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DETERMINATION OF OCCUPATIONAL EXPOSURE TO TOLUENE DIISOCYANATE BY BIOLOGICAL MONITORING

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SUMMARY

Exposure of workers to toluene diisocyanate monomer in a factory producing flexible polyurethane was measured using impinger and reagent-impregnated filter sampling. These techniques yielded highly concordant results so that the facile filter techniques can be recommended. The personal exposures were compared with the individual excretion of amine metabolites from the parent toluene diisocyanates. The excretion was linearly related to the product of sampling time and concentration. The biological half-lives of the amines proved to be short so that urine should be sampled 2 h after the exposure. The percutaneous absorption of the monomers could also be controlled.

INTRODUCTION

Organic isocyanates contain an NCO group attached to an organic molecule. Toluene diisocyanate (TDI), one of the commonest industrial isocyanates, is used primarily in the manufacture of flexible polyurethane foam. It is commercially available as a mixture of, *e.g.*, 80% 2,4- and 20% 2,6-isomers. The main risk to the health of workers is from inhalation of isocyanates in the form of vapour or aerosol. The workers may also be percutaneously exposed through skin contact with freshly cured foam or as result of spills or splashes. The highly reactive monomer is known as a sensitizer and as an irritant substance. Sensitive individuals show asthma-like symptoms when exposed to very low concentrations[1].

Most analytical methods for isocyanates deal with the detection and determination of the material in air. These include spectrophotometry[2,3], gas chromatography[4–7] and high-performance liquid chromatography (HPLC)[8–11]. The common sampling procedure in the most methods involves bubbling of air through an impinger containing the absorber solution. More convenient procedures for personal sampling include the collection of isocyanates in air by using solid sorbent media[12–14].

Urinary excretion of conjugated aromatic diamines derived from monomeric isocyanates has been demonstrated in rats[15,16]. Further, rapid excretion of urinary aliphatic diamines derived from parent isocyanates was detected in man after expo-

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sure to the aerosol[17]. These studies indicate that urinalysis of exogenous diamines could be suitable as a test for occupational exposure to organic isocyanates.

This paper reports a procedure for the determination of TDI in workplace air based on sampling on reagent-impregnated glass-fibre filters. The method employs as the derivatizing reagent 1-(2-methoxyphenyl)piperazine, which forms a stable derivative suitable for HPLC and electrochemical detection. We have improved the method for the separation and quantitation of TDI isomers and used it for personal monitoring of isocyanate exposure in a polyurethane plant. The results were compared with those obtained using filters and liquid impingers with N-[(4-nitrophenyl)methyl]propanamine as the derivatization reagent and UV detection.

In conjunction with this industrial hygiene study, urine was collected and analysed for toluenediamine as its perfluoroacylated derivative by gas chromatography–mass spectrometry (GC–MS), in order to develop a biological exposure test for man. 2,6-Toluenediamine, derived from 2,6-toluene diisocyanate, was excreted in a dose-dependent fashion in urine samples collected 2 h after exposure.

EXPERIMENTAL

Instrumentation

The liquid chromatographs consisted of a Pye Unicam LC3-XP pump, an LC-XP gradient programmer system coupled to a Metrohm 641 VA electrochemical detector and a Metrohm 656 detector cell, and a Kontron Model 600 system equipped with a Uvicon 720 LC variable-wavelength detector.

The GC–MS system consisted of a Hewlett-Packard 5990A mass-selective detector combined with a capillary gas chromatograph and a HP 9825B data system.

Chemicals

1-(2-Methoxyphenyl)piperazine was obtained from Ega-Chemie (Steinheim, F.R.G.), N-[(4-nitrophenyl)methyl]propanamine from Regis Chemicals (Morton Grove, IL, U.S.A.) and heptafluorobutyric anhydride (HFBA) from Pierce (Rockford, IL, U.S.A.). A mixture of 2,4- and 2,6-toluene diisocyanate (TDI) (80:20) was purchased from Merck-Suchardt (Münich, F.R.G.) and 2,4- and 2,6-toluenediamine from Fluka (Buchs, Switzerland). All other reagents were of analytical-reagent or HPLC grade.

Plant and process description

The factory involved in the field study produced flexible polyurethane foam in one continuous foam line. The generated foam had a cross-section of 1 m $\times$ 1.8 m. The plant used the common 80:20 mixture of 2,4- and 2,6-TDI. The monomer materials, isocyanate, polyol, resin activator and blowing agents were pumped from tanks and mixed in a foaming nozzle. The material was poured on to moving kraft paper, which was forwarded into a ventilated curing tunnel. The foam had reached its final degree of polymerization and size at the end of the tunnel, where the paper lining was stripped off and the slab was cut into pieces which were moved to a storage room for cooling before the final processing.

