

n-Hexane metabolism in occupationally exposed workers

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ABSTRACT Lung uptake and excretion of *n*-hexane were studied in ten workers in a shoe factory. Simultaneous samples of inhaled and alveolar air were collected with the aid of a Rhan-Otis valve, personal samplers, and charcoal tubes. Alveolar excretion was monitored during a six hour postexposure period. Uptake was calculated from lung ventilation, the retention coefficient, and environmental concentrations. The amount of exhaled *n*-hexane was calculated from the decay curve. According to the experimental data, alveolar retention was about 25% of the inhaled *n*-hexane, corresponding to a lung uptake of about 17%. The postexposure alveolar excretion was about 10% of the total uptake. The main metabolites of *n*-hexane were identified and measured by capillary GC/MS in spot urine samples collected before, at the end, and 15 hours after the same working shift. Urinary concentrations were low, though related to *n*-hexane in the air. 2,5-Hexanedione in the end of shift samples gave the best estimate of overall exposure. About 3 mg/g creatinine of 2,5-hexanedione would correspond to about 50 ppm of *n*-hexane in the air (mean daily exposure).

The use of *n*-hexane is widespread in industry. It is an excellent and inexpensive solvent used especially in glues, varnishes, and paints. Many mixtures are commercially available, and the most common among them contain different proportions of *n*-hexane and its isomers 2-methyl and 3-methyl pentane, cyclohexane, and methyl ethyl ketone. Occupational exposures have been reported to cause peripheral neuropathies in workers¹⁻³; electrophysiological changes have also been found in subjects exposed to relatively low airborne concentrations.⁴⁻⁸

The pharmacokinetics of *n*-hexane has been widely investigated in rats but much less is known for man. Among the published studies there is little agreement about the retention of inhaled *n*-hexane and about the proportion which is excreted through the lung during the postexposure period. Urinary metabolites of *n*-hexane in man have been shown by Perbellini *et al*⁹ after occupational exposure to solvent mixtures containing *n*-hexane.

The present study was undertaken to investigate

the metabolism of *n*-hexane in man, with the aim of evaluating more specifically: (a) the respiratory uptake and elimination in workers occupationally exposed under constant model ventilation conditions; (b) the possible relation between *n*-hexane uptake and urinary excretion of its metabolites; and (c) the usefulness of either alveolar concentration or urinary excretion of *n*-hexane metabolites for biological monitoring purposes.

Method

The study was performed on 10 young healthy subjects aged 18-30 years who continued their job during the experiment.

SAMPLING

n-Hexane concentrations in the breathing zone were monitored with personal samplers; inhaled and alveolar air were sampled with the aid of a Rhan-Otis valve (commercially available from PK Morgan, Chatham, UK). The samples were collected simultaneously for 5 min with two pumps, one for the inhaled air and the other for the last 100 ml of the tidal volume, which was taken as alveolar air.

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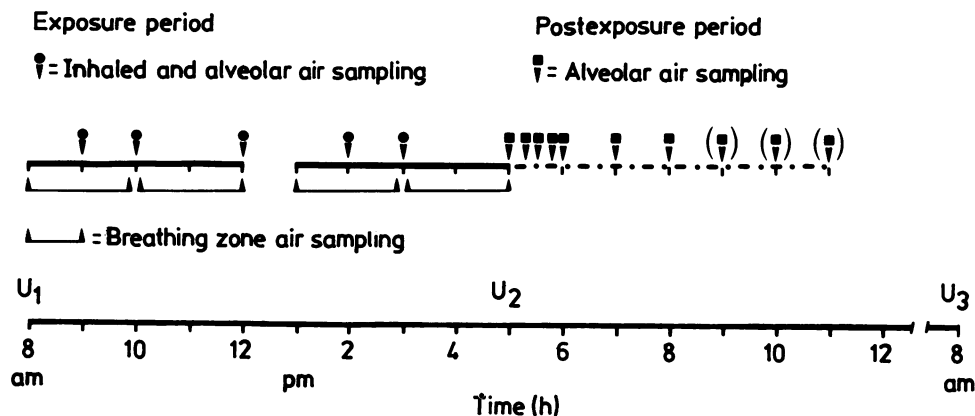


Fig 1 Experimental design: dots = sampling of alveolar and inhaled air; squares = sampling of alveolar air during postexposure period; squares in brackets = samples available for only two subjects. Distance between each double arrow indicates sampling time of environmental air in breathing zone. U = spot urine samples collected before, at end, and 15 hours after same working shift.

