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SIMULTANEOUS DETERMINATION OF BENZENE AND TOLUENE IN THE BLOOD USING HEAD-SPACE GAS CHROMATOGRAPHY

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SUMMARY

A head-space method for the simultaneous determination of benzene and toluene in blood using a gas chromatograph equipped with a photoionization detector was developed. Internal standards for benzene and toluene were fluorobenzene and *o*-xylene, respectively, and the detection limit was 5 nmol/l for both solvents. This method is sensitive enough for needs of biological monitoring of benzene and toluene in exposed workers. With automation it offers a possibility for routine measurements. An application of the method in monitoring exposed workers in the industry is presented.

INTRODUCTION

Measurement of benzene and toluene in the blood has been recommended as one of the biological monitoring methods for the workers exposed to these hazards [1]. In contrast to the analysis of urinary metabolites of benzene and toluene, phenol and hippuric acid, respectively, which are also found in the

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urine of non-exposed persons, these blood analyses offer a specific way of monitoring the exposure to low levels of benzene and toluene [2–4].

The requirements for the determination of blood benzene on the one hand and blood toluene on the other for the purpose of biological monitoring are quite different. The recommended limit values, TWA_{8h}, in the workplace air in various countries differ for benzene from 5 to 10 cm^3/m^3 [5,6] and for toluene from 100 to 200 cm^3/m^3 [6,7]. As the concentrations of benzene in the workplace are much lower, benzene levels are likely to be lower than toluene in the blood of exposed workers. Several analytical methods for the determination of toluene in blood have also been used to monitor exposure to toluene [3,4,8,9], and even for benzene in blood some methods have been described [10,11] using gas chromatography (GC) with flame ionization or mass spectrometric detection. The low sensitivity has been an important obstacle in the use of the blood benzene analyses for biological monitoring of workers exposed to low levels of benzene.

Head-space GC is a simple way to analyse volatile solvents in the blood. To make the head-space analyses more sensitive, and also to be able to determine benzene in blood of the exposed workers, we have developed a method using an automatic head-space injector, a wide-bore capillary column and a photoionization detector.

EXPERIMENTAL

Principles

Concentrations of benzene and toluene in blood samples were determined with a head-space method where volatile gases in the samples are vapourized by heating and then injected at elevated pressure directly onto the column. Quantification is made gas chromatographically with photoionization detection (PID).

Equipment

The gas chromatograph used was Sigma 2000, equipped with a flame ionization detector (Perkin Elmer, Norwalk, CT, U.S.A.) and a photoionization detector, UV light source 10.2 eV (HNU Systems Newton, MA, U.S.A.). A head-space injector, HS-100 (Perkin Elmer, Überlingen, F.R.G.) was used for automatic injection. Columns used were: HP-1, methylsilicone capillary column (30 m × 0.53 mm I.D.), film thickness 2.65 μm, Hewlett-Packard 19095Z-123; HP-5, 5% phenylmethylsilicone column, (30 m × 0.53 mm I.D.), film thickness 2.65 μm, Hewlett-Packard 19095J-123; HP-1 + HP-5 (i.e., the two capillary columns connected with shrinkable PTFE tubing, Stock No. 5801 (G09)77, Applied Science, Alltech Assoc. (Deerfield, IL, U.S.A.).

Chemicals

Benzene, toluene and fluorobenzene (p.a.) were purchased from Merck (Darmstadt, F.R.G.) and *o*-xylene (p.a.) from Riedel de Haen (Seelze, F.R.G.), 94% (vol.) ethanol from Oy Alko Ab (Finland). Deionized, distilled water was filtered through a Milli-Q system (Millipore, Bedford, MA, U.S.A.). Control blood for standard additions was collected from the blood left over from specimens sent to the laboratory for analyses other than benzene and toluene. Ethanol, water and control blood were checked by GC before use, as they sometimes contained other substances with the same retention times as benzene, toluene or the internal standards.