Both general and personal samples were collected at the pouring, paper stripping and cutting work stations during active production.

Sampling and analysis of TDI

1-(2-Methoxyphenyl)piperazine derivative. TDI vapour was collected on reagent-impregnated glass-fibre filters for personal monitoring. A 15-mg amount of 1-(2-methoxyphenyl)piperazine was dissolved in 40 ml of toluene and the solution was transferred into a beaker with 30 glass-fibre filters. The filters, 13 mm in diameter and without binders, were purchased from Millipore Iberica (Madrid, Spain). The solvent was evaporated to dryness with nitrogen in about 30 min. The dry filters were placed in filter holders (Swinnex, Cat. No. SX 001300; Millipore, Bedford, MA, U.S.A.). The reagent on the filters was stable for 3 months when stored at $-20°C$. Samples were taken at a flow-rate of 1 l/min for 15–30 min.

Reference standards of TDI 1-(2-methoxyphenyl)piperazine derivatives were prepared by the method developed by Warwick *et al.*[11]. A 100-mg amount of the isocyanate was added to 10 ml of toluene containing 600 mg of 1-(2-methoxyphenyl)piperazine and the mixture was allowed to stand for 1 h. The precipitated derivative was washed with toluene and appropriate concentrations of the dried derivative were prepared in methanol.

The collection efficiency and recovery from the filters were tested by evaporation of TDI. TDI dissolved in dichloromethane was injected into a silanized glass-wool plug in a glass tube (11 $\times$ 5 mm I.D.), which was heated to 40°C. The tube was coupled directly to the filter holder without PTFE tubing, the vaporized TDI was collected on the glass-fibre filter and air (15 l) was drawn through the system. The filters were placed in 1 ml of acetonitrile containing 0.5% of acetic anhydride. This solution was used for the chromatographic determination.

The general samples were collected in impingers and the method was essentially that described by Warwick *et al.*[11]. A 40-mg amount of 1(2-methoxyphenyl)piperazine was dissolved in 100 ml of toluene and a ten-fold dilution in toluene of the stock solution was used as an absorber solution. Sampling was carried out at a flow-rate of 1 l/min. Aliquots of 1 ml of the absorber solution were evaporated to dryness with nitrogen and the residue was dissolved in 1 ml of acetonitrile containing 0.5% of acetic anhydride.

A 20-μl volume of the acetonitrile solution was injected into the liquid chromatograph. The TDI isomers were separated on a Spherisorb S5 ODS2 (Phase Separations) reversed-phase column (150 $\times$ 4.6 mm I.D.) with acetonitrile–water (48:52) as the mobile phase. The aqueous phase contained 8 g/l of sodium acetate and its pH was adjusted to 5 with acetic acid. The eluent was pumped isocratically at 1.5 ml/min. The electrochemical detector was operated at $+0.8$ V (Ag/AgCl) at ambient temperature. The 2,6- and 2,4-isomers eluted at 3.6 and 4.8 min, respectively. The minimum detectable amount was 0.1 ng per injection with a detector setting of 5 nA.

N-[(4-Nitrophenyl)methyl]propanamine derivative. The glass-fibre filters were impregnated as described previously[14]. A stock solution was prepared by hexane extraction (2 $\times$ 25 ml) of 300 mg of N-[(4-nitrophenyl)methyl]propanamine dissolved in 25 ml of distilled water and 15 ml of 0.1 *M* sodium hydroxide solution. A 20-ml volume of a ten-fold dilution in hexane and 20 glass-fibre filters were placed in a beaker and the solvent was evaporated from the filters with nitrogen. The sampling flow-rate was 1 ml/min. The TDI derivatives were eluted from the filters with 1 ml of the chromatographic eluent.

Impinger samples were collected in 10 ml of a $2 \cdot 10^{-4}$ *M* solution of N-[(4-

nitrophenyl)methyl]propanamine in toluene[8] at a flow-rate of 1 l/min. For the chromatographic determination, the absorber solution was evaporated to dryness in a vacuum evaporator and the residue was dissolved in 1 ml of the eluent.

Aliquots of 50 μl were injected on to a Hypersil ODS column (125 × 4.6 mm I.D.) and eluted with acetonitrile–water–triethylamine (35:64:1). The isomers were eluted isocratically at 1.5 ml/min and the detector was operated awt 254 nm. The elution times were 4.3 and 5.1 min for the 2,6- and 2,4-TDI derivatives, respectively. The minimum detectable amount was 5 ng with a detector setting of 0.005 a.u.f.s.

Sampling of 1-(2-methoxyphenyl)piperine and N-[(4-nitrophenyl)methyl]propanamine derivatives were carried out simultaneously throughout the study.