The total sampled volume was about one litre at a flow rate of 200 ml/min. Vapours were absorbed on NIOSH approved charcoal tubes interposed between the valve and the pumps. Urinary samples were taken before, at the end, and 15 hours after exposure. The experimental design is summarised in fig 1. During each alveolar air sampling, pulmonary ventilation was measured by means of a volumetric counter (SIM Brunt AB) connected to the Rhan-Otis valve.

ANALYSIS

After desorption with CS₂, gas chromatographic analysis was performed using a Perkin Elmer Sigma 3B gas chromatograph equipped with an FID and a silicone OV-101 Column 50 m × 0.25 mm (id). Helium (1 ml/min) was used as the carrier gas. The column temperature was 40°C and the injector and detector temperatures were 160°C and 200°C respectively. Urinary metabolites of *n*-hexane were measured using a slightly modified version of the method of Perbellini *et al.*¹⁰ A silica capillary column (25 m × 0.25 mm id) was used, coated with Carbowax 20M. The column temperature was programmed after 2 min isothermal operation from 40°C to 150°C at a rate of 20°C per min and the final temperature was maintained for 5 min. The injector temperature was 200°C, the detector temperature 250°C, and the flow rate 1 ml helium per minute.

Particular interest was focused on the following metabolites: 2 and 3-hexanol, 2,5-hexanediol, methyl *n*-butyl ketone, 2,5 hexanedione, γ -valerolactone, and 2,5-dimethylfuran. Authentic

samples of each substance were obtained from Fluka AG (Buchs, Switzerland). The urinary metabolites were identified on the basis of gas chromatographic retention time, and their mass spectra were compared with those of authentic samples, using a Finnigan MAT 1020 mass spectrometer (Finnigan MAT, Cincinnati, OH, USA).

EQUATIONS

Intake, uptake, and alveolar excretion were calculated according to the following equations:

$$R = 1 - \frac{C_a}{C_i} \quad (1)$$

$$I = kC_{i(TWA)}V_e t \quad (2)$$

$$D = kC_{i(TWA)}V_a R t \quad (3)$$

$$E = KV_a \int_{t_0}^{\infty} C_a dt \quad (4)$$

where C_a = alveolar concentration of *n*-hexane (ppm); C_i = inhaled concentration of *n*-hexane (ppm); $C_{i(TWA)}$ = time weighted average of breathing zone levels of *n*-hexane (ppm); R = pulmonary retention coefficient; V_e = respiratory minute volume (l/min); V_a = alveolar ventilation, calculated according to Åstrand¹¹ (l/min); t = time (min); I = total respiratory intake (mg); D = alveolar uptake or dose (mg); E = alveolar excretion (mg); and k = factor converting ppm to mg/l (3.5×10^{-3}).

Results

Technical grade hexane is a mixture containing variable proportions of *n*-hexane, of its isomers, and of

Table 1 Concentration of organic solvents in the air (8h-TWA)

Solvent	8h-TWA* air concentration (mg/m ³)		Hygienic† effect	
	Median	Range	Median	Range
n-Hexane	243	8-1143	1.34	0.04-6.37
2-Methyl pentane	133	5-685	0.07	0-0.38
3-Methyl pentane	100	4-514	0.05	0-0.28
Cyclohexane	18	0-892	0.02	0-0.85
n-Heptane	65	0-136	0.04	0-0.08

*8h-TWA = Time weighted average.

†Hygienic effect is the ratio between the measured concentration of the compound and its threshold limit value. When specific information about synergism does not exist, the sum of the ratio does not exceed 1.0, which is the threshold limit value for the mixture.

