Urinary excretion of the metabolites of n-hexane and its isomers during occupational exposure

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ABSTRACT Environmental exposure to commercial hexane (n-hexane, 2-methylpentane, and 3-methylpentane) was tested in several work places in five shoe factories by taking three grab-air samples during the afternoon shift. Individual exposure ranges were 32-500 mg/m³ for n-hexane, 11-250 mg/m³ for 2-methylpentane, and 10-204 mg/m³ for 3-methylpentane. The metabolites of commercial hexane in the urine of 41 workers were measured at the end of the work shift. 2-Hexanol, 2,5-hexanedione, 2,5-dimethylfuran, and γ -valerolactone were found as n-hexane metabolites and 2-methyl-2-pentanol and 3-methyl-2-pentanol as 2-methylpentane and 3-methylpentane metabolites. The presence of metabolites in the urine was correlated with occupational exposure to solvents. n-Hexane exposure was correlated more positively with 2-hexanol and 2,5-hexanedione than with 2,5-dimethylfuran and γ -valerolactone. A good correlation was also found between total n-hexane metabolites and n-hexane exposure. 2-Methyl-2-pentanol and 3-methyl-2-pentanol were highly correlated with 2-methylpentane and 3-methylpentane exposure. The results suggest that the urinary excretion of hexane metabolites may be used for monitoring occupational exposure to n-hexane and its isomers.

Occupational exposure to commercial hexane (which contains n-hexane, 2-methylpentane, and 3-methylpentane) may be studied by means of environmental, alveolar, and blood tests^{1,2} but not by urinary tests, although research carried out on shoe factory workers exposed to commercial hexane has shown that numerous biotransformation compounds are excreted in the urine at the end of the work shift.³

2,5-Hexanedione, 2-hexanol, γ -valerolactone, and 2,5-dimethylfuran were found in connection with n-hexane exposure and 2-methyl-2-pentanol and 3-methyl-2-pentanol in connection with 2-methylpentane and 3-methylpentane exposure. The identification of these compounds in the urine of workers exposed to commercial hexane prompted us to test their urinary excretion to see if any correlation exists between environmental solvent exposure and metabolite excretion.

Materials and methods

REAGENTS

The following products were used to detect urinary

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metabolites: 2-methyl-2-pentanol, 3-methyl-2-pentanol, 2-hexanol, 2,5-hexanedione, 2,5-dimethylfuran, γ -valerolactone, trichloroethanol, and cyclohexanone. 2-Methyl-2-pentanol, 3-methyl-2-pentanol, 2,5-dimethylfuran and trichloroethanol were obtained from Aldrich Chemical Co, Milwaukee, Wis, USA; 2,5-hexanedione from Eastman Kodak Co, Rochester, New York; 2-hexanol and γ -valerolactone from Merck, Schuchardt, Honenbrunn; cyclohexanone, used as internal standard, from C Erba, Milan; and the "suc d'helix pomatia" containing 100 000 U of β -glucuronidase (Fishman) from Pharmindustrial IBF, Clichy.

ENVIRONMENTAL AND URINARY SAMPLING

Environmental solvent levels were measured at several work places in five shoe factories. In each work place three grab samples were taken from the breathing zone of workers during the afternoon shift. Workers stayed in their places during the whole shift. The air samples were taken by a manual pump into 75-ml glass tubes stoppered with screw caps with a Teflon septum at each end. The solvent concentrations of air samples were determined by gas chromatography within four hours of sampling time, as previously described.¹

At the end of the afternoon shift, samples of urine were collected from 41 workers whose en-

vironmental hexane exposure had been determined. The metabolites of hexane were tested in the urine samples, which were kept at -25°C until test time.

URINE ANALYSIS

Ten millilitres of urine placed in glass tubes stoppered with Teflon screw caps and adjusted to a pH 4.8 with HCl, were treated with 100 μl of β -glucuronidase. After an incubation of 24 hours at 37°C , the urine sample was adjusted to pH 2 with HCl and heated for 30 min in a steam bath. After cooling at room temperature, cyclohexanone (19 μg) as internal standard, and dichloromethane (3 ml) were added. The sample was shaken for one hour and then centrifuged for 15 min. The solvent recovered (80-90% of the amount added) was evaporated to 0.3 ml with a nitrogen flow and then injected into a gas chromatograph. The urinary metabolites of hexane were identified on the basis of gas chromatographic retention time, and their mass spectra were compared with those of authentic samples.³

