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Determination of toluenediamine isomers by capillary gas chromatography and chemical ionization mass spectrometry with special reference to the biological monitoring of 2,4- and 2,6-toluene diisocyanate

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ABSTRACT

The determination of 2,3-, 3,4-, 2,6-, 2,4- and 2,5-toluenediamine (TDA) in hydrolysed human urine and blood plasma was studied by GC–MS. The TDA isomers as their perfluoro-fatty acid anhydride derivatives were investigated. Chemical ionization with ammonia and isobutane as reagent gas and monitoring both positive and negative ions are studied. Negative ion monitoring using ammonia and the TDA pentafluoropropionic anhydride (PFPA) derivatives were chosen owing to the low detection limits and good separations of the isomers studied. The ions monitored were m/z 394 and 374 corresponding to the $(M-20)^-$ and $(M-40)^-$ ions and the $m/z = 397$ and 377 ions of the trideuterium-labelled TDA used as an internal standard. The performance of 2,4-, 2,5- and 2,6-TDA-PFPA in the ion source was studied by varying the ammonia pressure, temperature and electron energy. A 1-ml volume of human urine was added to 1.5 ml of 6 M HCl containing 0.5 μg/l of each of the trideuterated 2,6- and 2,4-TDA and the solution was hydrolysed at 100°C overnight. TDA was extracted into 2 ml of toluene by the addition of 5 ml of saturated NaOH solution. Derivatization was performed in toluene by the addition of 10 μl of PFPA. The excesses of the reagent and acid formed were removed by extraction with 1 M phosphate buffer solution (pH 7.5). Analyses of 2,6-, 2,4- and 2,5-TDA-spiked human urine (0.2–2.5 μg/l) were performed. The correlation coefficients were 0.999 ($n = 6$). The precision (R.S.D.) for human urine spiked at 1 μg/l was 1.6% for 2,6-TDA, 3,5% for 2,4-TDA and 3.2% for 2,5-TDA ($n = 10$). The detection limit, defined as twice the signal-to-noise ratio, was 1–5 fg injected, corresponding to less than 0.05 μg/l of TDA in human urine or plasma.

INTRODUCTION

The identification and determination of amines and isocyanates in air and biological samples such as urine and blood have been a subject of increasing interest in recent years owing to their environmental and occupational hazards. Kennedy and Brown [1] recently published a review on this topic. Toluene diisocyanate (TDI) is one of the main components in the production of polyurethane polymers. The commercially used TDI is a mixture of 2,4- and 2,6-TDI in the ratio 80:20 or 65:35 (v/v). The two TDI-related aromatic amines 2,4- and 2,6-toluenediamine (TDA) are mainly used as intermediates in TDI, elastomer and dye production [2].

Gas chromatography (GC) with electron-capture or mass spectrometric (MS) detection of amines has been studied in some detail during the last 25 years. Amines normally elute as tailing peaks when analysed as such. Analysis for amines as perfluoro-fatty acid derivatives reduces the adsorption and thereby the peak tailing. These derivatives also show very low detection limits using ECD. MS with chemical ionization

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(CI) monitoring of negative ions also gives low detection limits [3–5].

Several techniques have been used for the determination of aromatic amines in air and biological fluids, including liquid chromatography (LC) and UV [6] and electrochemical detection [7], GC with electron-capture [8] and thermionic specific detection (TSD) [9] and GC–MS using electron impact (EI) and positive ion monitoring [10].

Methods for the determination of low concentrations of TDA in hydrolysed human urine and plasma have been developed [11] at our laboratory. The methods have been used by Brorson *et al.* [12] and Skarping *et al.* [13] for the biological monitoring of exposure to TDI. The main interest has been focused on the 2,4- and 2,6-TDA isomers related to 2,4- and 2,6-TDI. 2,5-TDA, which is a component of hair dyes, is also of interest [14].

Capillary GC methods with thermionic specific detection of 2,4- and 2,6-TDA as acetyl, perfluoro-fatty acid and carbamate ester derivatives have been investigated in our laboratories [11]. In earlier studies, EI and the monitoring of the molecular ion of TDA pentafluoropropionic anhydride derivative (PFPA) using selected-ion monitoring (SIM) were considered [11]. The samples were enriched by a factor of 40 by evaporation of the solvent and the detection limit was *ca.* 8 pg injected, corresponding to a concentration of 0.1 μg/l of TDA in urine. In this paper, a GC–MS method based on monitoring of negative ions formed by CI with ammonia is presented. The determinations of the five isomers 2,3-, 3,4-, 2,6-, 2,4- and 2,5-TDA as their heptafluorobutyric (HFBA), pentafluoropropionic (PFPA) and trifluoroacetic (TFAA) derivatives in hydrolysed human urine and plasma samples are presented. The sixth TDA isomer, 3,5-TDA, was not commercially available and was therefore not included in this study.

EXPERIMENTAL

Apparatus

A VG-Quattro triple quadropole mass spectrometer (Fisons Instruments, VG-Biotech, Altrincham, UK) connected to a Carlo Erba Mega gas chromatograph equipped with an A200S autosampler (Fisons Instruments, Carlo Erba, Milan, Italy) was employed. Injection was performed using splitless injection. The syringe needle was heated in the injector for 10 s before injection (the hot needle technique) and the injector temperature was 290°C. The starting temperature of the column oven was isothermal at 110°C for 1 min, then raised at 15°C/min to 280°C, where it was kept for 2 min. The split-exit valve was kept closed for 1 min after injection. The temperature of the ion source was 230°C and the GC–MS interface temperature was 280°C. The capillary inlet pressure of helium was 0.8 kg/m^2. The solvent delay was set to 2.9 min. After injection, the syringe was cleaned by washing with eight 10-μl volumes of toluene. Before injection the syringe was washed by two 10-μl volumes of the sample.