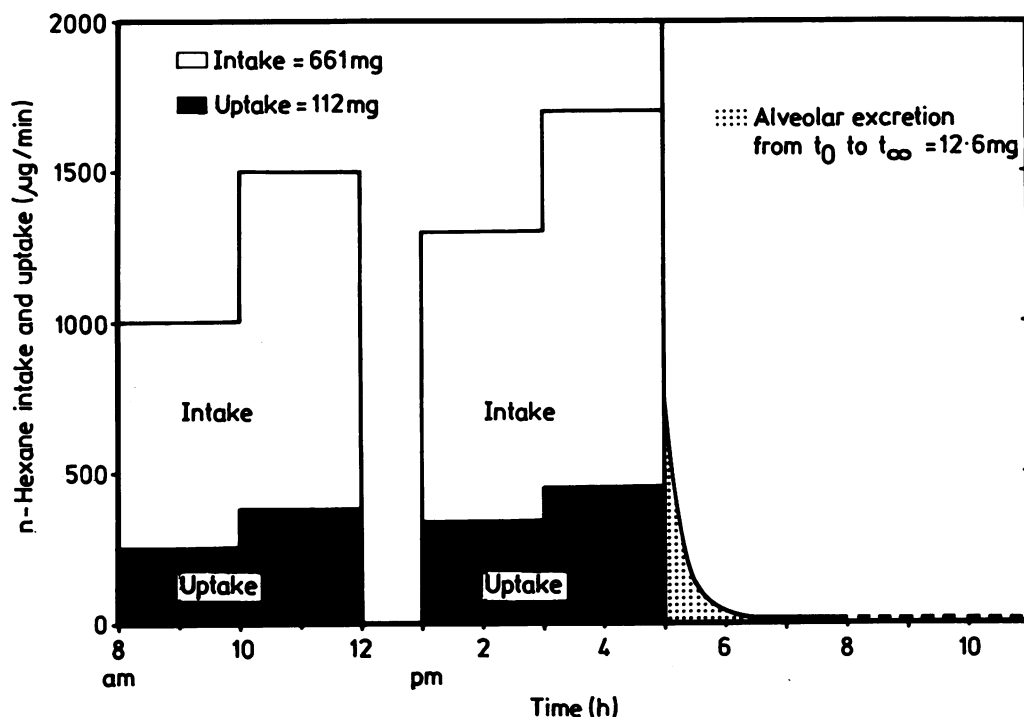


Fig 2 Time course of total respiratory intake and absorption of n-hexane during eight hours of exposure (left) and of alveolar excretion during the postexposure period (right) in a worker exposed to 243 mg/m³ of n-hexane in air (mean daily exposure).

other solvents. The main components of the mixture used by our study subjects and their 8 h time weighted average (TWA) concentrations in the air during the study are summarised in table 1. The solvent concentrations showed a wide scatter, the exposure to n-hexane ranging from a few to more than 1000 mg/m³.

Figure 2 shows the time course of n-hexane intake and uptake during exposure in a study subject and that of alveolar excretion during the postexposure period. From the difference between alveolar

uptake and excretion it seems reasonable to conclude that a considerable amount of absorbed n-hexane was either retained or metabolised. In fig 3 the time course of n-hexane alveolar excretion is shown in the same subject as in fig 2. During the postexposure period the alveolar excretion of unchanged n-hexane accounted for about 10% of the total uptake. Elimination from the lungs was biphasic, the median half lives of the fast and slow phases being 11 min and 99 min, respectively.

The results from the individual experiments are

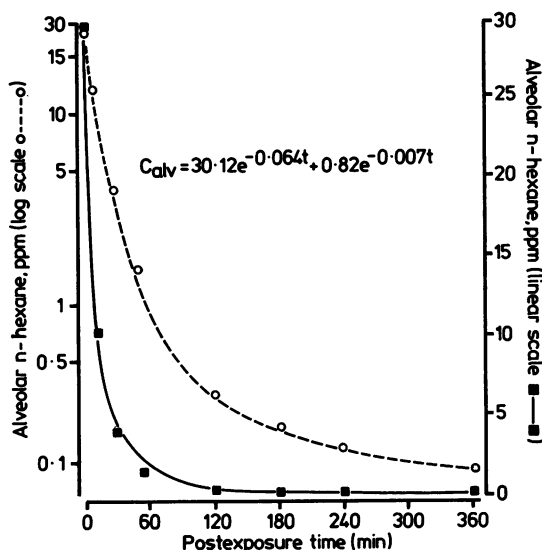


Fig 3 Time course of alveolar concentrations (ppm) of *n*-hexane during postexposure period in same subject as in fig 2. Measured values were plotted versus time both on linear (continuous line) and on lin-log scale (dashed line). Experimental curve was obtained by extrapolation to zero time. Fast phase (first hour) was calculated by subtracting slower component at each sampling period.