Sampling and analysis of urinary amines

Urine voided before work and during the work shift was sampled. A 1-ml volume of sulphuric acid–water (1 + 4) was added to the samples, which were immediately frozen and stored at −20°C until taken for analysis.

Aliquots (1 ml) were boiled with 50 μl of concentrated sulphuric acid to hydrolyse the acetyl conjugates of the amines[15]. The hydrolysate was cooled to room temperature and the liberated amines were extracted into toluene after the addition of of 0.5 g of sodium chloride and 2 ml of saturated sodium hydroxide solution. The toluene layer was taken and 20 μl of HFBA were added. The solution was shaken for 30 s and after 10 min the excess of acylating reagent was removed by extraction with 1 ml of 1 *M* dihydrogen phosphate buffer (pH 7). The toluene layer was retained and dried over sodium sulphate.

The perfluoroacylated toluenediamine isomers were analysed chromatographically on a fused-silica column (25 m × 0.2 mm I.D.) coated with 5% phenylmethylsilicone (cross-linked, Hewlett-Packard), which was connected directly to the mass spectrometer. Helium was used as the carrier gas with an inlet pressure of 110 kPa. Aliquots of 1 μl were injected with an on-column injector at 90°C. The column temperature programme was 1.5 min at 90°C with heating to 170°C at 5°C/min. The elution temperatures were 147 and 150°C for the derivatives of the 2,6- and 2,4-toluenediamine isomers, respectively.

The quadrupole mass selective detector was operated in the electron impact (70 eV) mode and mass spectra were recorded with full scanning (m/z 15–550) or multiple ion monitoring (m/z 345 + 514). Peak areas were integrated from the base peak m/z 345 for quantitation with m/z 514 as the peak identifier[15]. The detection limit was 2 pg for a 1-μl sample injected with a signal-to-noise-ratio of 5:1.

Aliquots of standard solutions of 2,6- and 2,4-toluenediamine in 0.05 *M* sulphuric acid were added to control urine samples. These standards (5–500 ng/ml) were analysed together with actual specimens and used for quantitation with the external standard method. The toluenediamine excretion was corrected for the excretion of creatinine determined by the alkaline picric acid method.

The urinary excretions of 2,6-toluenediamine were compared with those of the corresponding isocyanates in the ambient air using the least-squares method in a mathematical simulation.

RESULTS AND DISCUSSION

In order to investigate the collection efficiency of the 1-(2-methoxyphenyl)piperazine-impregnated glass-fibre filters. TDI was vaporized to yield an atmosphere ranging from 0.03 to 0.14 mg/m³ for 15-l samples. The upper level corresponds to common Europeàn exposure limits (0.14 mg/m³ for TDI in Finland)[18]. The average recoveries were virtually quantitative and no breakthrough was observed at the limit level. However, at levels approximately 1.5 times the limit breakthrough occurred. For higher concentrations, two filter holders should be connected in series during sampling. The sampling filters were stored awt −20°C and no breakdown was observed during a period of 3 weeks.

After having found a good collection efficiency for the 1-(2-methoxyphenyl)-piperazine-impregnated glass-fibre filters the procedure was compared in the field with 1-(2-methoxyphenyl)piperazine impinger and N-[(4-nitrophenyl)methyl]propanamine impinger and filter techniques.

The results for the different procedures correlated well (Tables I and II). The concentrations in general samples were on average 0.05 and 0.03 mg/m³ for the pouring and paper stripping work stations, respectively. The concentrations were considerably lower in the cutting area.

The 2,6-isomer constituted approximately 65% of the total result. This finding is in agreement with the hypothesis of an increased release of 2,6-TDI owing to its lower reactivity[19]. The same pattern was found in the personal samples, where the highest concentrations were found in the samples from the worker stripping off the paper lining at the end of the curing tunnel (Table II).

TABLE I

TDI VAPOUR CONCENTRATION IN GENERAL SAMPLES

Glass fibre filter *vs.* liquid impinger sampling procedure.

Work station	*Sample No.*	*TDI concentration (mg/m³)*		
		Filter procedure: 1-(2-Methoxyphenyl)piperazine	*Impinger procedure*	
			1-(2-Methoxyphenyl)-piperazine	*N-[(4-Nitrophenyl)-methyl]propanamine*
Pouring	1	0.055	0.043	0.031
	2	0.039	0.024	0.025
	3	0.032	0.033	0.029
	4	0.058	0.070	0.080
Paper stripping	1	0.023	0.022	0.021
	2	0.030	0.036	0.033
	3	0.058	0.021	0.038
	4	0.035	0.050	0.044
	5	0.023	0.011	0.014
Cutting	1	0.007	0.001	0.008
	2	0.001	0.001	0.001

TABLE II

TDI VAPOUR CONCENTRATIONS IN PERSONAL SAMPLES

Glass-fibre filter procedure, 1-(2-methoxyphenyl)piperazine *vs.* N-[(4-nitrophenyl)methyl]propanamine derivatives.