Standardization

Standard stock solutions in ethanol were further diluted in water and finally in blood by weighing. Concentrations of the diluted calibration standards were 0–0.2 μmol/l for benzene and 0–2.0 μmol/l for toluene. They were kept in closed head-space vials at +6°. Stock solutions of the standards in ethanol were kept in 2-ml vials in the freezer.

To prepare the internal standard (I.S.) solution, 4 μl of *o*-xylene and 1 μl of fluorobenzene were diluted in ethanol and further in water. Stock solutions in ethanol were kept in 2-ml vials in the freezer, and dilutions in water were prepared for each analytical series.

Preparation of the samples

Blood for the analysis was taken into tubes treated with heparin. Blood (2 ml) was pipetted into head-space vials containing 2 ml of the I.S. solution, and the vials were closed immediately. Standard solutions in blood were treated similarly as the blood samples to be analysed.

Chromatography

The head-space samples were injected via an HS-100 autosampler into a Sigma 2000 gas chromatograph. The samples were thermostatted at 80°C for 23 min. Pressurization and injection times were 1.5 and 0.1 min, respectively. The temperature programme for the (HP-1+HP-5) united column was as follows: 6 min at 50°C, then 10°C/min to 60°C, then 30°C/min to 100°C, then 10°C/min to 200°C, then hold for 1 min (Fig. 1). The flow-rate of the helium carrier gas was 12.5 ml/min. Quantification was achieved by measurement of peak heights by Perkin-Elmer Lims 2000 laboratory automation using multilevel calibration.

Stability tests

The stock solutions of benzene and toluene as well as I.S. solutions in ethanol were tested for stability at −20°C after two, four or seven months in 2-ml vials, with a Teflon screw-cap. The dilutions in water in bottles with plastic stoppers