summarised in table 2. The difference in the inhaled and alveolar concentrations indicates that about 25% of alveolar *n*-hexane is taken up by the blood. Absorption in relation to the total respiratory intake is only 17%, taking into account both the retention coefficient and alveolar ventilation. It should be emphasised that the physical load while working was

Table 2 Absorption and alveolar excretion of *n*-hexane assuming a mean ventilation of 8 l/min

Parameters	Median	Range
Intake (mg)	972	(32-4572)
Retention (%)	25.2	(22.1-28.7)
Uptake (mg)	166	(9-1146)
Excretion (mg)	14.7	(1-106)
Half lives (min):		
Fast phase	11	
Slow phase	99	

Table 3 Urinary excretion on *n*-hexane metabolites (mmol/mol creatinine)

<i>n</i> -Hexane metabolites	Preshift		End of shift		Next morning	
	Median	Range	Median	Range	Median	Range
2,5-Dimethylfuran	0.4	<0.01-5.2	2.6	<0.1-15.1	1.8	<0.1-8.1
2-Hexanol	0.2	<0.1-2.4	0.5	<0.1-5.3	0.3	<0.1-4.3
2,5-Hexanedione	0.6	<0.1-10.7	3.5	<0.1-17.9	2.4	<0.1-15.0
γ -Valerolactone	0.8	<0.1-7.5	2.9	0.1-16.4	2.0	<0.1-10.5

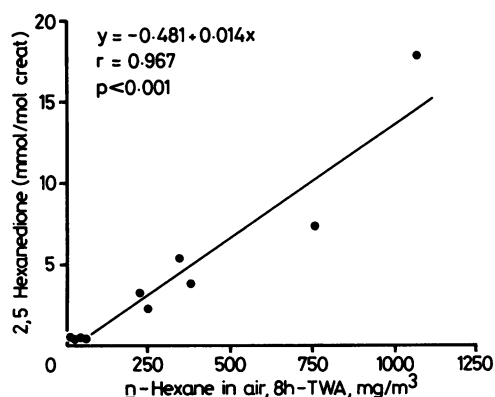
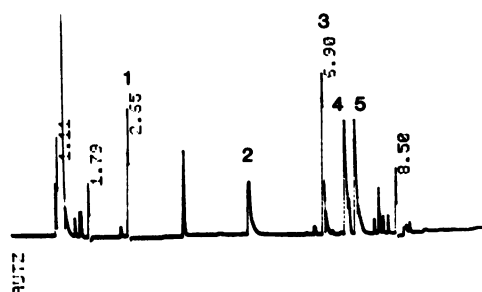


Fig 4 Relation between TWA concentration of *n*-hexane in air and 2,5 hexanedione in end of shift urine samples.



median uptake was 166 mg during the whole working shift.

The main urinary metabolite appeared to be 2,5-hexanedione, particularly in the end of shift sample. A good relation was found between exposure to n-hexane, as measured by its TWA concentration in the air, and the urinary excretion of 2,5-hexanedione at the end of the working day (fig 4). Nevertheless the scattergram suggests that the measurement of 2,5-hexanedione in the urine is not sensitive enough to detect exposures below 50 mg/m³.

Figure 5 shows a typical chromatogram of one end of shift urine spot sample. It can be seen that the identification of each metabolite and the interpretation of analytical results is a difficult task.

Discussion

A direct comparison with other field and experimental studies is not possible because of the differences in the methods used. There is, however, a reasonable agreement among the alveolar retention coefficients reported by Brugnone *et al.*¹² by Veulemans *et al.*¹³ and that found in the present study. In contrast to the retention data ranging from 0.164 to 0.2522 reported above a value of 0.056 was found by Nomiyama *et al.*¹⁴

