BIOMARKERS, 2002, VOL. 7, NO. 4, 299–305

Urinary 2,5 hexanedione as a biomarker of n-hexane exposure

OLGA MAYAN*, JOÃO PAULO TEIXEIRA, SANDRA ALVES and CONCEIÇÃO AZEVEDO

Center of Environmental and Occupational Health, Instituto Nacional de Saúde Dr. Ricardo Jorge, Porto, Portugal

Received 10 January 2002, revised form accepted 22 February 2002

n-Hexane is a saturated aliphatic hydrocarbon widely used in industry. In most cases it is used as a mixture with hexane isomers and various others solvents in the form of commercial hexane. n-Hexane is metabolized oxidatively to a number of compounds, including 2,5-hexanedione (2,5-HD), which is eliminated through the urine and is implicated in the neurotoxic effect of this solvent. The main objective of this study was to evaluate urinary 2,5-HD as a biomarker of n-hexane exposure. The study was carried out in seven industrial units. Post-shift urine samples from 111 workers who handled commercial hexane were collected and analysed for 2,5-HD by capillary gas chromatography. Air sampling was performed in the breathing zones of the workers, and the air samples were analysed using validated methods. Monitoring individual exposures showed that n-hexane exposure varied from 5 to 70 p.p.m. (mean ± SD = 15.24 ± 2.98 p.p.m.). Significant correlation was observed between exposure to n-hexane and urinary 2,5-HD levels, with high correlation coefficients ($\rho = 0.81$, $p = 0.000$), suggesting that urinary 2,5-HD is a good biomarker of occupational exposure to n-hexane. Urinary 2,5-HD is recommended as a better tool than air monitoring in the assessment of health risk, namely the early detection of n-hexane neurotoxicity.

Keywords: 2,5-hexanedione, n-hexane exposure, biomonitoring.

Introduction

n-Hexane (normal hexane), a saturated aliphatic hydrocarbon obtained from petroleum, is a colourless, high volatile and flammable liquid. Commercial hexane is a mixture of hexane isomers and various organic compounds and is one of the solvents widely used in industry (IPCS 1991). Pure n-hexane is used in laboratories, and several uses of commercial hexane have been reported, mainly in the manufacturing of adhesives, lacquers and cleaning solutions, in food processing such as vegetable oil extraction, as a rubber polymerization solvent and as a laboratory chemical (IPCS 1991, ILO 1996).

n-Hexane may penetrate the body by inhalation or absorption through the skin. It is absorbed rapidly through the lungs and distributed throughout the body. The principal metabolic pathways are shown in figure 1. n-Hexane is oxidized to 1-, 2- and 3-hexanol. 2-Hexanol is then hydrolysed to 2-hexanone and 2,5-hexanediol

* Corresponding author: Olga Mayan, Center of Environmental and Occupational Health, Instituto Nacional de Saúde Dr. Ricardo Jorge, Largo 1º Dezembro, 4049-019 Porto - Portugal. Tel: (+351) 22 3326705; Fax: (+351) 22 2005323; e-mail: olgamayan@mail.telepac.pt

* Corresponding author:

Biomarkers ISSN 1354–750X print/ISSN 1366–5804 online © 2002 Taylor &
http://www.tandf.co.uk/journals
DOI: 10.1080/13547500210136796

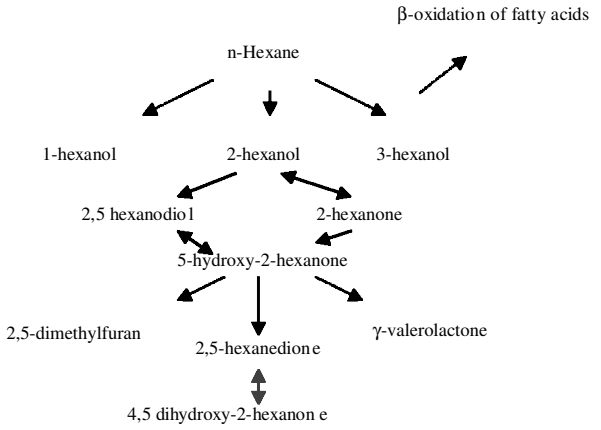

Figure 1. Biotransformation of n-hexane in humans.

and further to 5-hydroxy-2-hexanone. This substance is then metabolized to 2,5-hexanedione (2,5-HD), which is the main metabolite of n-hexane (IPCS 1991).