CALIBRATION CURVE OF URINARY METABOLITES

Standard solutions of 2-hexanol, 2,5-hexanedione, 2-methyl-2-pentanol, 3-methyl-2-pentanol, 2,5-dimethylfuran, and γ -valerolactone were prepared in dichloromethane. Increasing amounts of the products were added to 10 ml of control urine adjusted to pH 2. After addition of cyclohexanone (19 μl) and dichloromethane (3 ml), the samples were shaken and centrifuged as described above. The following minimum concentrations of metabolites were detectable: 2-hexanol 0.1 mg/l; γ -valerolactone 0.25 mg/l; 2,5-dimethylfuran 0.2 mg/l; and 2,5-hexanedione 0.4 mg/l.

The mean recovery rate of the metabolites from urine when in a concentration of between 0.5-20 mg/l was: 2,5-hexanedione 77%, 2-hexanol 94%, 2,5-dimethylfuran 78%, γ -valerolactone 86%, 2-methyl-2-pentanol 81%, and 3-methyl-2-pentanol 86%.

The coefficients of variation for the amount of 15-20 mg/l of each metabolite added to urine were: 2,5-hexanedione 2%, 2-hexanol 4%, γ -valerolactone 3%, 3-methyl-2-pentanol 2%, 2-methyl-2-pentanol 3%, and 2,5-dimethylfuran 10% ($n=5$). The urinary concentrations of hexane metabolites were corrected to a specific gravity of 1024.

GAS-CHROMATOGRAPHIC CONDITIONS

The determination was performed with a Perkin Elmer F 17 gas chromatograph equipped with FID and two columns. The separation column consisted of 2-m long stainless steel tubing (ID 2 mm) filled

with Carbowax C 80-100 mesh, coated with 0.2% Carbowax 1500. Nitrogen was used as the carrier gas at a flow rate of 20 ml/min. The column temperature was programmed from $125-140^{\circ}\text{C}/\text{min}$, the initial temperature remaining constant for 4 min. The injector and detector temperature was 225°C . The detectors were connected to a recorder Perkin Elmer model 56 (chart speed; 5 mm/min).

Results

In the environmental air of the shoe factories studied the following main solvents were found: *n*-hexane, 2-methylpentane and 3-methylpentane. Six other solvents (methylcyclopentane, cyclohexane, 2,3-dimethylbutane, acetone, dichloromethane, and trichloroethylene) were occasionally found at very low concentrations. The mean concentration of the main solvents determined in the three environmental samples collected for each worker individually was considered as representing the individual workers' environmental exposure. The distribution of environmental concentrations with regard to individual workers' exposure to the three main solvents is given in fig 1.

Fig 1 Distribution, mean, standard deviation (SD), median and range of environmental concentrations of *n*-hexane, 2-methylpentane, and 3-methylpentane.

Fig 2 Gas chromatogram of urinary extract of GC.

Mean, standard deviation (SD), median and range of hexane metabolite concentration in workers' urine. Concentrations corrected to a specific gravity of 1024

Metabolites	Mean (mg/l)	SD (mg/l)	Median (mg/l)	Range (mg/l)
2-Methyl-2-pentanol	1.28	1.30	0.95	<0.1- 5.15
3-Methyl-2-pentanol	0.16	0.24	<0.1	<0.1- 1.0
2,5-Hexanedione	5.4	4.9	3.9	<0.4-21.7
2-Hexanol	0.19	0.26	0.12	<0.1- 1.2
2,5-Dimethylfuran	3.7	4.1	2.1	0.7-17.5
γ-Valerolactone	3.3	2.7	2.2	0.7-11.5

This figure also shows the mean, median, and range of concentrations of the three solvents tested. The environmental exposure to solvents ranged from 32 to 500 mg/m³ for n-hexane, 11 to 250 mg/m³ for 2-methylpentane, and 10 to 204 mg/m³ for 3-methylpentane. The mean concentration of n-hexane was 182 mg/m³ and its median 160 mg/m³. 2-Methylpentane and 3-methylpentane were present in lower concentrations than n-hexane. The mean concentration of 2-methylpentane was 77 mg/m³ and its median 69 mg/m³; the mean concentration of 3-methylpentane was 63 mg/m³ and its median 50 mg/m³.