In most of the quantitative investigations the instrument was used in the CI mode with negative ion monitoring using ammonia as the reagent gas. When determining the TDA-PFPA derivatives the ions monitored were m/z 394 and 374, corresponding to the $(M-20)^-$ (M = molecular ion) and the $(M-40)^-$ ions, and the m/z 397 and 377 ions of the trideuterium-labelled internal standard. The dwell time for each of the ions was 0.2 s and the inter-scan delay was 0.02 s. The pressure in the ion source, in the case of CI with ammonia and negative ion monitoring, was kept at *ca.* $2 \cdot 10^{-4}$ mbar (source readout pressure, not the actual pressure in the ionization cavity). The emission current was 200 mV and the electron energy 60 eV. The tuning of the instrument was optimized and performed using perfluorotributylamine (PFTBA) as a calibrant. The resolution was set by monitoring the fragments at m/z 633 and 634. The valley between the fragments was set to 10% of the peak height of the m/z 634 fragment. Mass spectra were obtained by scanning ions between 50 and 600 u for 0.7 s with an inter-scan delay of 0.1 s.

For the continuous monitoring of TDA derivatives in the MS system, an inlet device was designed at VG-Biotech. A few micrograms of the sample were injected into a heated reservoir kept under vacuum. The reservoir was in contact

with the ion source via a capillary strainless-steel tube of I.D. < 0.1 mm. The inlet device was connected to the MS instrument similarly to the solid sample inlet apparatus. This inlet device makes it possible to optimize the system faster and in much more detail. A Sigma 3E-1 centrifuge (Sigma, Hartz, Germany) was used for phase separation.

Columns

Fused-silica capillary columns with DB-5 chemically bonded stationary phase (J&W Scientific, Folsom, CA, USA) (30 m × 0.25 mm I.D.) with a film thickness of 0.25 μm were used.

Chemicals

The chemicals used were 2,4- and 2,6-TDA from Fluka (Buchs, Switzerland), 2,5-TDA and 3,4-TDA from Janssen (Geel, Belgium) and 2,3-TDA from Aldrich-Europe (Steinheim, Germany). Trifluoroacetic anhydride (TFAA), pentafluoropropionic anhydride (PFPA) and heptafluorobutyric anhydride (HFBA) were obtained from Pierce (Rockford, IL, USA), toluene from Lab-Scan (Dublin, Ireland), HCl, NaOH, perfluorotributylamine (PFTBA) and K_2HPO_4 from Merck (Darmstadt, Germany) and trideuterated 2,6- and 2,4-TDA, 2,6- and 2,4-TDA-PFPA derivatives and 2,6- and 2,4-HFBA derivatives from Syntelec (Lund, Sweden). The synthesis of these derivatives has been described previously [11].

Procedure

Preparation of standard solutions. Standard solutions of the five investigated TDA isomers were prepared by dissolving accurately weighted amounts in 0.1 *M* hydrochloric acid. The solutions were further diluted to appropriate concentrations.

Sampling and storage of biological samples. All urine samples were collected in polyethylene bottles. Urine samples were acidified by addition of 5 ml of 6 *M* HCl per 100 ml of urine. The density, creatinine concentration, pH and total volume of the urine samples were determined. The urine samples were stored in a refrigerator until analysis. Blood was sampled in heparinized tubes (Venoject). Plasma was separated within 8 h and transferred to a new tube. Blood plasma was kept frozen at −20°C until analysis.

Work-up procedure. To a 1-ml urine or plasma sample, 1.5 ml of 6 *M* HCl, containing the trideuterated 2,6- and 2,4-TDA internal standards each at a concentration of 0.5 μg/l, was added and the samples were hydrolysed at 100°C overnight. A 5-ml volume of saturated NaOH and 2 ml of toluene were then added and the mixture was shaken for *ca.* 10 min and then centrifuged at 1500 *g* for 10 min. A 1.5-ml volume of the organic layer was transferred to a new test-tube and 10 μl of the anhydride derivatization reagent were added. The mixture was immediately shaken vigorously for *ca.* 10 min. The excesses of the reagent and acid formed were removed by extraction with 2 ml of 1 *M* phosphate buffer solution (pH 7.5). A 1-ml volume of the toluene layer containing the amide derivative and the internal standard was transferred into an 1.5-ml autosampler vial and was then ready for injection into the GC–MS system.

Internal standard

Trideuterated 2,6- and 2,4-TDA were used as internal standards and were added to the urine samples prior to hydrolysis.

RESULTS AND DISCUSSION

Standards

The identities of the perfluoro-fatty acid amides of the TDAs were confirmed by GC–MS and purities were determined using capillary GC–TSD and GC with flame ionization detection. Elemental analysis of the amide derivatives of HFBA and PFPA gave values differing by less than 0.3% from the calculated values.

The isotopic purities of 2,6- and 2,4-TDA and the trideuterated 2,6- and 2,4-TDA were also checked. When determining 2,6- and 2,4-TDA-PFPA derivatives by GC–SIM using CI with ammonia and negative ion monitoring, the most abundant fragments were m/z $(M-20)^-$ (394). Less than a 0.3% relative abundance was observed for the m/z 397 compared with the m/z 394 fragment. Trideuterated 2,6- and 2,4-TDA-PFPA derivatives were monitored in the same way with the most abundant ions of $m/z =$

$(M-20)^-$ (397). Less than a 0.3% relative abundance was observed for the m/z 394 compared with the m/z 397 fragment.

Internal standard

All determinations were performed using trideuterated 2,6- and 2,4-TDA as the internal standards. The three hydrogens in the methyl group were exchanged with deuterium. The use of trideuterated 2,6- and 2,4-TDA as internal standards has been discussed previously [11].

TDA and trideuterated TDA show very similar fragmentation patterns. Several fragments [$(M-20)^-$, $(M-40)^-$ and $(M-60)^-$] could be monitored simultaneously.

Work-up procedure

Storage and treatment of samples. Urine samples spiked with 2,6- and 2,4-TDA were found to be stable after acidification. No noticeable degradation of the samples was found after storage for several weeks in the dark at room temperature. Plasma samples were kept frozen until analysis. On analysing the same plasma samples on three different occasions during a 5-week period, no noticeable degradation of the sample was found. In the present study all standards were freshly prepared every other week. The derivatives in toluene solution were found to be stable for several weeks without any noticeable degradation.

