

AN INVESTIGATION OF URINARY 2,5-HEXANEDIONE EXCRETION IN SHOE-
MANUFACTURE WORKERS

AYAKKABI İMALATINDA ÇALIŞAN İŞÇİLERDE İDRARLA 2,5-HEKSANDİON ATILIMININ
ARAŞTIRILMASI

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2,5-Hexanedione (2,5-HD) is the major metabolite of n-hexane and is considered responsible for n-hexane-induced polyneuropathy. Therefore, urinary 2,5-HD excretion has been measured for biological monitoring of exposure to n-hexane. In the present study, we determined urinary 2,5-HD levels in shoe-manufacture employees by gas chromatographic method. None of the urinary 2,5-HD levels were over the biological exposure index recommended by ACGIH. Results were compared by the urinary 2,5-HD levels of healthy subjects not exposed to n-hexane. The difference between the groups has been found statistically significant (p<0.0001). Correlation between urinary 2,5-HD concentrations and age or working period was not found.

2,5- Heksadion (2,5-HD), n-heksan'ın temel metabolitidir ve n-heksan'a bağlı nöropatiden sorumlu olduğu kabul edilmektedir. Bu nedenle n-heksan'a maruziyetin biyolojik izlemesi için idrar 2,5-HD atılımı ölçülmektedir. Bu çalışmada ayakkabı imalatında çalışan işçilerde idrar 2,5-HD düzeylerini gaz kromatografisi yöntemi ile tayin ettik. İdrar 2,5-HD düzeylerinin hiçbirisi ACGIH tarafından önerilen biyolojik maruziyet sınırının üzerinde değildi. Sonuçlar n-heksan'a maruz kalmayan sağlıklı kişilerin idrar 2,5-HD düzeylerinde karşılaştırıldı. Gruplar arasındaki farklılık istatistiksel olarak anlamlı bulundu (p<0.0001). İdrar 2,5-heksandion konsantrasyonları ile yaş veya çalışma süresi arasında korelasyon bulunmadı.

Keywords : *n-Hexane ; 2,5-hexanedione ; Urinary excretion ; Gas chromatography ; Occupational exposure*

Anahtar kelimeler: *n-Heksan; 2,5-heksandion; İdrarla atılım; Gaz kromatografisi; Mesleki maruziyet*

Introduction

n-Hexane is a widely used solvent for shoe adhesives as well as other industrial products such as glues, lacquers, paints, printing, etc. (1,2). Therefore, occupational exposure to n-hexane is frequent among workers in shoe factories (3,4). Several studies on the toxicity of this solvent have shown that n-hexane exposure causes polyneuropathy in both human and animals and that this effect is directly related to the occurrence of 2,5-HD, which is the main metabolite of n-hexane in several species (5,1). The excretion of 2,5-HD in urine of workers exposed to n-hexane has been extensively studied and a statistically significant correlation between intensity of exposure to this solvent and urinary excretion of 2,5-HD has been shown (3, 6-9). Therefore the analysis of urinary 2,5-HD excretion has been suggested for biological monitoring of exposure to n-hexane (3, 6,7,10,11).

In the present study, our aim was to evaluate the exposure to n-hexane in small shoe-manufactories located in İzmir. For this purpose

we measured total urinary 2,5-HD excretion of workers exposed to n-hexane and compared with those of healthy subjects not occupationally exposed to this solvent.

Materials and Methods

All chemicals used were analytical grade and as follow: Methanol, hydrochloric acid, acetonitrile, dichloromethane, 2,5-hexanedione, cyclohexanone (Merck; Darmstadt, Germany), sodium hydroxide (atabay; Istanbul, Turkey), picric acid (Riedel de Haen; SeelzeHannover, Germany), C18 cartridges were purchased from Alltech (Deerfield, U.S.A.).

Urine collection, processing and instrumentation: The spot urine samples were obtained from workers of two different shoe-manufactories and from healthy volunteers who did not occupationally expose to n-hexane. Exposed subjects were 48 males aged 15-54 years and unexposed volunteers were 17 males aged 14-52. Each worker had been handling 3 different type of adhesives without any protective device. Adhesives had n-hexane at the concentration of 30% of total solvent and associated with 30% toluene in one type of products.