Figure 2 shows the gas chromatographic analysis of the urinary extract from one worker. 2-Methyl-2-pentanol and 3-methyl-2-pentanol are detectable, together with the n-hexane metabolites: 2,5-hexanedione, 2-hexanol, γ-valerolactone, and 2,5-dimethylfuran. Figure 2 also shows a trichloroethanol peak that indicated a trichloroethylene exposure. In all the workers' urine the concentration of trichloroethanol, when present, was always lower

than 25 mg/l, which suggest that exposure to trichloroethylene exposure was low.⁴

The mean, median, and range of urinary metabolite concentrations are given in the table. Although

Fig 3 Distribution of urinary concentration of n-hexane metabolites.

the mean concentrations of 2-methylpentane and 3-methylpentane in the environmental air were similar (77 and 63 mg/m³), the mean urinary concentration of 2-methyl-2-pentanol (1.28 mg/l) was almost 10 times higher than that of 3-methyl-2-pentanol (0.16 mg/l). The urinary concentration of 2-methyl-2-pentanol was lower than 0.1 mg/l in four out of 41 workers, that of 3-methyl-2-pentanol lower than 0.1 mg/l in 23 workers.

As regards n-hexane metabolites, 2-hexanol showed the lowest mean and median urinary concentrations (0.19 and 0.12 mg/l), while 2,5-hexanedione showed the highest (5.4 and 3.9 mg/l). The urinary concentrations of 2,5-dimethylfuran and γ -valerolactone showed a similar mean and median: 3.7 and 2.1 mg/l, and 3.3 and 2.2 mg/l respectively.

Figure 3 shows the distribution of n-hexane metabolite concentrations. The urinary concentration of 2-hexanol was lower than 0.1 mg/l in 15 out of 41 workers, that of 2,5-hexanedione lower than 0.4 mg/l in five workers; while 2,5-dimethylfuran and γ -valerolactone were always higher than their detectable limits in all the 41 workers' urine. The mean urinary concentration of 2-hexanol, 2,5-hexanedione, 2,5-dimethylfuran, and γ -valerolactone corresponded to 1.1% (SD 1.3), 36.5% (SD 21.3), 30.5% (SD 16.8), and 31.9% (SD 16.1) of the total n-hexane metabolite concentration respectively. A wide variability in the percentage concentration of n-hexane metabolites was found. Nevertheless,

Fig 4 Relation between 2,5-hexanedione and 2-hexanol concentrations in workers' urine ($y = 0.04x - 0.028$; $r = 0.7448$; $n = 41$; $p < 0.001$).

Fig 5 Relation between 2,5-hexanedione and 2,5-dimethylfuran concentrations in workers' urine ($y = 0.295x + 2.11$; $r = 0.3461$; $n = 41$; $p < 0.05$).

2,5-hexanedione was in most cases the main metabolite.

The urinary concentration of 2,5-hexanedione was correlated with both 2-hexanol and 2,5-dimethylfuran (figs 4 and 5). No correlation was found among the other n-hexane metabolites.

The urinary products turned out to be correlated with the environmental concentrations of the parent solvents. Figure 6 shows that correlation between 2-methylpentane and 2-methyl-2-pentanol was highly significant ($r = 0.7588$, $p < 0.001$). Figure 7 shows the correlation between environmental 3-methylpentane and urinary 3-methyl-2-pentanol ($r = 0.6513$, $p < 0.001$). Since the

Fig 6 Correlation between environmental 2-methylpentane concentration and urinary concentration of 2-methyl-2-pentanol ($y = 0.018x - 0.1$; $r = 0.7588$; $n = 41$; $p < 0.001$).

Fig 7 Correlation between environmental 3-methylpentane concentration and urinary concentration of 3-methyl-2-pentanol ($y = 0.0035x - 0.06$; $r = 0.6513$; $n = 41$; $p < 0.001$).

Fig 8 Correlation between environmental n-hexane concentration and urinary concentration of total n-hexane metabolites ($y = 0.054x + 2.7$; $r = 0.7858$; $n = 41$; $p < 0.001$).

