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MASS FRAGMENTOGRAPHIC DETERMINATION OF URINARY AMINE METABOLITES IN RATS EXPOSED TO DEGRADATION PRODUCTS FROM HEATED RIGID POLYURETHANE

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SUMMARY

A mass fragmentographic method was developed for the urinalysis of amine metabolites in rats resulting from inhalation of thermally induced degradation products of rigid polyurethane synthesized from the aromatic diisocyanate 4,4'-bis(carbonylamino)diphenylmethane. The urinary excretion of acetylated 4,4'-bis(amino)-diphenylmethane was determined after acid hydrolysis, followed by alkaline extraction in toluene and derivatization with heptafluorobutyric acid anhydride. The excretion was linearly correlated to the inhaled parent isocyanate at three different degradation temperatures. Mass spectrometric detection of the amines also allowed comparison with the degraded polymer, which should be of considerable forensic significance. The sensitivity (5 pg/μl) also enables biological monitoring of occupational exposure in the production and engineering of polyurethane articles.

INTRODUCTION

Polyurethane polymers are formed through the reaction of organic diisocyanates and polyhydroxyl compounds. The polymers have found widespread use as foams, coatings, elastomers and fibres. Rigid polyurethane foam, based on 4,4'-bis-(carbonylamino)diphenylmethane, has a very small thermal conductivity and its major application has been in thermal insulation. The articles for which it is used include, for example, refrigerators and pipe-lines in urban heating systems. The expense of energy has also brought about greater demands for efficient insulation in houses. Its widespread and ever increasing use as a construction material has caused greater fire risks, as very toxic decomposition products may be formed at relatively low temperature ranges even before actual combustion[1]. The generation of heat, smoke and toxic gases during combustion has been studied for some years[2,3]. The toxicity of off-gases has been focused especially on acute effects due to carbon monoxide or hydrogen cyanide[4–6]. The decomposition of polyurethane has been proposed to proceed at a low temperature (300°C) through the loss of nitrogen-rich material, with little generation of free parent isocyanate[7]. At higher temperatures, more isocyanate

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monomer is released[8] and absorbed by the exposed subjects. Urinary isocyanate-derived amine has been detected in rats percutaneously exposed to toluene diisocyanate[9].

In our study, rats were exposed to decomposition products from rigid polyurethane degraded by heating in air at three different temperatures. The parent isocyanate 4,4′-bis(carbonylamino)diphenylmethane concentration in the atmosphere was monitored together with the hydrogen cyanide and carbon monoxide concentrations. The amine, 4,4′-bis(amino)diphenylmethane, derived from the parent isocyanate was excreted in the urine in a dose-dependent fashion. To detect the amine at the required low pg/μl levels, a gas chromatography–mass spectrometry method was developed involving derivatization with heptafluorobutyric acid anhydride.

EXPERIMENTAL

Materials

All reagents were of analytical-reagent or HPLC grade unless stated otherwise. 4,4′-Bis(amino)diphenylmethane was obtained from Fluka (Buchs, Switzerland) and 4,4′-bis(carbonylamino)diphenylmethane from Merck-Schuchardt (mixture of di- and triisomers, Münich, F.R.G.). N-[(4-Nitrophenyl)methyl]aminopropane was from Regis Chemicals (Morton Grove, IL, U.S.A.) and heptafluorobutyric acid anhydride (HFBA) from Pierce Chemicals (Rockford, IL, U.S.A.). Stock solutions for the amine were prepared in 0.1 *N* sulphuric acid at the mg/ml level and standards were diluted to the desired concentration before use. The pure isocyanate monomer was isolated by vacuum distillation before use as a standard.

Methods

Inhalation exposure. Twenty-four three-month-old male Wistar rats (250–300 g) divided into three groups were exposed to thermooxidative decomposition products using a dynamic degradation technique[10]. Strips (0.05 g/cm) of rigid polyurethane foam were placed in a quartz tube (150 cm × 1.7 cm). The tube was heated by a tubular ring oven (10 cm × 1.8 cm I.D.) which moved along the tube propelled by a programmable step-motor at 10 cm/h. The generated fumes were passed into an exposure chamber (32 × 50 × 70 cm) by flowing air at a velocity of 0.5 l/min through the tube and diluting the primary stream before the inlet with fresh air flow at 8 l/min. The oven temperatures were 350, 410 or 500°C. The three groups of eight rats at each temperature were exposed for 3 h. The rats were given free access to water during the exposure. Three additional groups of two rats were sham-exposed and served as controls.