Hydrolysis. When performing the work-up procedure with urine samples spiked with the five TDA isomers to a concentration of 20 μg/l, no losses of 2,6-, 2,4- and 2,5-TDA were found. However, more than 70% of the 2,3- and 3,4-TDA isomers were lost. The losses were related to the hydrolysis step and increased with the hydrolysis time. On prolonging the hydrolysis time losses were also seen for 2,4- and 2,6-TDA. A hydrolysis time of *ca*. 240 h gave a 10–20% loss in plasma and a 40% loss in urine. The hydrolyses of pooled humane urine and pooled human plasma from exposed TDI workers were studied under acidic and alkaline conditions. Acidic hydrolysis for 24 h was studied for 4 *M* and 6 *M* HCl at 100°C, 4 *M* and 6 *M* HCl at 110°C and 6 *M* HCl under vacuum with freeze-dried urine at 110°C. Alkaline hydrolysis was studied for 24 h for 5 *M* NaOH at 100 and 110°C. The amounts of both 2,6- and 2,4-TDA released increased by 20–30% with increasing hydrolysis temperature for acid hydrolysis. Hydrolysis under alkaline conditions released about twice as much 2,6- and 2,4-TDA. On varying the hydrolysis time up to 240 h in 4 *M* HCl at 100°C of pooled humane urine and pooled human plasma from exposed workers, the amounts of 2,4- and 2,6-TDA increased with time and no optimum was found. On working up the samples without the hydrolysis step no 2,4- and 2,6-TDA were obtained, within experimental error. For practical reasons and comparison with our earlier studies [12], hydrolysis with 4 *M* HCl at 100°C overnight was chosen and the high sensitivity of the method presented does not necessitate further release of TDA from the biological samples.

The results indicate that the TDAs in plasma and urine are covalently bonded. The bonds seems stronger than peptide bonds. Peptide bonds are expected to break up under acidic hydrolysis. The investigation of the chemical form of TDI/TDA in human urine and plasma is in progress. In a controlled exposure study [12] of human volunteers, 15–19% of 2,4-TDA and 17–23% 2,6-TDA of the estimated dose of the TDI isomers were found in the excreted urine. A linear relationship between urinary excreted amount and the dose was obtained.

Choice of reagent

Several anhydride and chloroformate derivatization reagents were tested. The carbamate ester derivatives formed with TDA after derivatization with chloroformates such as methyl, ethyl and isobutyl chloroformate elute at higher elution temperatures, giving longer elution times than the perfluoro-fatty acid amide derivatives. The carbamate esters are thermolabile and all the carbamate derivatives formed are decomposed in the injector [10]. The cold on-column injection technique is therefore the most suitable injection technique for these kinds of compounds. With the present GC–MS systems available at our laboratory, no automatic on-column injector with an autosampler was available. No thermal decomposition of the perfluoro-fatty TDA

amides was observed. The detection limits achieved for the carbamate esters, using CI and monitoring positive ions, are at least two orders of magnitude higher than those for perfluoro-fatty acid amides when using CI with ammonia and monitoring negative ions. It seems that derivatives giving low detection limits using ECD also give low detection limits using GC–MS with CI with negative ion monitoring [10]. All the tested perfluoro-fatty acid anhydrides gave a *ca.* 100% recovery for the single-phase derivatization. The elution time and temperature varied surprisingly little (less than 10%) with variation in the perfluoro-fatty acid anhydride reagent.

The chromatographic separation achieved for the five TDA isomers studied is almost the same for the three derivatization reagents. The sensitivity when monitoring the $m/z = (M-20)^-$ fragments is virtually the same for PFPA and HFBA derivatives but 40–200 times less for the TFA derivatives. The sensitivity for the TFA derivatives also varies with the isomer studied and *ca.* ten times higher sensitivities for the 2,3- and 3,4-TDA compared with the 2,6-, 2,4- and 2,5-TDA derivatives are observed. The PFPA derivatization reagent was chosen owing to the 5–10% shorter retention times of the amides formed and because this reagent has been the most frequently used in our earlier studies on the determination of aliphatic and aromatic amines.

Chromatography

TFAA, PFPA and HFBA derivatives of 2,3-, 3,4-, 2,6-, 2,4- and 2,5-TDA were studied. All derivatives showed excellent chromatographic behaviour. Using the DB-5 capillary column, baseline separations of the five TDA amides were obtained with the three derivatization reagents tested. Chromatograms for diluted standards in toluene of the five TDA derivatives are shown in Fig. 1. No interfering peaks were observed in urine from ten unexposed persons when monitoring the $m/z = (M-20)^-$ fragments. After *ca.* 1000 injections of biological samples the TDA-PFPA peaks started to show some tailing. When about 0.5 m of the capillary column inlet end was cut off, the column performance was restored.

Detection

For the analysis of environmental samples containing traces of aromatic amines, very sensitive determination is necessary. This can be achieved using TSD, ECD and MS detection. Earlier, for air samples, we achieved very sensitive determinations using ECD and TSD. TDI in air was determined below 1 $\mu g/m^3$. The present Swedish Threshold Limit Value (TLV) is 40 $\mu g/m^3$. When monitoring isocyanates in a more complex matrix such as air containing thermal decomposition products, only TSD demonstrated satisfactory selectivity. Initial attempts to analyse biological samples containing TDA using TSD and ECD resulted in chromatograms with an enormous background of peaks giving detection limits several orders of magnitudes higher than required. Using capillary GC–MS very sensitive and selective determinations were possible.

Mass spectrometry

EI and CI with ammonia and isobutane monitoring positive (CI^+) and negative ions (CI^-) were studied. Using EI and determining the TDA-PFPA derivatives the molecular ion (m/z = 414) and the $M - C_2F_5$ ($m/z = 295$) fragment are the most abundant ions, which were selected for quantification. Compared with CI monitoring negative ions, the detection limit was *ca.* 50 times higher. When determining low nanograms per litre concentrations of TDA, interferences relative the matrix were seen. In Table I the mass spectra obtained for the TFAA, PFPA and HFBA derivatives of 2,3-, 3,4-, 2,6-, 2,4- and 2,5-TDA using CI with positive and negative ion monitoring with ammonia and isobutane as the reagent gases are shown.