2,5 HD, 2,5-dimethylfuran and (γ-valerolactone as well as small amounts of n-hexanol have been identified in urine samples from workers exposed to n-hexane (Perbellini *et al*. 1981, Mutti *et al*. 1984). These compounds were present as conjugates, together with some free 2,5-HD and 2,5-dimethylfuran. Fedtke and Bolt (1987) have also identified 4,5-dihydroxy-2-hexanone as a major metabolite in the urine of a male volunteer exposed to n-hexane. These authors point out that the acid hydrolysis commonly used in urine analysis may actually lead to the production of 2,5-HD and 2,5-dimethylfuran from 4,5-dihydroxy-2-hexanone glucuronide (Fedtke and Bolt 1987). Other study developed by Fedtke and Bolt (1986) also demonstrated that the major part of the 2,5-HD detected in urine was produced from 4,5-dihydroxy-2-hexanone as a result of the hydrolysis to which the samples were subjected in the analytical process (Fedtke and Bolt 1986).

These findings need to be taken into account when measuring 2,5-HD for biological monitoring. Almost all the reported studies have used acid hydrolysis of the urine, and good correlations had been found between exposure to n-hexane and urinary 2,5-HD excretion (Perbellini *et al*. 1981, Mutti *et al*. 1984, Iahonen and Schimberg 1988, Mayan *et al*. 2001). Thus the analysis of urinary 2,5-HD was suggested by the American Conference of Government Industrial Hygienists (ACGIH) for the biological monitoring of n-hexane exposure. A biological exposure index (BEI) for total urinary 2,5-HD of $5\,mg\,g^{-1}$ creatinine has been put forward by the ACGIH, and a notice of intended change to $4\,mg\,l^{-1}$ of free 2,5-HD is proposed (ACGIH 2001).

The main effect of n-hexane exposure is on the nervous system. Although effects on the central nervous system have been noted, the principal toxic effect of n-hexane is a peripheral neuropathy, typically causing paralysis of the muscles of the lower limbs and, less often, the upper limbs as well (Ruff *et al*. 1981, Nakajima and Murayama 1985, IPCS 1991). Contact with n-hexane causes irritation, burning eyes and damage to the skin. In laboratory studies, exposed animals showed lung irritation and kidney and liver damage, as well as effects on embryo development and reduced fertility (IPCS 1991).

Epidemiological and toxic-kinetic studies performed in experimental animals have shown that the main metabolite of n-hexane, 2,5-HD, is the primary cause of n-hexane-induced peripheral neuropathy (Perbellini *et al.* 1982, Couri and Milks 1982). Thus, measuring urinary 2,5-HD concentrations in workplaces handled n-hexane products provides information about neurotoxic hazards.

The aim of this study was to evaluate the urinary 2,5-HD as a biomarker of n-hexane exposure.

Materials and methods

Study participants

The study population consisted of workers who handled commercial hexane and were included in the biological monitoring programme of the occupational health services of seven industrial units: four shoe manufacturers, one plastic factory, one synthetic resins and emulsions polymer plant, and one cork unit. The workers included in the study sample were healthy and showed no abnormalities on routine clinical examination.

The study, approved by the ethical committee of the Instituto Nacional de Saúde, was developed in accordance with the Helsinki Declaration of 1975 (revised in 1989). All subjects gave their informed consent and completed a short questionnaire giving details of age, smoking status, alcohol intake, current medication and other information (including hobbies involving possible contact with organic solvents). Biological monitoring data from subjects who reported taking medication up to 1 week before or who had had other contact with organic solvents outside the workplace were not included in the results. The number of study participants was 111 (99 males, 12 females), with a mean age of 34.7 years (range 20–52 years).