Task	*Case No.*	*Isomer*	*TDI concentration (mg/m³)*	
			1-(2-Methoxy-phenyl)-piperazine	*N-[(4-Nitro-phenyl)methyl]-propanamine*
Pouring	1	2,6-	0.021	0.015
		2,4-	0.012	0.009
		Total	0.033	0.024
	2	2,6-	0.027	0.027
		2,4-	0.013	0.016
		Total	0.040	0.043
	3	2,6-	0.015	0.015
		2,4-	0.036	0.034
		Total	0.051	0.049
Paper stripping	1	2,6-	0.022	0.025
		2,4-	0.020	0.020
		Total	0.042	0.045
	2	2,6-	0.047	0.050
		2,4-	0.031	0.030
		Total	0.078	0.080
	3	2,6-	0.019	0.012
		2,4-	0.009	0.005
		Total	0.028	0.017

For the urinalysis of toluenediamine, acylation with HFBA to the corresponding amide was chosen as the derivatization procedure. A stable derivative was formed that was suitable for trace analysis by capillary gas chromatography. Mass fragmentography of the base peak at m/z 345 (M − C_3F_7) made it possible to detect the diamines at the low picogram level. The molecular ion at m/z 514 of the acylated diamine appeared with relative abundances of 41 and 37% for the 2,6- and 2,4-isomers, respectively. Thus m/z 514 was used as a peak identifier to obtain further structural confirmation. The recovery in extraction and analysis was 96 ± 3% (± S.D., n = 10).

The low vapour pressure of the TDI monomers implies that the airborne material may be significant only near the warm, freshly polymerized resin and that the vapour settles rapidly, which was confirmed by the low concentrations in the general samples (Table I). However, workers handle the fresh resin frequently and, *e.g.*, the stripping of the side paper is initiated manually.

The excretion pattern of the toluenediamine isomers reflected the exposure conditions, *i.e.*, the share of the 2,6-isomer was 66 ± 10% (± S.D., n = 10). We chose the 2,6-isomer as the indicator compound as it clearly predominates in exposure

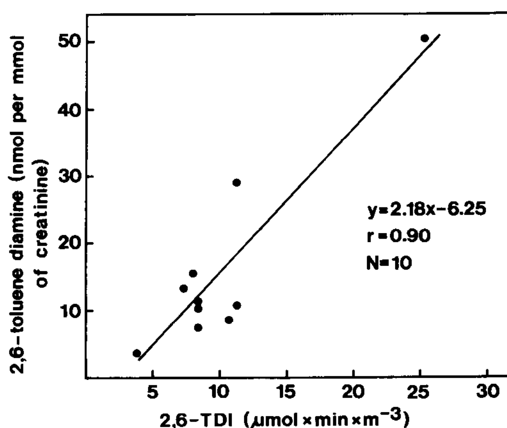

Fig. 1. Urinary excretion of 2,6-toluenediamine as a function of product of detected 2,6-toluene diisocyanate and sampling time. The exposure profile to 2,6-TDI contains several peaks so that averaging over 8 h abolishes the linear relationship. Urine must also be sampled 2 h after the exposure peak owing to the rapid biological half-life of the exogenous amines in man.

air. Partly because of the characteristics of TDI and partly because of the nature of the tasks, the exposure of workers is far from stable. The exposure pattern includes several peaks during the shift so that averaging over time may not be justified. To characterize the exposure accurately, we multiplied the sampling time by the detected concentrations. This product predicted the urinary excretion of the metabolites accurately (Fig. 1), so that urinary amines could be used as a biological index of the exposure. However, we have shown earlier that, *e.g.*, hexamethylene diisocyanate-derived amines in man are excreted within 4 h after exposure[17]. This necessitates the use of urine specimens obtained 2 h after exposure. The correlation was poor with later samples.

Another complicating factor is that unreacted monomers are occasionally spilled on bare hands. We have shown that TDI is absorbed through the skin[15]. This might explain very high urinary TDI-derived amines in the absence of significant atmospheric TDI. A clue to this would be a higher presence of 2,4-isomer, as it would point to contact with unreacted monomer mixtures as this monomer is more reactive than 2,6-TDI, which is more prevalent in the air samples.

In conclusion, glass-fibre filter samples are very good substitutes for impinger techniques. Personal exposure to TDI monomer in the field can also be monitored by the analysis of their amine metabolites. The hygienic and urinary analyses are sensitive enough to detect exposure during the normal operation of production lines.

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