Chemical ionization with ammonia monitoring positive ions. As can be seen in Table I, positive ions formed with ammonia show simple and easy to interpret spectra and very little fragmentation is seen. The $m/z = (M+18)^+$ fragment is the most abundant for all the compounds studied.

Chemical ionization with ammonia monitoring negative ions. The negative fragments formed vary with the isomer and derivative studied and a more complicated fragmentation pattern is seen compared with positive ion monitoring. The relative abundance of the molecular ion was less

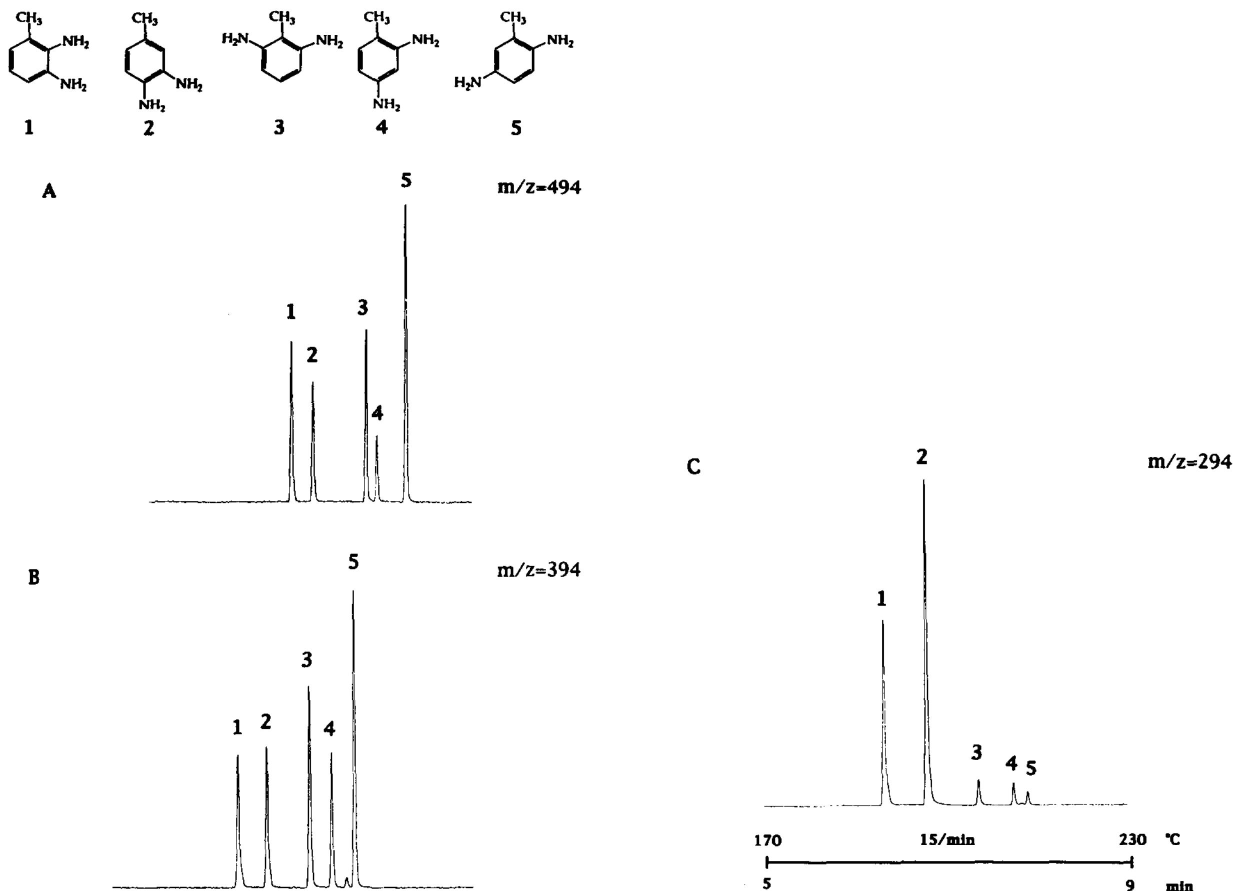

Fig. 1. Selected-ion monitoring of perfluoro-fatty acid amides with TDA in toluene using CI with ammonia and monitoring negative ions of $m/z=(M-20)^-$. (A) TDA-HFBA derivatives (m/z 494). The peaks correspond to 100 fg of TDA. (B) TDA-PFPA derivatives (m/z 394). The peaks correspond to 100 fg of TDA. (C) TDA-TFAA derivatives (m/z 294). The peaks correspond to 1 pg of TDA. Peaks: 1 = 2,3-TDA; 2 = 3,4-TDA; 3 = 2,6-TDA; 4 = 2,4-TDA; 5 = 2,5-TDA. Column, fused silica coated with DB-5 bonded stationary phase (30 m × 0.243 mm I.D.); film thickness, 0.25 μm; inlet pressure of the carrier gas (helium), 0.8 kg/cm^2; splitless injection (1 min) of 1 μl of toluene; temperature programme, isothermal at 110°C (1 min), raised at 15°C/min to 280°C, maintained for 2 min.

than 0.1% and the most abundant fragment was the $m/z=(M-20)^-$ fragment. This fragment probably originates from the molecular ion with the neutral loss of HF. In the spectra of 2,6-TDA-HFBA, further neutral loss ($m/z=M-n\times 20)^-$ of HF may occur ($n=1$–4). For 2,6- and 2,4-TDA it is convenient to monitor more than one ion to minimize the influence of interfering compounds. Ammonia as the reagent gas can be used continuously for several weeks, giving reproducible results without the necessity to clean the ion source. Negative ion monitoring with ammonia is by far the most sensitive technique.

Chemical ionization with isobutane monitoring positive ions. CI with positive ion monitoring with isobutane shows, compared with CI$^+$ with ammonia, simple spectra with very little fragmentation. The $m/z=(M+1)^+$ fragment is the most abundant fragment in all instances. All isomers and derivatives give similar spectra.

Chemical ionization with isobutane monitoring negative ions. On monitoring negative ions formed with isobutane, similar spectra to those with ammonia are obtained. The intensities of the ions formed are lower, resulting in higher detection limits.