Smoking status was classified as not smokers (score 1), 1–4 cigarettes/day (score 2), or $\geq$5 cigarettes/day (score 3). Subjects were asked about their average weekly alcohol consumption of spirits, wine and beer. Consumption of different beverages was totalled in terms of units of alcohol, and subjects were classified as non-drinkers (score 1), $<$7 units/week (score 2), 7–27 units/week (score 3) or $>$27 units/week (score 4). One unit of alcohol was defined as 10 g of ethanol.

All subjects performed cleaning tasks using cleaning solutions based on n-hexane and hexane isomers. Some of the workplaces (42%) had appropriate local extraction at the point of origin of vapours, and 92% of the workers used gloves to eliminate dermal absorption.

Exposure monitoring

All the workplaces were monitored for n-hexane exposure as part of the occupational hygiene programme. Individual air samples were collected by drawing air through standard size coconut shell charcoal tubes (SKC 226-01) using SKC constant low flow personnel pumps (MODEL 222-3). The sampling flow rate, 200 ml min^{-1}, was calibrated before and after each sample. The sampling charcoal tubes were analysed following the National Institute of Occupational Safety and Health (NIOSH) analytical method for n-hexane (method 1500), using a gas chromatography technique (NIOSH 1994).

Worker exposure to n-hexane was expressed as the time-weighted average (TWA) of solvent vapour concentrations for an 8 h work shift.

Occupational hazards were evaluated using the ACGIH criteria, which recommend a threshold limit value (TLV) for exposure to each particular substance (ACGIH 2001).

To evaluate exposure to organic compounds other than n-hexane (co-exposure), the additive effects formulae was applied and reported as the exposure index (EI). The EI is the sum of the ratios between the measured concentration of each compound; its TLV according to the ACGIH is 1.0.

Post-shift urine samples from workers were collected and stored at -4°C until analysis using capillary gas chromatography technique. Urinary 2,5-HD was measured using a slightly modified version of the method of Saito *et al.* (1991). A sample of 5.0 ml urine was acidified with 0.2 ml of concentrated hydrochloric acid. The mixture was heated in a water bath (90–100°C) for 30 min, then cooled with water. Sodium chloride (1.5 g) and 1.0 ml of dichloromethane containing 20.0 mg l^{-1} of cyclohexanone as an internal standard were added. The samples were shaken vigorously for 3 min and centrifuged at 3000 r.p.m. for 5 min. The dichloromethane layer was separated. An aliquot of the resulting extract (2 µl) was injected into a gas chromatograph (Philips PU-UNICAM 4400) equipped with a 30 m $\times$ 0.32 mm DB1 capillary column (JW Scientific, USA) and a flame ionization detector. The working conditions were an injection block temperature of 120°C, a detector temperature of 200°C, an initial oven temperature of 60°C for 2 min, a temperature gradient of 15°C min^{-1} until 140°C and then 20°C min^{-1} until 180°C, and a final oven temperature of 180°C for 3 min; the carrier gas was nitrogen.

The concentrations of urinary 2,5-HD were expressed relative to the creatinine concentration ($mg\,g^{-1}$ creatinine). The urinary creatinine was measured on an auto-analyser (Hitachi 704) using the Jaffé method (Rock *et al.* 1986).

The biological monitoring results were assessed using the BEIs proposed as guidance values by the ACGIH (2001). The BEI for 2,5-HD in urine collected at the end of a shift is $5\,mg\,g^{-1}$ creatinine.

The laboratory involved in analytical measurements adheres to the current quality assurance procedures and participates in the external quality programme organized by the Finnish Institute of Occupational Health (FIOH).

Statistical analysis

Since the results of the 2,5-HD concentrations and n-hexane exposures showed skewed distributions, their log distribution was checked with the Kolmogoroff–Smirnov one-sample test. The geometric mean, standard deviation and range were used to describe the distribution of air n-hexane and 2,5-HD concentrations. The Spearman correlation coefficient was used to study the relationship between urinary metabolite concentrations and n-hexane exposure. The Mann–Whitney U test was used to examine the differences in urinary 2.5-HD concentrations between groups (no smokers versus smokers, and no drinkers versus drinkers). The relationship between levels of urinary 2,5-HD and n-hexane and others variables was investigated using multiple linear regression; levels of metabolite and n-hexane exposure were log-transformed to normalize their distribution before regression analysis.