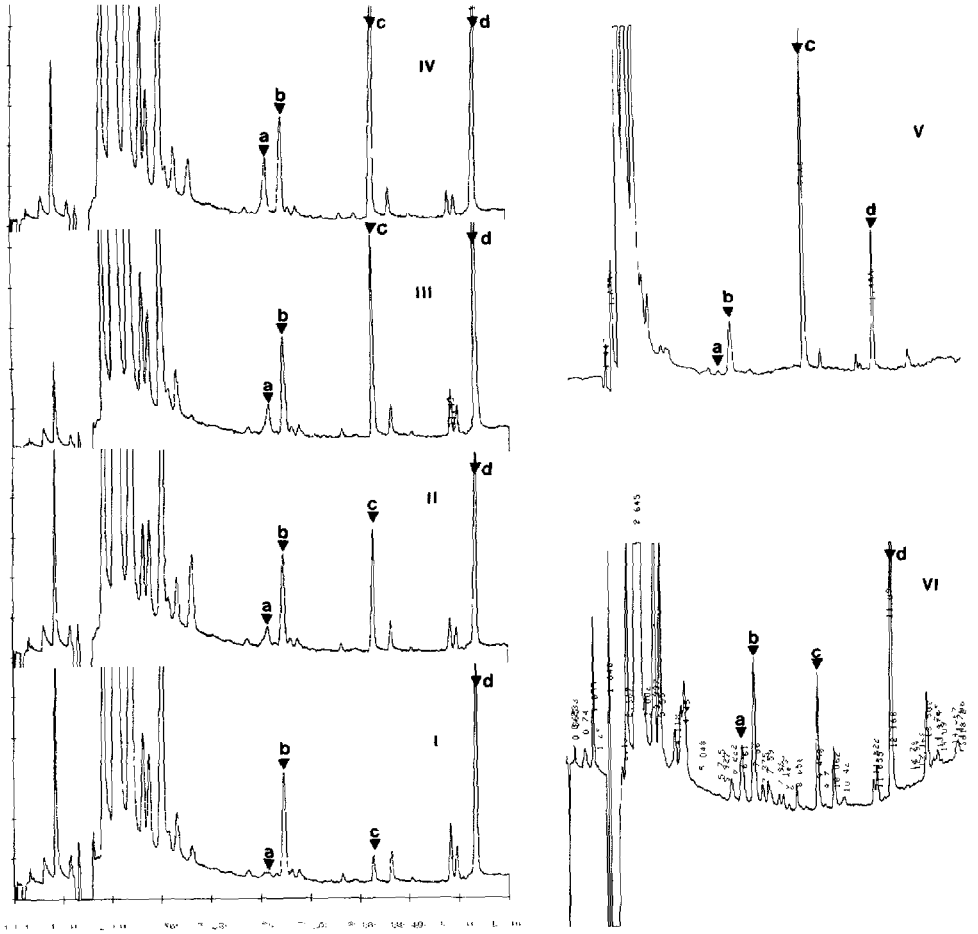

Fig. 1. Head-space determination of benzene (a) and toluene (c) in blood; internal standards were fluorobenzene (b) and *o*-xylene (d). Two columns (HP-1 + HP-5, both 30 m × 0.53 mm I.D.) connected with shrinkable Teflon tubing. Added amounts of benzene and toluene in blood: (I) a, 0 nmol/l; c, 0 μmol/l; (II) a, 7 nmol/l; c, 0.054 μmol/l; (III) a, 13 nmol/l; c, 0.108 μmol/l; (IV) a, 26 nmol/l; c, 0.215 μmol/l; (V) blood of a photogravure printing worker exposed to toluene; (VI) blood of a pumpman in petroleum transport exposed to benzene and toluene.

and the final calibration standards in blood in the closed head-space vials were also tested after two weeks.

RESULTS AND DISCUSSION

Stability tests

The stock solutions of benzene and toluene standards in ethanol were reasonably stable in the freezer. The yield of a toluene stock solution stored for

two months was 100.4% (S.D. 5.5) ($n=6$) and that of a benzene solution after four months 98.3% (S.D. 9.1) ($n=3$). When stored for seven months the yields of toluene and benzene were ca. 90%. The diluted standards in water stored in the cold room (+6°C) did not remain stable for many days, probably due to leaks from the flasks with plastic stoppers [12,13]. Final calibration standards of toluene and benzene stored at +6°C for five days in head-space vials gave yields of 93.2% (S.D. 6.1) ($n=6$) and 92.4% (S.D. 6.9) ($n=3$), respectively. Hence calibration standards from stored stock solutions were prepared weekly and kept in closed head-space vials until analysed.

Effect of pressurization and injection times on the peak height

Fig. 2 shows the effect of the injection time on the peak height when the pressurization time is held constant and conversely the effect of the pressurization time when injection time is constant.

When either the injection or the pressurization time was increased with the other parameter held constant, the peak height increased. However, when the maximum injection and pressurization times were used simultaneously the peaks appeared broadened, owing to insufficient carrier gas pressure. This led to decreased sensitivity. It was concluded that with the carrier gas flow-rate of 12.5 ml/min the injection time should be shorter than 0.14 min and the pressurization time should not exceed 1.5 min.

Gas–liquid equilibration in the head-space samples

Gas–liquid equilibration was investigated by changing the thermostatting time from 5 to 70 min and analysing the head space for benzene, toluene, fluorobenzene, and *o*-xylene (Fig. 3).

All four solvents reached equilibrium in the head-space vials at 80°C in 1 h. However, for practical reasons, we have used a thermostatting time of 23.0 min, as the sensitivity of benzene or fluorobenzene did not increase much with increasing thermostatting time up to 60 min, and with toluene and *o*-xylene there was sufficient sensitivity at 23 min, when the repeatability was also good.