Isobutane contaminates the ion source, notice-

TABLE I

MASS SPECTRA OBTAINED FOR TFA-, PFPA- AND HFBA AMIDE DERIVATIVES OF 2,3-, 3,4-, 2,6-, 2,4- AND 2,5-TDA

The injected amount was 10 ng of each TDA isomer. The ion source temperature was 230°C and the capillary inlet pressure of helium was 0.8 kg/m^3. The pressure in the ion source was *ca.* 1×10^{-4} mbar for CI with ammonia and positive ion monitoring and *ca.* 2×10^{-4} mbar for CI with ammonia and negative ion monitoring. The emission current was 200 mV and the electron energy 60 eV. Ions of *m/z* 50–600 were monitored for under 0.7 s with an inter-scan delay of 0.1 s. Values of *m/z* and relative abundance (332/100 etc.) are arranged in ascending order. Only fragments with relative abundances >4% are included.

Method	TDA	*m/z* and relative abundance (%)
CI with ammonia monitoring positive ions of TFAA amides	2,3-TDA	332/100, 201/85, 219/5, 283/5
	2,4-TDA	332/100, 219/15, 236/10, 149/5
	2,5-TDA	332/100, 201/35, 283/5, 219/5
	2,6-TDA	332/100, 236/15, 314/10, 219/5
	3,4-TDA	332/100, 219/10, 236/5
CI with ammonia monitoring negative ions of TFAA amides	2,3-TDA	294/100, 177/85, 279/70, 217/25, 313/5
	2,4-TDA	294/100, 298/30, 274/25, 278/20, 313/15
	2,5-TDA	294/100, 298/65, 313/20, 69/10, 217/8
	2,6-TDA	294/100, 298/45, 313/13
	3,4-TDA	294/100, 217/30, 177/30, 199/15, 314/15
CI with ammonia monitoring positive ions of PFPA amides	2,3-TDA	432/100, 251/20, 148/10
	2,4-TDA	432/100, 286/10, 269/10
	2,5-TDA	432/100, 251/15, 148/10
	2,6-TDA	432/100, 286/10, 414/10
	3,4-TDA	432/100, 269/5, 287/5
CI with ammonia monitoring negative ions of PFPA amides	2,3-TDA	394/100, 379/45, 128/45, 230/45, 275/20, 293/10, 413/5
	2,4-TDA	394/100, 374/100, 128/55, 354/35, 228/10, 248/10, 413/5
	2,5-TDA	394/100, 128/55, 344/15, 248/10, 274/5, 413/5
	2,6-TDA	394/100, 374/55, 128/45, 248/10, 413/5
	3,4-TDA	394/100, 275/70, 128/40, 230/35, 413/5
CI with ammonia monitoring positive ions of HFBA amides	2,3-TDA	532/100, 301/20, 148/10
	2,4-TDA	532/100, 336/5, 319/5
	2,5-TDA	532/100, 301/15, 148/10, 319/5
	2,6-TDA	532/100, 336/10, 514/5
	3,4-TDA	532/100, 319/5, 336/5
CI with ammonia monitoring negative ions of HFBA amides	2,3-TDA	494/100, 325/35, 178/25, 280/25, 479/20, 343/20, 513/5
	2,4-TDA	474/100, 254/80, 494/75, 178/40, 278/10, 513/5
	2,5-TDA	494/100, 178/30, 298/10, 474/10, 324/10, 513/5
	2,6-TDA	494/100, 474/75, 178/30, 454/15, 298.10, 513/5
	3,4-TDA	494/100, 325/90, 178/30, 280/25, 157/10, 146/10, 513/5

able even after 1–2 h of use, and rinsing of the ion source must be performed frequently. Isobutane is therefore not recommended as a reagent gas for automatic "routine" determinations that may take several days to complete.

The positioning of the capillary column outlet in the ion source was critical; moving the column 2–3 mm changed the sensitivity 50-fold. Optimum sensitivity was obtained when the column outlet was only *ca.* 2 mm from the centre of the ion source. At the beginning of the study, changing the ionization mode from CI^- or CI^+ to EI was troublesome, with drifting optimum tuning parameters. Typically, the EI sensitivity was lowered a factor of 10 after using CI for 1–2 weeks. When the same column had been used for several weeks the decrease in EI sensitivity on changing from CI to EI became less. It was also

observed that about 5 mm of the major part of the polyimide polymer coating of the end of the capillary outlet had vanished. By connecting a capillary column after burning off *ca.* 5 mm of the capillary outlet with a micro-flame or a heat gun in order to remove the polyimide layer, switching between CI and EI could be done without any problems. The ion source could be used for weeks without the need for rinsing and with very good repeatability.

Ion source optimization. An inlet device for the continuous introduction of sample molecules into the ion source was tested. Stable conditions (amount of sample introduced into the ion source per unit time) were obtained for more than 10 h. The inlet device makes it possible to optimize the MS conditions in more detail for the compounds under study. Optimum settings for lenses and electron energy and conditions such as reaction gas pressure, temperature and resolution were established. The ion source pressure read out only gives a "good hint" about the real pressure in the source. It was not possible to measure the real pressure inside the source where the chemical reactions take place. In Fig. 2 it can be seen that the pressure giving optimum abundance of the $m/z = (M-20)^-$ (394) fragment was about $1.8 \cdot 10^{-4}$ mbar, which is 40% less than with optimization using PFTBA.

In Fig. 3 the optimum electron energy was *ca.* 80 eV, which is the same as with PFTBA. The temperature of the ion source was varied between 140 and 350°C and standard solutions

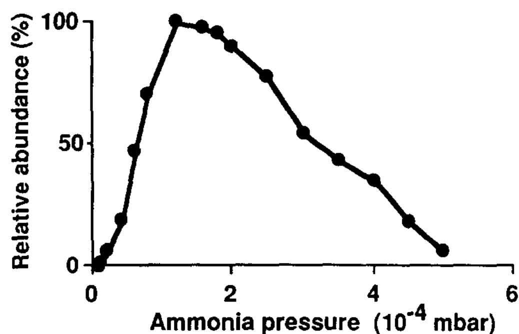

Fig. 2. Variation of the relative abundance of the m/z 394 fragment with source pressure. The ion source pressure readout is an indirect measure which was performed outside the source.