Sampling. All voided urine was collected in metabolic cages for 3, 8 and 24 h after each exposure. Because of the small urinary volumes, two rats were put in one cage, which produced four urine specimens from the eight rats per degradation temperature point. Samples were stored frozen until analysis. Air samples for the determination of isocyanate concentration in the chamber were collected during the exposure in impingers filled with 10 ml of $2 \cdot 10^{-4}$ *M* N-[(4-nitrophenyl)methyl]aminopropane in toluene at a collection rate of 1 l/min[10]. Air samples for hydrogen cyanide determination were also collected in impingers filled with 10 ml of 0.1 *N* sodium hydroxide at a rate of 1 l/min[11]. The carbon monoxide concentration was determined with indicator tubes (CO 5/c, Dräger, Lübeck, F.R.G.).

Analysis of urine samples. The isocyanate-derived amines in the urine were determined after hot acid hydrolysis. The urine aliquots (0.3–1 ml) were hydrolysed at 100°C for 60 min by the addition of 50 μl of concentrated sulphuric acid[9]. The hydrolysate was cooled to room temperature and the liberated amine recovered into toluene after alkalinization and derivatization with heptafluorobutyric acid anhydride as described previously[10]. A 500-μl volume of the final extract containing the acylated amine was evaporated to dryness under a gentle nitrogen stream and the residue was dissolved in 50 μl of toluene. These samples were used in the mass fragmentography. Quantitation was done by the external standard method. Standards were prepared by adding variable amounts of the amine (0.01–0.2 μl/ml) in a urine matrix, analysed together with the actual specimens.

Analysis of air samples. The isocyanate concentration was determined as previously described[10]. The absorption solution containing the isocyanate was evaporated to dryness and the residue was dissolved in the eluent used for the liquid chromatographic determination. The hydrogen cyanide concentration was determined according to the NIOSH method[11] with an ion-selective electrode.

Instrumentation

A Stanton Redcroft TG-770 thermobalance was used for thermogravimetric analysis to study the degradation profile of the rigid polyurethane foam. The experiment was carried out under air-flow (7 ml/min). The maximum sample size was 10 mg and the heating rate 20°C/min.

A Hewlett-Packard 5990A combination gas chromatograph–mass spectrometer with a Hewlett-Packard 9825B data system was used for the amine determination. The quadrupole mass-selective detector was operated in the electron impact (70 eV) mode using full scan (*m/z* 15–600) or multiple-ion monitoring (*m/z* 132 + 302). The instrument was equipped with a 25 m × 0.2 mm I.D. Ultra-2 (Hewlett-Packard, cross-linked 5% phenylmethylsilicone) fused-silica column, which was connected directly to the mass spectrometer ion source. Helium was used as the carrier gas at 32 cm/s linear velocity. For each run, 1 μl of the final toluene extract was injected with an on-column injector at 90°C. The column temperature was programmed to 265°C at 15°C/min. The elution temperature was 257–258°C for the perfluoroacylated 4,4′-bis(amino)diphenylmethane under these conditions.

A Kontron Model 600 liquid chromatograph, equipped with a Uvicon 720 LC variable-wavelength detector was used for the isocyanate determination. A Hypersil ODS (5 μm, 125 × 4.6 mm I.D.) column was used for the isocratic reversed-phase chromatography (1.5 ml/min). The eluent was acetonitrile–water–triethylamine mixture at pH 3 with *o*-phosphoric acid (40:59:1, v/v/v).

RESULTS AND DISCUSSION

The thermal stability of polyurethanes varies and depends on the chemical composition of the principal subunits used in the formulation[12]. The rigid foam used is degraded by thermal energy in two distinct steps (Fig. 1). The first step began after 300°C and ended at *ca.* 400°C with a 50% weight loss. The remainder of the weight loss was more gradual and over 95% of the weight was lost at *ca.* 800°C.

The carbon monoxide concentrations were 1.2, 4 and 12 μmol/l at the degra-

TABLE I

DOSE-DEPENDENT URINARY 4,4'-BIS(AMINO)DIPHENYLMETHANE IN RATS EXPOSED TO RIGID POLYURETHANE-DERIVED THERMOOXIDATIVE DEGRADATION PRODUCTS

The amine figures are the means of four determinations ± S.D. No urine was obtained at the highest temperature immediately after the exposure; n.d. = not detectable (limit of accurate determination 5 nmol/l); n.a. = no sample available. Amines in urine (y) collected 3–8 h after the exposure were linearly correlated to the inhaled isocyanate (x): $y = 48.05x - 85.52$ ($r = 0.93$).