Chromatographic separation

The tested chromatographic phases, methylsilicone and 5% phenylmethylsilicone and their combination, gave baseline separation for pure benzene and toluene. Similarly, the separation of toluene from the blood matrix peaks was sufficient with all columns tested. The benzene peak was not totally separated from the matrix peaks when using the non-polar methylsilicone phase, but with the more polar phenylmethylsilicone phase (HP-5) the separation was sufficient when the starting oven temperature was 10–25°C. When the two columns were connected with shrinkable Teflon tubing we obtained a good separation starting at 50°C (Fig. 1). Thus cooling of the column, necessary at temperatures below 50°C, could be avoided.

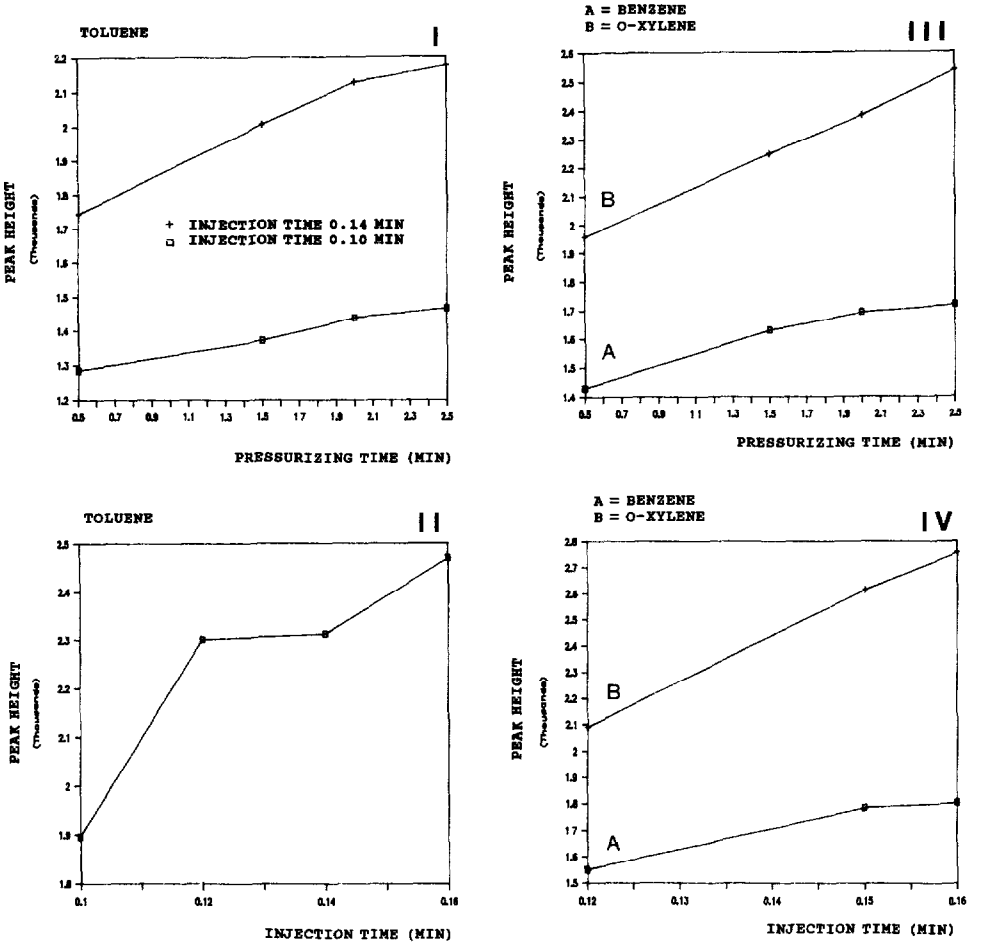

Fig. 2. Effect of pressurization and injection times on the chromatographic peak heights of benzene, toluene and *o*-xylene. (II, IV) Pressurization time, 1.5 min; (III) injection time, 0.14 min.

