AN INVESTIGATION OF URINARY 2,5-Hexanediane EXCRETION IN SHOE-MANUF
ACTURE WORKERS

AYAKKABI İMALATINDA ÇALIŞAN İŞÇILERDE İDRARLA 2,5-HEKSANDİON
ATILIMININ ARASTIRILMASI

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2,5-Hexanediane (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.

Keywords : n-Hexane; 2,5-hexanediane; Urinary excretion; Gas chromatography; Occupa-
tional exposure

2,5-Hexandion (2,5-HD), n-heksan'ın temel meta-
bolitidir ve n-heksan'a bağlı nöropatiden sorumlulu kabul edilmektedir. Bu nedenle n-heksan'a maruziyetin
biyolojik izlenmesi için idrar 2,5-HD atımı ölçülmemektedir. Bu çalışmadan ayakkabı imalatında çalışan işçilerde idrar 2,5-HD düzeylerini gaz kromatografisi yöntemi ile tavan etik. İdrar 2,5-HD düzeylerinin hafif ve orta ACGIH tarafından önerilen biyolojik maruziyet sınırının üzerinde değildi. Sonuçlar n-heksan'a maruz kalan sahlak
kişilerin idrar 2,5-HD düzeylerile karşılaştırıldıkları,
Gruplar arasındaki farklılık istatistiksel olarak anlamalı bulundu (p<0.0001). İdrar 2,5-hexandion konsan-
trasyonları ile yaş veya çalışma süresi arasında ko-
relasyon bulunmadı.

Anahtar kelimeler: n-Heksan; 2,5-heksandion; İdrarla atım; Gaz kromatografisi; Meslekel
maruziyet

Introduction

n-Hexane is a widely used solvent for shoe adhesives as well as other industrial products
such as glues, lacquers, paints, priting, 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 bi-
ological 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 Izmir. For this purpose
we measured total urirnary 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, dichloro-
methane, 2,5-hexanediane, cyclohexanone (Merck;
Darmstadt, Germany), sodium hydroxide (Atabay;
Istanbul, Turkey), picric acid (Riedel de Haen; Seel-
ze-Hannover, 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. Adhesive had n-hexane
at the concentration of 30% of total solvent and associated
with 30% tolustone in one type of products.

Urine samples were taken at the second half of a
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 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<and20 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.

Urinary 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 (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 cyclohexane (22 µg ml−1) as internal standard. After centrifugation for 1 min at 3000 x 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 0.25µm) 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°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−2. Injection volume was 1 µl and detection limit was 12 µg 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 |
|-----------------|-----------------|-----------------|
| Age1            | 31.58(15-54)    | 28.59(14-52)    |
| Duration of employment1 (year) | 16.48(5*-40) | - |
| Urinary 2,5-HD (mg l−1) | 1.170±1.04** | 0.050±0.02 |
| Urinary 2,5-HD (mg g−1 creatinine) | 1.564±1.48** | 0.058±0.02 |
| Smokers3 | (0.136-5.720) | (0.028-0.098) |
| >20 cigarettes/day3 | 29 | 5 |
| 20 cigarettes/day3 | 4 | - |
| Alcohol consumption4 | 25 | 5 |

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 μg l⁻¹. The recovery rate was 91.65±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.

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

Table 2. Frequency of common complaints of subjects 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

<table>
<thead>
<tr>
<th>Complaints</th>
<th>Unexposed subjects (%)</th>
<th>Exposed subjects (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>0.17</td>
<td>81.25</td>
</tr>
<tr>
<td>Dizziness</td>
<td>0.06</td>
<td>54.17</td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
<td>18.75</td>
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<tr>
<td>Stupor</td>
<td>0</td>
<td>37.50</td>
</tr>
<tr>
<td>Weakness</td>
<td>0.06</td>
<td>25.00</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.06</td>
<td>25.00</td>
</tr>
<tr>
<td>Anorexia</td>
<td>0</td>
<td>14.58</td>
</tr>
<tr>
<td>Weight loss</td>
<td>0</td>
<td>22.92</td>
</tr>
<tr>
<td>Palpitation</td>
<td>0</td>
<td>22.92</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>0.06</td>
<td>37.50</td>
</tr>
<tr>
<td>Pallor</td>
<td>0</td>
<td>25.00</td>
</tr>
<tr>
<td>Blurred vision</td>
<td>0</td>
<td>35.42</td>
</tr>
</tbody>
</table>
shown that the workers had significant electoneuromyographic 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, sleepiness, 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.

Acknowledgement

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References


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