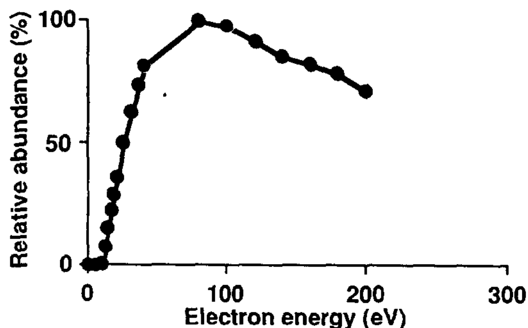

Fig. 3. Variation of the relative abundance of the m/z 394 fragment with electron energy.

containing *ca.* 10 ng/μl of the TFAA, PFPA and HFBA derivatives of 2,3-, 3,4-, 2,6-, 2,4- and 2,5-TDA were repeatedly injected. Mass spectra were obtained and evaluated. The relative abundances of the $m/z = (M - n \times 20)^-$ ($n = 1$–4) fragments *versus* ion source temperature are shown in Fig. 4 for the 2,6- and 2,4-TDA TFAA, PFPA and HFBA derivatives. The typical fragmentation pattern involves increased neutral losses of HF with increase in temperature. The optimum sensitivity was at 140°C, but at this low temperature tailing chromatographic peaks were obtained. It can be seen that 230°C is the optimum temperature for the $m/z = (M-20)^-$ fragment in the PFPA and the HFBA plots. The TFAA plots have an optimum at 250°C.

Quantitative analysis

Recovery. When performing the work-up procedure with urine samples spiked with the five TDA isomers at a concentration of 20 μg/l, for 2,6-, 2,4- and 2,5-TDA the recovery was 100 ± 2% (with a 95% degree of confidence, $n = 5$). However, significant losses, which varied, of >70% of 2,3- and 3,4-TDA was found. It was established that the losses occurred during the hydrolysis of the urine. To apply the present method for the determination of 2,3- and 3,4-TDA in human urine further development is necessary.

Calibration graphs. Human urine were spiked with 2,6-, 2,4- and 2,5-TDA at six different concentrations in the range 0.2–2.5 μg/l and the work-up procedure was then performed. The $m/z = (M-20)^-$ fragments of the TDA and the

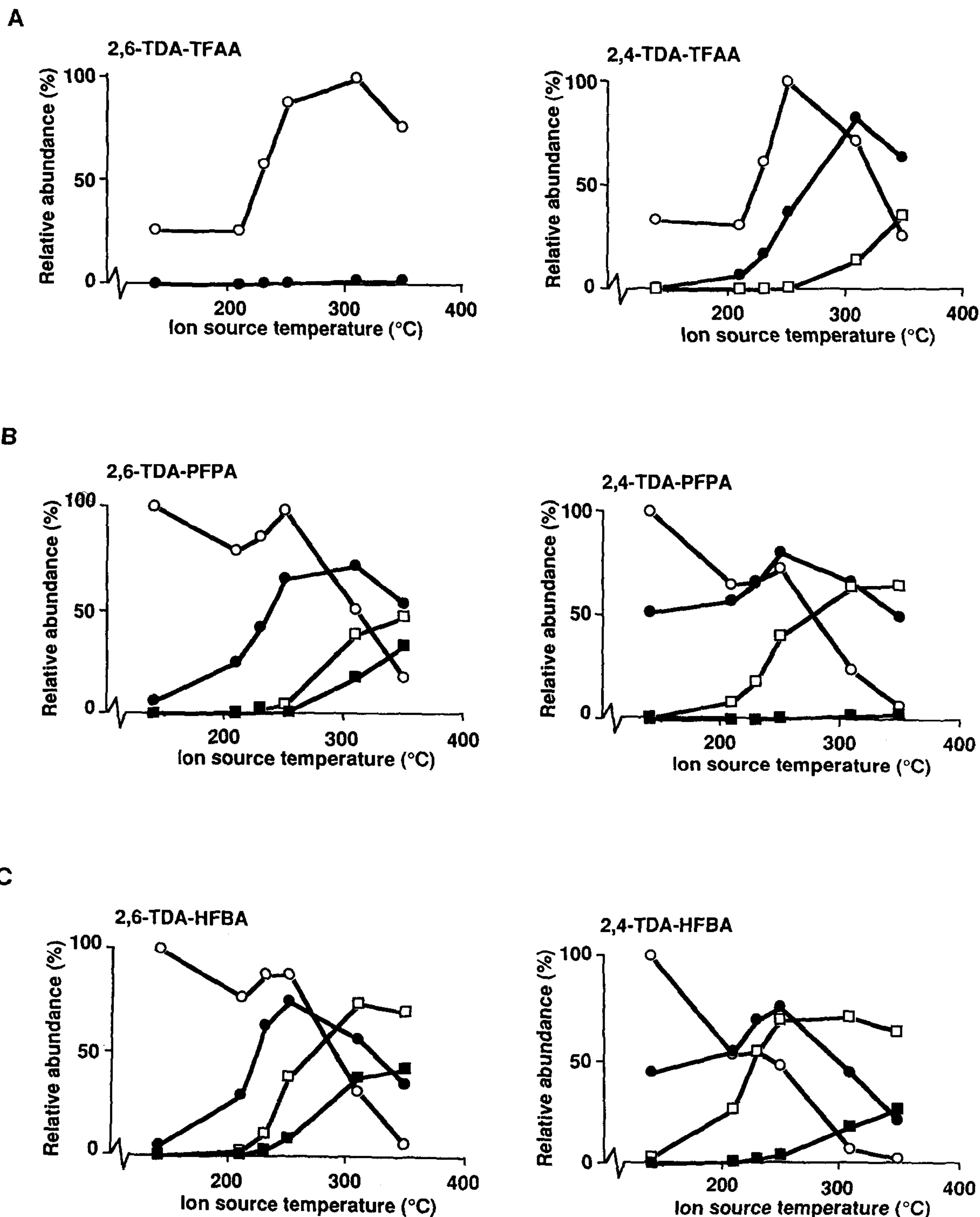

Fig. 4. Variation of the relative abundance of the $m/z = (M - n \times 20)^-$ ($n = 1$–4) fragments with the temperature of the ion source. Amounts of 10 ng of 2,6- and 2,4-TDA derivatized with (A) TFAA, (B) PFPA and (C) HFBA. Mass spectra were obtained by scanning ions between 50 and 600 u for 0.7 s with an inter-scan delay of 0.1 s. Chromatographic conditions as in Fig. 1. ○ = $m/z = (M - 20)^-$; ● = $m/z = (M - 40)^-$; □ = $m/z = (M - 60)^-$; ■ = $m/z = (M - 80)^-$.