Urine samples were taken at the second half of a

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working week and were kept at -20°C before analysis by gas-chromatography. Creatinine levels of each sample were also determined by Jaffe method (12). Each employee responded to a standard questionnaire form regarding age, length of employment, general health conditions, alcohol consumption, smoking habits and lifestyle. There were not any subject with metabolic disturbances such as alcohol abuse and diabetes, and with other disease which may effect neurophysiological functions in this study. Smokers were classified as 2 groups, in regard to cigarette consumption by subjects per day ($20 <$ and 20 cigarettes/day). Alcohol consumption by subjects was evaluated by using the scale as follows: 1=none; 2=less than one serving per week; 3=one serving per week; 4=two-six servings per week; 5=one serving per day; 6=more than one serving per day.

Urine samples were processed according to the method of Perbellini et al. (13). Briefly, 5 ml-portion of urine samples were taken into glass tubes with Teflon screw caps and treated with 1 ml concentrated hydrochloric acid to bring the pH to <0.1 . The mixtures were heated for 45 min in an oven at 100°C , then allowed to cool down to room temperature. For extraction of 2,5-HD, urine samples were applied to C18 cartridges which were prewashed with 3 ml methanol and 5 ml acidified water ($\text{pH}<1$), respectively. Cartridges were eluted with 3 ml of 5% (v/v) acetonitrile in water and the eluate was extracted with dichloromethane (2 ml), containing cyclohexanone ($22 \mu\text{g ml}^{-1}$) as internal standard. After centrifugation for 1 min at $3000\times g$, dichloromethane phase was evaporated to about 0.3 ml with nitrogen flow. Concentrated samples were applied to gas chromatograph for 2,5-HD analysis. The gas chromatograph used was a Shimadzu GC-14 model equipped

with flame ionisation detector (FID) for capillary columns and Shimadzu C-R 6A Chromatopac integrator. A SE 54 Capillary column (30 m long, 0.32 mm id, coated with 5% methyl, 95% methyl polysiloxane at the thickness of $0.25 \mu\text{m}$) was used.

Running conditions were as follow: Injector and detector temperature 220°C ; initial oven temperature 70°C for 10 min, followed by increments of $10^{\circ}\text{C min}^{-1}$ up to 200°C and 200°C for 5 min. Hydrogen, air, and nitrogen (carrier) gases were used at 0.7 kg cm^{-3} . Injection volume was 1 μl and detection limit was $12 \mu\text{g l}^{-1}$.

The concentrations of 2,5-HD in urine samples were calculated by a calibration curve which was obtained by injection of 2,5-HD standard solutions prepared in dichloromethane, at the concentrations of 0.45, 0.9, 2.9, 6.8, 9.7, 30 mg l^{-1} .

The recovery rate was estimated by adding 2,5-HD standard solutions to the urine samples of unexposed subjects, at the concentrations of 0.9, 2.9, 6.8 mg l^{-1} . 2 different samples of each concentration were used and the spiked samples were subsequently analysed.

The urinary 2,5-HD concentrations were corrected in regard to the recovery rate and expressed as both mg l^{-1} and mg g^{-1} creatinine (Table 1).

Statistical analysis: The differences between groups were evaluated by non-parametric Mann-Whitney Wilcoxon-U test. Associations between variables were assessed by Pearson correlation coefficient.