Detection

In chromatographic analysis benzene and toluene are often detected by universal flame ionization detection (FID) [3,4,8–10,14] or specific mass detection [4,11,15,16]. The sensitivity for benzene and toluene with these two detectors is rather similar. Campbell et al. [4] gave a detection limit of 0.3 μmol/l for toluene in blood and Drummond et al. [10] a limit of 0.05 μmol/l for benzene using FID. We have got a detection limit of 0.05 μmol/l for benzene and toluene when using packed column and FID with head-space analysis. Recently, Mølhave and Pedersen [17] reported analysis of benzene in exhaled air using PID. With PID the detection limit for benzene and toluene in blood

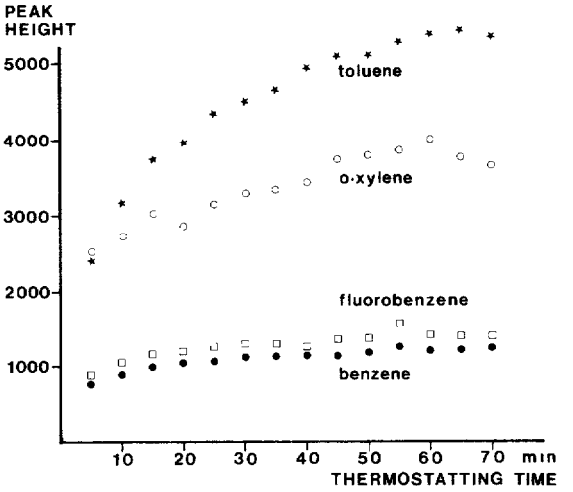

Fig. 3. Effect of thermostatting time on the concentrations of benzene, fluorobenzene, toluene and *o*-xylene in the head-space phase.

with our method is 5 nmol/l, measured as a smallest addition of the compound in the control blood that gives a peak height twice that of reagent blank. However, from time to time we have detected small peaks (less than 2 nmol/l) cochromatographing with benzene or toluene even from empty head-space vials. If analyses of reagent blank in a routine analysis contained such a peak, the result was corrected by reducing this height from the peak height of the specimens. Interference from matrix and reagents is less marked with PID, which is sensitive to double bond and aromatic ring, than with FID. For ionization of benzene and toluene the energy of 10.2 eV is sufficient, and thus interference from compounds with higher ionization potentials may be avoided [18].

Effect of the carrier gas flow-rate on the peak height

With concentration-sensitive detection like PID, the response of the detector is increased as the carrier gas flow-rate is decreased, to the point where the pressure is no longer sufficient to move the sample smoothly. In our head-space apparatus the minimum flow-rate with the column of 0.53 mm I.D. that gives a peak of maximum height is 12.5 ml/min. If the flow-rate is increased the peak becomes smaller and if the flow-rate is decreased injection system will produce split peaks (Fig. 4).

Linearity

Calibration graphs from spiked blood samples were found to be linear over the concentration ranges 0–0.2 μmol/l for benzene and 0–2.0 μmol/l for toluene ($r \geq 0.99$).

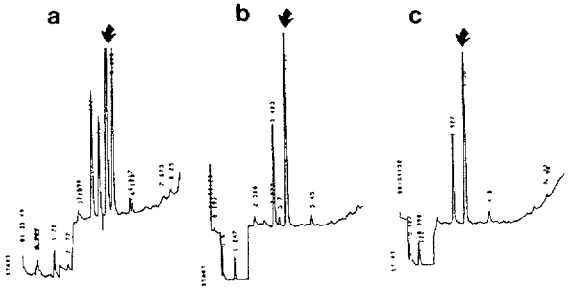

Fig. 4. Effect of the carrier gas flow-rate on the detection of benzene: (a) 10 ml/min; (b) 12.5 ml/min; (c) 14.0 ml/min.

TABLE I

COEFFICIENTS OF VARIATION IN THE ANALYSES OF BENZENE AND TOLUENE CALCULATED FROM DUPLICATE DETERMINATIONS WITHIN A RUN

Compound	Concentration range (μmol/l)	Number of duplicates	C.V. (%)
Toluene	0.1 -4.1	10	0.06
Benzene	0.003-0.017	10	0.30
	0.050-0.010	10	0.08
	0.200-0.400	10	0.06

Repeatability

Coefficients of variation (C.V.) calculated from duplicate determinations are presented in Table I. At concentrations close to the detection limit the C.V. for benzene was 30%, but at concentrations close to 0.2 μmol/l it was 6–8%, similar to that for toluene.