Results

A total of 464 individual air samples were collected (three to five for each worker) and the TWA was calculated. Analysis of organic solvent vapours in individual air samples showed that the main organic compounds to which the workers were exposed were n-hexane and hexane isomers. Other aliphatic hydrocarbons were also detected, but at a very low levels. Table 1 shows the organic solvent exposure profile.

The n-hexane concentration as a whole was lower than the threshold limit, although some workers (11.7%) were exposed up to this limit value (50 p.p.m.). Biological monitoring indicated that the 2,5-HD concentration ranged from 0.12 to $14.25\,mg\,g^{-1}$ creatinine (mean $\pm$ SD $= 1.99 \pm 2.13\,mg\,g^{-1}$ creatinine), and 9.9% of the workers had a urinary excretion of 2,5-HD higher than the BEI.

A significant correlation was observed between exposure to n-hexane and urinary 2,5-HD levels, with a high correlation coefficient ($\rho = 0.81$, $p = 0.000$). A scatter diagram showing the correlation between TWA n-hexane concentrations in air and 2,5-HD concentrations in post-shift urine samples of workers is given in figure 2.

The relationship between co-exposure to hexane isomers and other hydrocarbons and urinary excretion of 2,5-HD was investigated. The results showed that there was no significant association between the metabolite concentrations and the EI value.

Table 1. Exposure profile.

	Mean $\pm$ SD	Range	TLV
n-Hexane	19.24 ± 2.98 p.p.m.	5–70 p.p.m.	50 p.p.m.
Hexane isomers	55.81 ± 15.61 p.p.m.	10–180 p.p.m.	100 p.p.m.
EI[a]	0.10 ± 0.81	0–0.4	1

[a] EI to hydrocarbons other than n-hexane; calculated from the additive effects formula (sum of the ratios between the measured concentration of each compound and the corresponding threshold limit [8]).

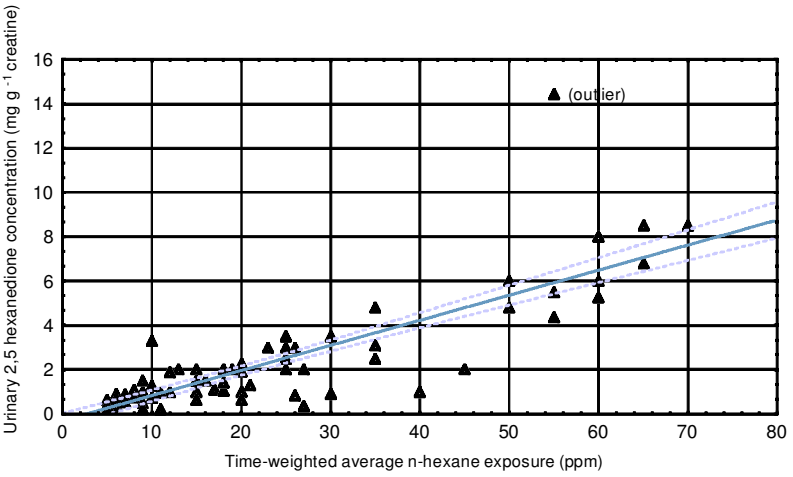

Figure 2. 2,5-HD excretion versus n-hexane exposure.

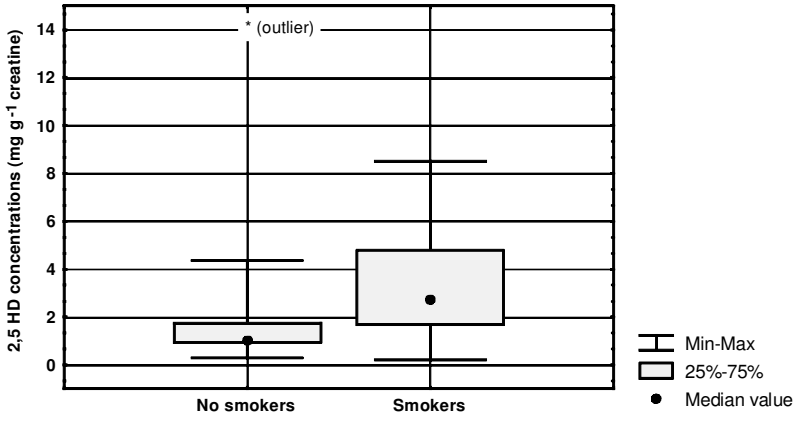

Figure 3. Urinary 2,5-HD concentrations in smokers and non-smokers.