3-methyl-2-pentanol in 23 out of 41 urine samples was lower than the minimum concentration detectable it is evident that many further tests are needed to confirm our results. All the n-hexane metabolites considered together were well correlated with n-hexane exposure (fig 8). Even individual n-hexane metabolites were correlated with environmental n-hexane concentrations (figs 9-12). 2-Hexanol and 2,5-hexanedione were the metabolites best correlated with environmental exposure to n-hexane ($r = 0.6851$ and $r = 0.6725$). 2,5-Dimethylfuran and γ -valerolactone showed a lower correlation coefficient ($r = 0.5012$ and $r = 0.3991$) than 2-hexanol

Fig 9 Correlation between environmental n-hexane concentration and urinary concentration of 2-hexanol ($y = 0.0015x - 0.08$; $r = 0.6851$; $n = 41$; $p < 0.001$).

Fig 10 Correlation between environmental n-hexane concentration and urinary concentration of 2,5-hexanedione ($y = 0.027x + 0.49$; $r = 0.6725$; $n = 41$; $p < 0.001$).

Fig 11 Correlation between environmental n-hexane concentration and urinary concentration of 2,5-dimethylfuran ($y = 0.017x + 0.59$; $r = 0.5012$; $n = 41$; $p < 0.001$).

and 2,5-hexanedione. Nevertheless, their correlation with n-hexane environmental concentrations was significant ($p < 0.001$ and $p < 0.01$ respectively).

Discussion

in animals exposed to methyl-n-butyl ketone;⁶ 2,5-hexanedione was found in the serum but not in the urine of people experimentally exposed to methyl-n-butyl ketone. On the other hand all four of the metabolites were found after exposure to n-hexane both in animals (N Frontali *et al* at the International Congress of Neurotoxicology in Varese, Italy, 27-30 September 1979) and in man.³

Examination of our data (fig 8) shows that at the 95% confidence level an n-hexane exposure of 90 mg/m³ (TLV proposed by ACGIH 1979) corresponded with a urinary excretion of 5.5-9.7 mg/l of total n-hexane metabolites. For each of the individual n-hexane metabolites, the urinary excretion corresponding to an n-hexane exposure of 90 mg/m³ was 0.1-3 mg/l for 2-hexanol, 0.8-3.7 mg/l for 2,5-dimethylfuran, 1.5-3.5 mg/l for γ -valerolactone, and 1.5-4.4 mg/l for 2,5-hexanedione (figs 9-12).

As regards the correlations between the environmental exposure to n-hexane and its urinary metabolites, our data show that 2-hexanol and 2,5-hexanedione correlated more positively ($r = 0.6851$ and $r = 0.6725$) than 2,5-dimethylfuran and γ -valerolactone ($r = 0.5012$ and $r = 0.3991$). The latter compounds are probably not "true metabolites" but products formed by cyclisation of hydroxylated precursor during gas-chromatographic analysis.⁷

Thus it seems reasonable that 2-hexanol and 2,5-hexanedione might be used as reliable indicators for monitoring environmental exposure to n-hexane. From the practical point of view, 2,5-hexanedione, which shows higher urinary concentrations than 2-hexanol, must probably be considered as more reliable than 2-hexanol. The urinary concentration of total n-hexane metabolites correlated well with environmental n-hexane concentrations ($r = 0.7858$).

The urinary excretion of 2-methyl-2-pentanol and 3-methyl-2-pentanol and their statistical correlations to environmental exposure to 2-methylpentane and

Fig 13 *Main metabolic pathway of n-hexane in men and in animals and its link with methyl-n-butyl ketone biotransformation.*

3-methylpentane respectively suggest that occupational exposure to the latter may also be monitored by means of urinary tests.

By comparing the slope of the regression line in figs 6, 7, and 8, it emerges that with the same environmental concentrations of the three solvents the concentration of all n-hexane metabolites taken together is three and 15 times higher than that of 2-methyl-2-pentanol and 3-methyl-2-pentanol respectively.

As regards the n-hexane exposure, our results showed that 2-hexanol and 2,5-hexanedione were the least and the most important metabolites respectively. This finding differs from the results from animal experiments in which 2-hexanol is the main metabolite of n-hexane.^{8,9} This could suggest a higher susceptibility to n-hexane neurotoxicity in man than in animals. Experimental studies showed that 2,5-hexanedione causes an inhibition of glycolytic enzyme activity (phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase and α -glycerophosphate dehydrogenase) in nerve fibres, which could be the primary cause of hexacarbon neuropathies.¹⁰

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