Internal standard

The internal standards for biological monitoring methods must be substances that do not usually occur in the blood of exposed workers. For benzene a suitable I.S. is fluorobenzene and for toluene one can use *o*-xylene. These compounds have not been found in the blood of Finnish workers exposed to benzene and toluene. The two internal standards were chosen because their volatilization was similar to benzene and toluene (Fig. 3). They did not improve the repeatability in the analyses, when the head-space vials were properly closed. C.V. values for analyses of toluene and benzene were 7.0 and 18.5% with I.S., and 5.5 and 9.3%, without I.S., respectively. However, the use of an I.S. is of great importance. If the caps of the head-space bottles are loose one can immediately notice it as the peaks of the volatile internal standards, benzene and toluene nearly disappear and the analyser does not record the mini-

mum concentration of benzene and toluene as a true value of the sample in question.

Application

The biological monitoring method for the workers exposed to toluene based on the determination of toluene in blood, as a manual method using a packed column (10% SP 2100 on Supelcoport) and GC–FID instrumentation with a separate thermostatting system, has been used in Finland since 1979. With the method described here we have been able to perform routine measurements for both benzene and toluene in blood. The requests for biological monitoring analyses of benzene and toluene in blood have come from the occupational health care of workplaces in Finland where there is a known or suspected exposure to benzene and toluene. However, the specimens are by no means representative of the whole industry in Finland. The frequency distribution of the results obtained in 1987 is depicted in Fig. 5.

About 60% of the determinations of toluene in blood in the Finnish workers were smaller than 0.1 μmol/l and more than 80% were below 0.3 μmol/l (Fig. 5). We collected the specimens for blood toluene analyses towards the end of the week in the morning before exposure, which provides under fairly constant exposure conditions an idea of an approximate index of accumulated weekly exposure [19] or under variable exposure conditions an idea of the previous day's exposure. According to Campbell et al. [4] exposure to 100 ppm (375 mg/m^3) of toluene for 8 h results in a mean value of 21 μmol/l toluene in blood and of 580 nmol/l toluene in expired air in specimens collected at the end of the working shift. Approximated from the disappearance rate of toluene from exhaled air [4], the level of toluene in the blood in the morning will be ca. 2 μmol/l. Brugnogne et al. [3] have shown that a mean environmental exposure to ca. 150 mg/m^3 (40 ppm) during the working day yields a blood toluene concentration of ca. 0.3 μmol/l the following morning. Taking into account the accumulation of daily toluene exposure [20], we may assume that the exposure of the persons who gave specimens for our analyses (Fig. 4) was much lower than the Finnish hygienic limit value (375 mg/m^3).

Approximately 60% of benzene determinations in blood were below 0.005, 89% below 0.010 and 96% below 0.020 μmol/l when specimen collection was carried out 1 h after the daily exposure. Sato et al. [21] have reported that after exposure to benzene (25 ppm, 2 h), blood benzene concentrations were ca. 0.5–0.6 μmol/l, when the specimen collection took place 1 h after the exposure. Perbellini et al. [11] have presented a simulation model of variations of blood benzene concentrations during and after exposure to benzene of 1–5 mg/m^3 (0.3–1.7 ppm) for 8 h, where the expected benzene level in blood ca. 1 h after the exposure varies between 0.02 and 0.10 μmol/l and 16 h after the exposure between 2 and 15 nmol/l, respectively. In the exposure chamber test performed in the Institute of Occupational Health in Finland, we obtained