In agreement with the experimental study of Veulemans *et al.*¹³ we found that the postexposure alveolar excretion was biphasic and the half lives were similar to those of Veulemans despite the different exposure levels. According to the same authors, the short half life of the fast phase would cause a high variability in the measurements of the concentration of n-hexane in breath samples taken at specified time intervals. As a result, the alveolar concentration does not give better information about the absorbed dose during the whole exposure period than the measurement of the environmental concentration. As shown earlier by Iwata *et al.*¹⁵ and Perbellini *et al.*¹⁶ a close relation was found between the mean daily exposure to n-hexane and the urinary excretion of 2,5-hexanedione in the end of shift sample. In the present study individual exposure was accurately defined and covered a wide range (2–317 ppm); regression was linear over this range. Nevertheless, at least three false negative urines were found at exposure levels lower than 50 mg/m³, or about one third of the current TLV. Furthermore, for practical reasons such an indicator cannot be recommended for the routine biological monitoring of occupationally exposed workers. Biological monitoring permits an accurate estimation of the absorbed dose, whatever the absorption route, the physical work load, and the changes in the biotrans-

formation of a given toxic chemical. Nevertheless, practical reasons should also be considered when implementing a biological monitoring programme. When evaluating the usefulness of a biological indicator for monitoring purposes these reasons might represent a serious drawback in terms of analytical difficulties and data interpretation. As a result, a great difference may occur between statistically significant and biologically significant relationships between exposure and biological indicators.

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Notices

5th International Conference on Heavy Metals in the Environment, Athens, 10-13 September 1985

Themes for the Athens conference are environmental pathways, fate and effects, exposure and health effects, disposal, control, and legislation with emphasis on: marine and estuarine environments; fate of heavy metals in sludge treatment/disposal and problems with dredged materials; aquatic chemistry; atmospheric particulates and dispersion; microbial interactions and availability; speciation—in soil, water, and organisms; health effects—case studies, specific effects—neurotoxic, immunological, renal, human studies of combined exposures, remobilisation, and excretion; metal cycling and effects on forest ecosystems—evolution and mechanisms of metal tolerance, ecosystems as sinks for heavy metals; detoxification of soils and water by plants; control strategies and legislation; and clean technology in metal processing industries. Papers for the conference will be welcome, and authors should submit an abstract of 200-300 words to the secretariat before 1 February 1985. Further information is available from Heavy Metals Secretariat, CEP Consultants Ltd, 26 Albany Street, Edinburgh EH1 3QH, UK. (Tel: 031 557 2478).

Impact of Biotechnology on Diagnostics, Rome, 16-18 April 1985

At this international symposium the list of topics will be: production and genetic manipulation of monoclonal antibodies, tumor detection by immunocytochemical and radioimmunometric methods, recombinant DNA and hybridoma technology, DNA probes in diagnostics, biotechnology in animal and human vaccines, and antibodies to synthetic antigenic determinants. Further details available from the organising secretariat at Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23-20121 Milan, Italy. Tel: (02) 783868-702267, Cable: Lorenzfond.

International Symposium on Hexachlorobenzene (HCB), Lyon, France, 24-28 June 1985

The symposium is jointly sponsored by the International Agency for Research on Cancer, World Health Organisation, International Programme on Chemical Safety WHO-UNEP-ILO, US Environmental Protection Agency, US National Cancer Institute, and Commission of the European Communities, Health and Safety Directorate, Luxembourg. The sponsoring organisations are concerned about the hazards associated with exposures to HCB. The major aim of the symposium is to provide an up to date evaluation of both known exposures and biological effects of HCB. In particular, the symposium will focus on the following objectives: presentation of available data on HCB and its impact on human health and the environment—sources, distribution, exposures, and effects; comparability of HCB toxicity to that of other environmental chemicals of similar structure and toxicity; and recommended future research and risk management options. For further details contact the symposium secretariat: Dr JRP Cabral, IARC, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France. Tel: (7)875-81-81, telex: 380023 F.

1st International Conference on Environmental Contamination, London, 10-13 July 1984

The published proceedings of the first conference are now available. The proceedings contain over 150 papers from 23 countries covering: environmental asbestos; organic and inorganic contaminants in agriculture; contaminants in drinking water, river waters, stormwaters and sediments; contaminants in wastewaters and sludges; hazardous and toxic wastes; airborne contaminants; land contamination and reclamation; marine contaminants; and radioactive wastes. Published by CEP Consultants Ltd, 26 Albany Street, Edinburgh EH1 3QH, UK. Price (inc p&p) UK: £50.00, Americas: US\$75.00, Elsewhere: £55.00. Available direct from the publishers CEP Consultants Ltd. Payment is required with order.