internal standards trideuterated 2,6- and 2,4-TDA were monitored. Trideuterated 2,4-TDA was used as the internal standard for the determination of 2,5-TDA. The ratios of the peak area of TDA to that of the internal standards were calculated. For PFPA and HFBA derivatives the slopes of the calibration graphs were *ca.* 2.3 for 2,6-TDA and 2,4-TDA and were *ca.* 4.5 and *ca.* 10, respectively, for 2,5-TDA. TFAA derivatives of 2,6-, 2,4- and 2,5-TDA were studied in the concentration range 2–25 μg/l. Ten times higher concentrations of the internal standards were used. The slopes of the calibration graphs were *ca.* 0.23 for 2,6-TDA and 2,4-TDA and *ca.* 0.14 for 2,5-TDA. Duplicate work-up with duplicate injections were made for each concentration. The linear calibration graphs obtained passed almost through the origin and the correlation coefficients were in the range 0.998–0.999.

Precision. The overall precision for human urine spiked at a concentration of 1 μg/l was found to be 1.6% for 2,6-TDA, 3.5% for 2,4-TDA and 3.2% for 2,5-TDA determined as the PFPA derivatives ($n = 10$).

Detection limit. For the GC–MS system the detection limit, monitoring the $m/z = (M - 20)^-$ fragment, defined as twice the signal-to-noise ratio, is in the range 1–5 fg for the five TDA isomers derivatives with PFPA or HFBA. For the TFAA derivatives the detection limit is 40–200 times higher. The detection limits for biological samples are defined by the matrix. The detection limit defined as the blank plus three standard deviations of the blank was 0.05 μg/l for human urine and plasma. This could be lowered by a factor of 40 if an enrichment step was included.

When processing raw data from the chromatograms in the computer, with the use of a filtering technique, etc., considerably lower detection limits are achievable. In our experience the detection limit when analysing real biological samples can also be improved in the same way, but it is much more difficult to define the conditions.

Application

A male worker in a polyurethane foam factory was exposed to *ca.* 10 μg/m^3 of TDI. The peaks in the chromatogram in Fig. 5 corresponds to a concentration of 2,6-TDA of *ca.* 1 μg/l and of 2,4-TDA of *ca.* 0.7 μg/l in urine. The concentration of TDA in urine varies with time and exposure. The chromatogram in Fig. 6 is for a hydrolysed plasma sample from another TDI worker. The peaks corresponds to a concentration of *ca.* 5.7 μg/l of 2,6- and 0.6 μg/l of 2,4-TDA in plasma. The concentration of TDA in plasma was found to vary by less than 10% from the average and no variations were seen with time (50 h) and exposure. This study has been described in more detail elsewhere [15].

Analytical considerations

The uptake of TDI by oral, dermal and inhalation administration is not known in detail. The metabolism in humans is also unknown. For ethical reasons it is not possible to study the metabolism using ^{14}C-labelled TDI in humans. The high toxicity of TDI results in very low TLVs and therefore only low concentration are found in biological samples from workers. Only during the very infrequent accidents in the industry can high exposure be expected. It has also been suggested that different metabolism may occur at very high exposure compared with exposure under normal working conditions below the TLV levels. The determination of TDA in hydrolysed biological samples described here not only monitors the exposure to TDI but also possibly to TDA or other TDI/TDA related compounds that will release TDA during the hydrolysis. Owing to the carcinogenic properties of 2,4-TDA, this compound is very little used in most countries. The method is well suited for routine determinations of TDA in hydrolysed urine or plasma.

In the work-up procedure, aliphatic and aromatic primary and secondary amines will be derivatized. However, lower extraction recoveries of aliphatic amines from an aqueous phase into toluene have been found owing to the pK_a values of the amines. MDA (4,4′-methylenediphenyldiamine) and HDA (1,6-hexamethylenediamine) can also be determined by the same method by monitoring different mass fragments. Details concerning the determination of these compounds will be presented elsewhere. For the biological monitoring of organic com-