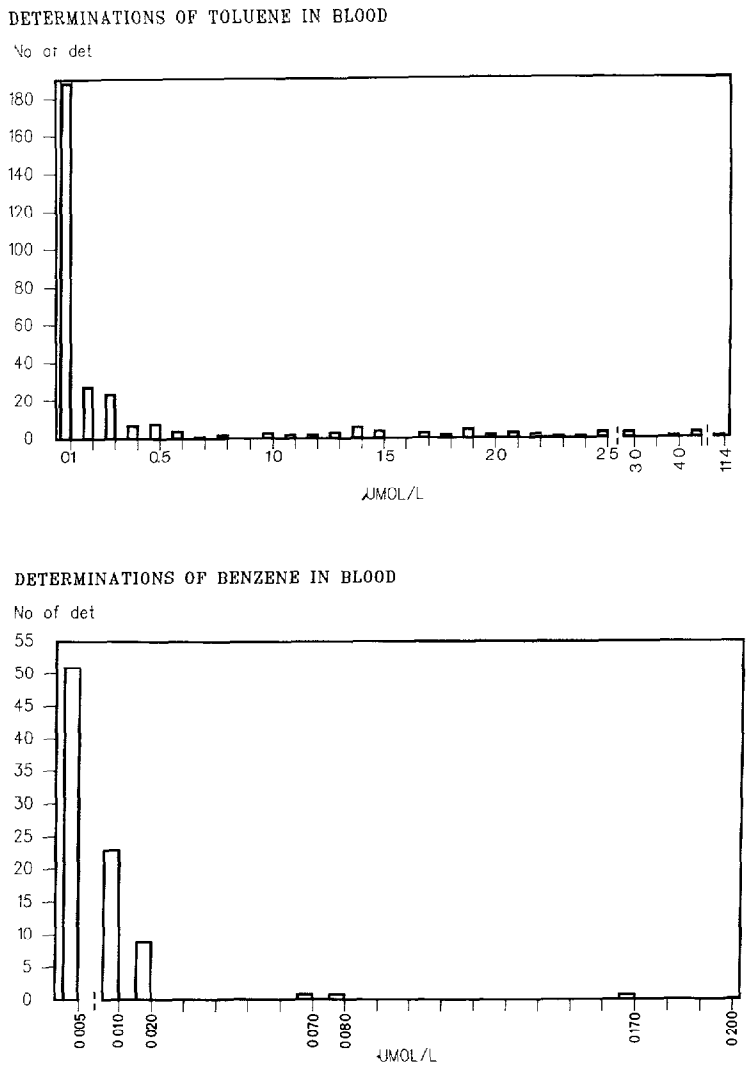

Fig. 5. Frequency distribution of the concentrations of benzene and toluene in blood of exposed workers. Number of benzene and toluene analyses in 1987 were 82 and 312, respectively. The specimens for toluene analysis were collected in the morning, before the daily exposure (and thus reflect exposure on preceding days). Those for the benzene analysis were collected 1 h after cessation of the exposure.

comparable results for benzene levels in blood after exposure of 10 ppm for 4 h and 1.7 ppm for 4 h, when specimen collection was 1 h after the exposure. Blood benzene levels were then 0.2–0.3 and 0.03 μmol/l, respectively [22]. Smoking before specimen collection leads to an overestimation of occupational

exposure to benzene [11] and should be controlled, especially if the collection time is on the morning before the exposure.

CONCLUSIONS

The head-space method described provides good selectivity and sensitivity for the determination of benzene and toluene. A single analysis for both benzene and toluene, which may occur simultaneously in the work environment, is a rapid way of analysing the two compounds. Another important advantage is also the smaller consumption of control blood, which may not be readily available. Benzene is a compound that may cause leukaemia in humans [23]. Toluene may cause narcotic and neurotoxic effects in humans, and it has shown some potential reproductive hazard as well [24,25]. It is important that, for the protection of workers exposed to these two solvents, there is a possibility of monitoring the amount taken up in the body at the relatively low exposure levels prevalent in today's industry.

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