-exposure of hexane and toluene could inhibit hexane metabolism in workers. In fact, in our study, co-exposure to toluene might cause some degree of metabolic interference in workers. However, from the limited details of toluene exposure reported, it may not be possible to establish any definite conclusion.

For an environmental exposure to *n*-hexane of 50 ppm (Threshold Limit Value–TLV), the analytical method used in this study yielded a urinary concentration of 2,5-HD of 1.20 mg g^{-1} creatinine. The urinary concentration of 2,5-HD corresponding to the TLV was closer to the values found in some studies, especially in Japanese populations (Iwata *et al.* 1983, Ahonen and Schimberg 1988, Saito *et al.* 1991, Takeuchi 1993) than other studies (Perbellini *et al.* 1981, Mutti *et al.* 1984, Periago *et al.* 1993). We are also aware that some variables other than those mentioned above, such as kinetic factors, metabolic clearance, alveolar ventilation, genetic differences as well as previous exposure may contribute to the inter- and intra-individual variability of urinary 2,5-HD levels. Our figure for urinary 2,5-HD was far below the Biological Exposure Index (BEI) value (5 mg l^{-1}, ACGIH 1991) and we feel that 5 mg l^{-1} of 2,5-HD might be a little high as an indicator value corresponding to 50 ppm hexane exposure. The Ministry of Labor in Turkey has not yet adopted urinary 2,5-HD as a biological indicator. Nevertheless, irrespective of its

relationship with hexane in air, urinary 2.5-HD levels may give useful results when monitoring environmental exposure to *n*-hexane. In addition, based on controversial findings of previous studies, we agree that both air monitoring and urinalysis should be standardized so that a reliable dose–response relationship can be determined for effective monitoring of *n*-hexane exposure.

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