A gender effect on the urinary excretion of 2,5-HD could not be investigated because only a few of the workers were women, and all of them were working in shoe manufacturing units with similar n-hexane exposure (range 14.5–19 p.p.m.).

The effect of the drinking was investigated. Thirty five workers had reported no alcohol consumption (score 1). Thirty three workers usually consumed alcoholic beverages: 20 were classified as score 2, 12 as score 3 and two as score 4. Therefore the subjects were divided into two groups: no drinkers versus drinkers. The results showed that drinking had no significant effect on urinary 2,5-HD levels.

Urinary 2,5-HD excretion levels were compared for no smokers (score 1) (n = 39) and smokers (score 2 and score 3 considered together) (n = 37). The results showed a significant difference in urinary 2,5-HD excretion between the two groups (p = 0.011), with smokers having higher levels of 2,5-HD (figure 3).

Multiple regression analysis indicated that occupational exposure to n-hexane and smoking habits were significant predictors concentrations of urinary 2,5-HD (table 2).

Table 2. Multiple regression: regression summary for dependent variable 2,5-HD concentration.

Variable	B^a	SE(B)^b	$t(74)^c$	p
Intercept	−1.040	0.113	−9.136	0.000
n-Hexane exposure[d]	0.917	0.092	10.017	0.000
Smoking effect	0.113	0.475	2.386	0.024

Multiple correlation coefficient (R^2) = 0.856; adjusted R^2 = 0.846; $F(2,74) = 86.148$, $p < 0.000$.

[a] Non-standardized regression coefficient for the respective variable when entered into the regression equation as an independent variable.

[b] Standard error of the B coefficient.

[c] Student's t-test.

[d] Log-transformed TWA average of n-hexane concentration.

Discussion and conclusions

The results of this study provided evidence that exposure to n-hexane increased the excretion of 2,5-HD. A linear correlation was demonstrated between the concentration of 2,5-HD in urine collected at the end of a work shift and the level of n-hexane exposure.

Good agreement was found between high urinary 2,5-HD levels and poor environmental conditions at the workplace, such as poorly ventilated rooms, no local extraction and inappropriate storage of cleaning solutions. Almost all the workers used gloves, so inhalation of vapours was the main pathway of exposure.

The correlation coefficient obtained is similar to those in previous studies of n-hexane exposure (Perbellini *et al.* 1985, Iahonen and Schimberg 1988, Saito *et al.* 1991, Mayan *et al.* 2001).

The effect of social habits does not interfere with the significant correlation between urinary 2,5-HD concentration and n-hexane exposure. The results showed that there was no significant effect of drinking on the metabolite excretion. Smoking tended to increase urinary 2,5-HD concentrations, but the correlation coefficient obtained was s small (0.113), although it was statistically significant ($p = 0.024$). Therefore n-hexane exposure was the most powerful determinant of urinary 2,5-HD excretion, indicating that urinary 2,5-HD concentrations were linearly related to n-hexane exposure even under the effects of smoking.

The results suggest that 2,5-HD, the urinary metabolite of n-hexane, could be a useful biomarker of n-hexane exposure. Workplace air monitoring is useful for estimating exposure, but it does not always accurately reflect the intake of the individual worker. Biological monitoring could measure the interindividual variation in n-hexane biotransformation. In addition, 2,5-HD is the primary cause of n-hexane-induced peripheral neuropathy and is implicated in the other neurotoxic effects of the solvent, so it would be advisable to use biological monitoring for the early detection of neuropathy. In Japan since 1989 there has been a legal obligation to measure urinary 2,5-HD in workers exposed to n-hexane (Saito *et al.* 1991).