Temperature (°C)	*Hydrogen cyanide in air (μmol/l)*	*Isocyanate in air (nmol/l)*	*Time after the exposure (h)*					
			Concentration (nmol/l)			*Excretion rate (pmol/h ml)*		
			0–3	*3–8*	*8–24*	*0–3*	*3–8*	*8–24*
350	0.03	2	38 ± 14	13 ± 3	n.d.	13	2.6	–
410	0.1	3	113 ± 41	55 ± 16	13 ± 2	38	11	0.8
500	1.0	4.3	n.a.	123 ± 25	20 ± 4	–	25	1.3

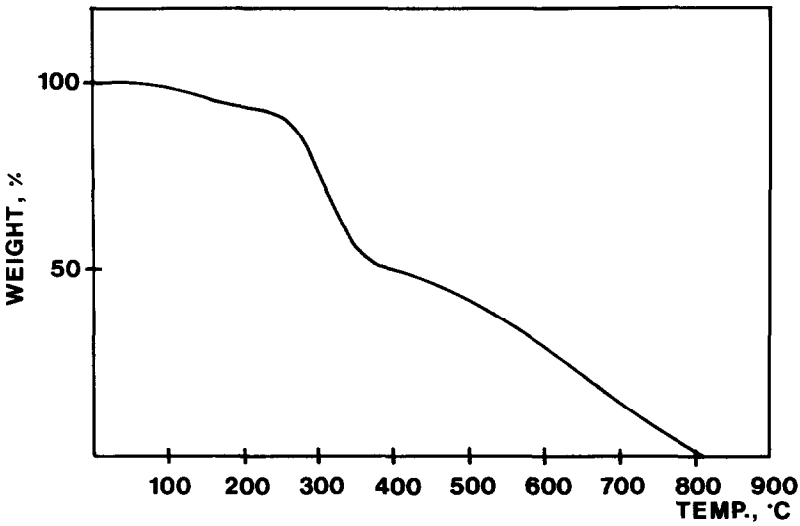

Fig. 1. Thermogram of 4,4'-bis(carbonylamino)diphenylmethane-based rigid polyurethane foam under air-flow. Note the biphasic degradation profile given as loss of weight. The rapid degradation begins at 250°C.

dation temperatures 350, 410 and 500°C, respectively. The isocyanate and cyanide concentration were similarly dependent on the degradation temperature (Table I).

The reason for derivatizing primary amines is to improve their chromatographic behaviour. Underivatized amines exhibit strong adsorption on glassware, with low reproducibility in the chromatographic runs. A frequently used derivatization procedure is the acylation with perfluorobutyric acid anhydride to form an amide[13].

Electron impact mass spectrometry was used to confirm the structure of the derivatized 4,4'-bis(amino)diphenylmethane, with HFBA as the acylating agent. The mass spectrum of the acylated compound and the total ion chromatogram are given in Fig. 2. The molecular ion of m/z 590 was obtained with a relative abundance of 25%. The base peak m/z 132 $(C_7H_5)^+NHCO$ is considered a tropylium ion produced by a rearrangement of the basic aromatic structure[14]. The fragment m/z 302 $(C_7H_5)^+NHCOC_3F_7$ produced by splitting off m/z 288 $(C_6H_4NHCOC_3F_7)^+$ from the molecular ion appeared at 34% relative abundance. The aforementioned ions are characteristic of the acylated amine, so mass chromatograms can be used to obtain structural information. Consequently, the base peak m/z 132 was monitored for quantitation together with m/z 302 as the peak identifier. Peak areas were integrated and the detection limit was 5 pg per 1-μl sample, injected with a signal-to-noise ratio of 5:1. Although electron capture detection would give comparable sensitivity, the structural information would be lacking. The nitrogen-specific detection would provide some selectivity but the sensitivity would be much less than in electron capture detection or mass fragmentography.