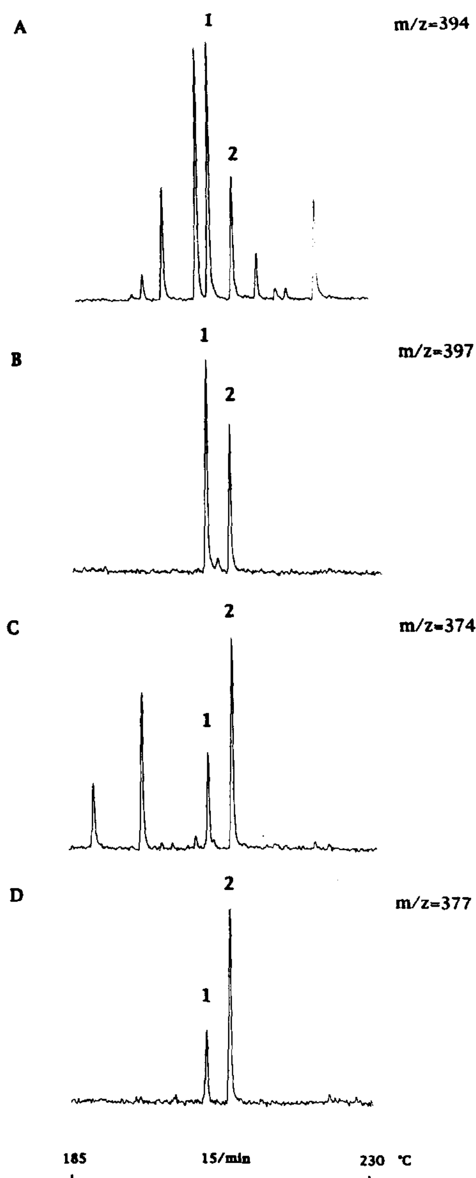

Fig. 5. Chromatograms of a hydrolysed urine sample from a worker exposed to an air concentration of *ca.* 10 $\mu g/m^3$ of 2,4- and 2,6-TDI. SIM using CI with ammonia and negative ion monitoring. (A) $m/z = (M-20)^-$ (394) ion of the TDA-PFPA derivative. (B) $m/z = (M-20)^-$ (397) ion of the trideuterated TDA-PFPA derivative. (C) $m/z = (M-40)^-$ (374) ion of the TDA-PFPA derivative. (D) $m/z = (M-40)^-$ (377) ion of the trideuterated TDA-PFPA derivative. The peaks correspond to a concentration of *ca.* 1 μg/l of 2,6- and *ca.* 0.7 μg/l of 2,4- in urine. Peaks in (A) and (C): 1 = PFPA derivative of 2,6-TDA; 2 = PFPA derivative of 2,4-TDA. Peaks in (B) and (D): 1 and 2 are the corresponding deuterium-labelled internal standards. Chromatographic conditions as in Fig. 1.

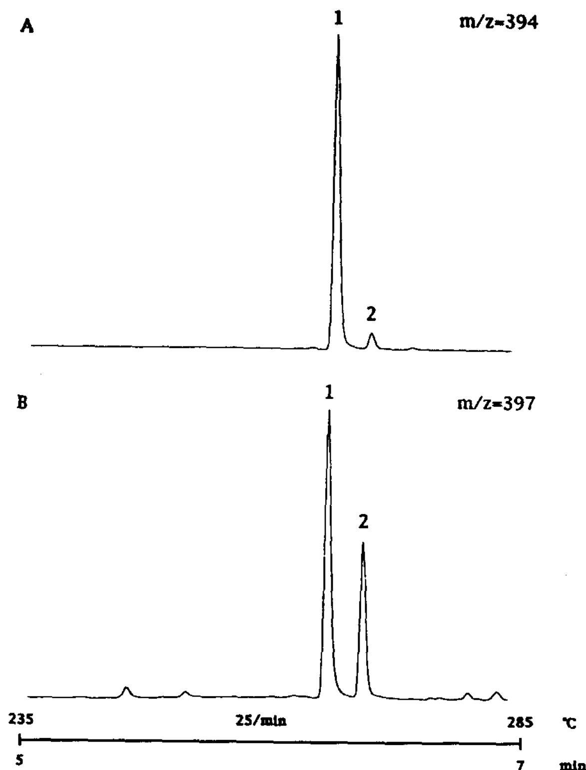

Fig. 6. Chromatograms of a hydrolysed plasma sample from a worker exposed to 2,4- and 2,6-TDI. (A) m/z 394 ion of the TDA-PFPA derivative. (B) $m/z = 397$ ion of the trideuterated TDA-PFPA derivative. The peaks correspond to a concentration of *ca.* 5.7 μg/l of 2,6- and *ca.* 0.57 μg/l of 2,4- in plasma. Peaks: 1 = 2,6-TDA; 2 = 2,4-TDA. Column: fused silica coated with DB-5 bonded stationary phase (25 m × 0.25 mm I.D.); film thickness, 0.25 μm; temperature programme, isothermal at 110°C (1 min), raised at 25°C/min to 280°C. Other conditions as in Fig. 1.

pounds a proper choice between selectivity and sensitivity must be made. It is possible to monitor exposure to TDI at levels several orders of magnitude below the Swedish TLV by the proposed method. The method cannot differentiate between exposure to TDI or the corresponding amine TDA or related derivatives of TDA. The nature of the TDA-releasing compounds, during hydrolysis, is not known. For the complete speciation of the nature of exposure, further research is necessary. The proposed method was developed for assessing occupational exposure to TDI. Selective and sensitive determinations of TDA isomers in hydrolysed human urine and plasma, at the low μg/l level, are possible using GC–SIM. The use of trideu-

terated TDA as internal standard has been demonstrated to give accurate and precise determinations.

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REFERENCES

1 A.L. Kennedy and W.E. Brown, *Isocyanates and Lung Disease: Experimental Approaches to Molecular Mechanisms*, Vol. 7, *Occupational Medicine: State of the Art Reviews*, No. 2, Hanley & Belfus, Philadelphia, 1992, p. 301.
2 World Health Organization, *Environmental Health Criteria 75, Toluene Diisocyanates*, WHO, Geneva, 1987.
3 D.E. Bradway and S. Talaat, *J. Chromatogr. Sci.*, 15 (1977) 322.
4 T.M. Trainor and P. Vouros, *Anal. Chem.*, 59 (1987) 601.
5 H.-B. Lee, *J. Chromatogr.*, 457 (1988) 267.
6 C. Sangö, *J. Liq. Chromatogr.*, 2 (1979) 763.
7 M. Dalene, L. Mathiasson, G. Skarping, C. Sangö and J.F. Sandström, *J. Chromatogr.*, 435 (1988) 469.
8 G. Skarping, C. Sangö and B.E.F. Smith, *J. Chromatogr.*, 208 (1981) 313.
9 G. Skarping, L. Renman and M. Dalene, *J. Chromatogr.*, 270 (1983) 207.
10 G. Skarping, L. Renman, C. Sangö, L. Mathiasson and M. Dalene, *J. Chromatogr.*, 346 (1985) 191.
11 J.F. Sandström, G. Skarping and M. Dalene, *J. Chromatogr.*, 479 (1989) 135.
12 T. Brorson, G. Skarping and C. Sangö, *Int. Arch. Environ. Health*, 63 (1991) 253.
13 G. Skarping, T. Brorson and C. Sangö, *Int. Arch. Environ. Health*, 63 (1991) 83.
14 H. Tokuda, Y. Kimura and S. Takano, *J. Chromatogr.*, 367 (1986) 345.
15 P. Persson, M. Dalene, G. Skarping, M. Adamsson and L. Hagmar, *Br. J. Ind. Med.*, 50 (1993) 1111–1118.