In order to safeguard workers' health, it is advisory to reduce n-hexane exposure. This can be done by the substitution of n-hexane in the cleaning products used by hexane isomers or other less toxic hydrocarbons, and by equipping cleaning desks with a hood and an extraction device. In order to prevent dermal absorption all the workers must used protective equipment such as gloves.

Acknowledgements

We are grateful to the factories' owners, occupational physicians and workers for their helpful cooperation.

References

ACGIH. 2001, *Threshold Limit Values (TLVs®)* for Chemical Substances and Physical Agents and Biological Exposure Indices (BEIs®) (Cincinnati: American Conference of Governmental Industrial Hygienists).

Couri, D. and Milks, M. 1982, Toxicity and metabolism of neurotoxic hexacarbons n-hexane, 2-hexanone and 2,5-hexanedione. *Annual Review of Pharmacology and Toxicology*, **22**, 145–146.

ILO. *1996, Encyclopaedia of Occupational Health and Safety*, 4th edition, edited by L. Parmeggianni (Geneva: International Labour Office).

Fedtke, N. and Bolt, H. M.1986, Methodological investigations on the determination of n-hexane metabolites in urine. *International Archives of Occupational and Environmental Health*, **57**, 149–158.

Fedtke, N. and Bolt, H. M. 1987, The relevance of 4,5-dihydroxy-2-hexanone in the excretion kinetics of n-hexane metabolites in rat and man. *Archives of Toxicology*, **61**, 131–137.

Iahonen and Schimberg, R. W. 1988, 2,5-Hexanedione excretion after occupational exposure to n-hexane. *British Journal of Industrial Medicine*, **45**, 133–136.

IPCS. 1991, *n-Hexane* (Geneva: World Health Organization/International Programme on Chemical Safety).

Mayan, O., Teixeira, J. P. and Pires, A. 2001, Biological monitoring of n-hexane exposure in Portuguese shoe manufacturing workers. *Applied Occupational and Environmental Health*, **16**(7), 736–741.

Mutti, A., Falzoi, M., Lucertini, S., Arfini, G., Zignani, M., Lombardi, S. and Franchin, I. 1984, n-Hexane metabolism in occupationally exposed workers. *British Journal of Industrial Medicine*, **41**, 533–538.

Nakajima, T. and Murayama, N. 1985, Polyneuropathy caused by n-hexane used under the commercial name of benzine. *Industrial Health*, **27**, 340–341.

NIOSH. 1994, *NMAM-NIOSH Manual of Analytical Methods* (Cincinnatti: National Institute for Occupational Safety and Health, US Department of Health and Human Services – Centers for Disease Control and Prevention).

Perbellini, L., Brugnone, F., Silvertri, R. and Gaffury, E. 1981, Measurement of urinary metabolites of n-hexane and their isomers by gas chromatography. *International Archives of Occupational and Environmental Health*, **48**, 99–106.

Perbellini, L., Amantini, M. C., Brugnone, F. and Frontali, N. 1982, Urinary excretion of n-hexane metabolites. *Archives of Toxicology*, **50**, 203–215.

Perbellini, I., Bartolucci, G. B., Brugnone, F., De Rosa, E. and Valentini, F. 1985, Il 2,5-esanodione nel controllo biologico dell'esposizione ptofessionale a n-esano. *La Medicina del Lavoro*, **76**(1), 35–43.

Rock, R. C., Water, G. W. and Jennings, D. C. 1986, Nitrogen metabolites and renal functions. In *Textbook of Clinical Chemistry*, edited by M. W. Tietz (Philadelphia: WB Saunders), pp. 1270–1281.

Ruff, R. L., Petito, C. K. and Acheson, L. S. 1981, Neuropathy associated with chronic low level exposure to n-hexane. *Clinical Toxicology*, **18**(5), 515–519.

Saito, I., Shibata, E., Huang, J. M., Hisanaga, N., Ono, Y. and Takeuchi, Y. 1991, Determination of urinary 2,5 hexanedione concentration by an improve analytical method as an index of n-hexane exposure. *British Journal of Industrial Medicine*, **48**(8), 568–574.