Table 1. Subject characteristics and urinary 2,5-HD excretion

	Exposed (n=48)	Unexposed (n=17)
Age ¹	31.58 (15-54)	28.59 (14-52)
Duration of employment ¹ (year)	16.48 (5*-40)	-
Urinary 2,5-HD ² (mg l^{-1})	$1.170 \pm 1.04^{**}$ (0.120-3.200)	0.050 ± 0.02 (0.026-0.089)
Urinary 2,5-HD ² (mg g^{-1} creatinine)	$1.564 \pm 1.48^{**}$ (0.136-5.720)	0.058 ± 0.02 (0.028-0.098)
Smokers ³	29	5
>20 cigarettes/day ³	4	-
20 cigarettes/day ³	25	5
Alcohol consumption ⁴	2.2 ± 1.9	-

¹mean(range), ²mean SD(range), ³number of subjects, ⁴Mean SD of consumption by subjects using the scale described in material and methods, *month, ** $p < 0.0001$, compared with unexposed subjects

Results and Discussion

The method used in this study allowed us to detect 2,5-HD concentrations in all urine samples with a detection limit of 12 $\mu\text{g l}^{-1}$. The recovery rate was $91.65 \pm 0.66\%$. Therefore the method used in this study appears very sensitive for determination of urinary 2,5-HD excretion as well as physiological levels. Table 2 shows the general characteristics and urinary 2,5-HD excretion of subjects. We did not find any significant effect of smoking and/or alcohol consumption on 2,5-HD excretion. Also, no significant correlation has been found between urinary 2,5-HD excretion and age or length of employment.

Table 2. Frequency of common complaints of subjects

Complaints	Unexposed subjects (%)	Exposed subjects (%)
Headache	0.17	81.25
Dizziness	0.06	54.17
Nausea	0	18.75
Stupor	0	37.50
Weakness	0.06	25.00
Fatigue	0.06	25.00
Anorexia	0	14.58
Weight loss	0	22.92
Palpitation	0	22.92
Shortness of breath	0.06	37.50
Pallor	0	25.00
Blurred vision	0	35.42

Although the difference of 2,5-HD excretion between exposed and unexposed subjects was statistically significant ($p < 0.0001$), none of the 2,5-HD concentrations in urine samples from exposed workers was over the biological exposure index recommended by ACGIH (5 mg l^{-1}). In literature, the urinary 2,5-HD excretion in workers exposed to n-hexane were demonstrated as 0.1-17.9 mg l^{-1} (7,11), 0.2-24.2 mg l^{-1} (14,11), and 0.5-19.0 mg l^{-1} (14,7). Therefore, it was surprising for us to find that the urinary 2,5-HD excretion levels of exposed

subjects were low, particularly in regard to the working conditions. The workers worked at least 8 hours a day, 6 days per week and each worker had been handling the glues without any protective devices. The working areas of manufactures were very small. We did not determine TWA n-hexane concentrations, but a good correlation has been shown between n-hexane exposure levels and urinary 2,5-HD excretion in literature (15). It was demonstrated by the manufacturers of the adhesives used that n-hexane content of each adhesive was 30%. This value is lower than that of reported by Governa et al (14). The study was also conducted in spring, when it was hot in Izmir. Therefore, all doors and windows were left open and workers were working with frequent breaks.

As a consequence, air n-hexane levels were possibly low, although working places were very small. The workers were also not eating and sleeping in the working place because of hot weather.

It has been shown that simultaneous exposure to other solvents may effect the excretion of 2,5-HD and toxicity of n-hexane (15-19). Toluene, another common solvent present in glues, is known to suppress n-hexane metabolism and reduce some toxic effects of n-hexane (11, 16, 18,19). In our study, 30% of toluene was present in one type of adhesives. But it is not possible to predict any effect of solvent interaction on 2,5-HD excretion.

In some cases which 2,5-HD excretion was under the threshold values, it has been shown that the workers had significant electroneuromyographic changes, although it has been suggested that the urinary 2,5-HD concentration over an established threshold value can be predictive for early detection of neurotoxic lesions (14,9). In our study, headache, vertigo, sleepness, and blurred vision were established as the most common complaints (Table 2). However, these were non-specific central nervous system symptoms and our data is not adequate to evaluate any neurological changes in workers because we did not examine the functions indicative of neuropathy.

In conclusion, the exposure levels of n-hexane determined by urinary excretion of 2,5-HD in shoe-manufacture workers, were

found under the biological exposure index recommended by ACGIH. Further studies such as collecting of urine samples at different periods of working shift and different periods of year, associated with determination of neurophysiological changes would be complementary for a better evaluation of the occupational exposure risk.

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