A calibration plot for the HFBA derivative of 4,4'-bis(amino)diphenylmethane demonstrated a linear response in the 10–200 pg range with a 1-μl sample injected ($y = 4.97 \cdot 10^{-5} x + 1.78 \cdot 10^{-3}$, $r = 0.999$). The sensitivity of the method was increased ten times by the evaporation of the final toluene extract. The recovery of

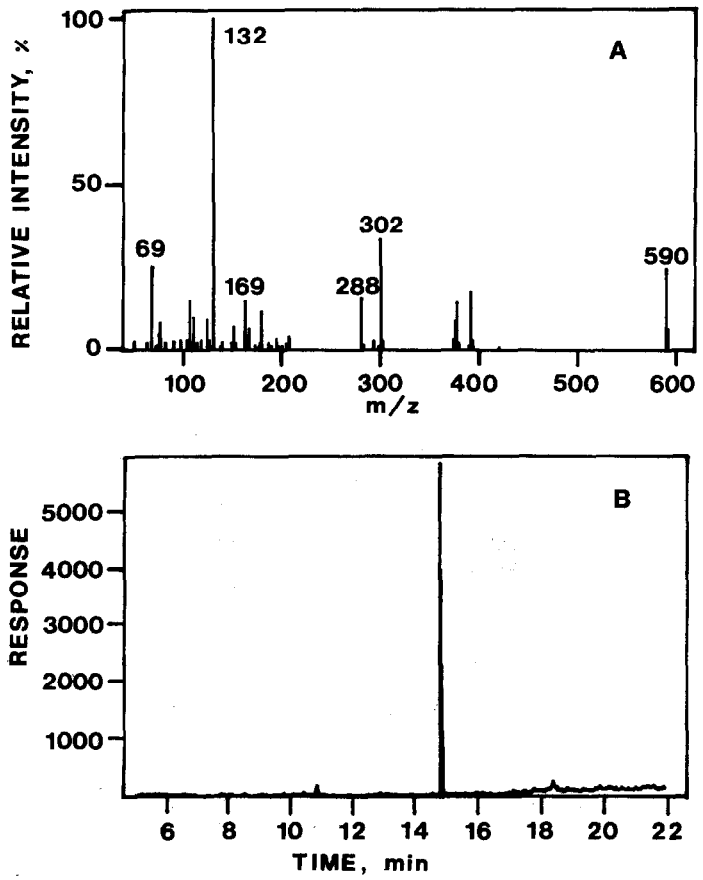

Fig. 2. Mass spectrum (A) and total ion chromatogram (B) of HFBA-acylated 4,4'-bis(amino)diphenylmethane (100 ng/μl) obtained by electron impact ionization and positive-ion monitoring [*m/z* 590 $(M)^+$, *m/z* 302 $(C_7H_5)^+NHCOC_3H_7$, *m/z* 288 $(C_6H_4NHCOC_3F_7)^+$, *m/z* 132 $(C_7H_5)^+NHCO$, *m/z* 169 $(C_3F_7)^+$, *m/z* 69 $(CF_3)^+$]. Column, Ultra-2 (cross-linked 5% phenylmethylsilicone) 25 m × 0.2 mm I.D.; column temperature programmed from 115°C (1.5 min) to 275°C at 15°C/min splitless injection.

added amine from urine was 87 ± 5% (± S.D., n = 10). The hydrolysis of the urine sample was included as primary aromatic amines are acetylated for their excretion in the urine[9]. No unconjugated amine was detected in the urine samples.

The condition of rats exposed to the degradation fumes at 500°C was very poor. They made no active movement and did not urinate for 3 h, although all eventually survived. This is in good agreement with the elevated carbon monoxide and very high hydrogen cyanide concentrations (Table I). As the monomer is released as a function of the degradation temperature, its inhaled amount also varies. The body burden of exogenous amines is reflected in the excretion of their metabolites in the urine. One of the determinants of the removal of the amines is the rate of their acetylation in the liver[15]. Although the excretion rate is also dose-dependent (Table I), the amine metabolites in the urine collected between 3 and 8 h after the exposure linearly reflect the inhaled concentration.

The urine test can thus be used as a method for personal monitoring of the exposure, which is often a desirable objective, *e.g.* in occupational health care. Another aspect is that the mass spectral identification of the urinary amines also reveals the original degraded polyurethane. The putative polymer, upon heating, should release the same monomeric isocyanate structure and its derivative amine found in the urine of the exposed subjects, an important element in the toxicological evaluation of fire victims.

CONCLUSION

The mass fragmentographic method described in this paper offers a sensitive and selective means of detecting exogenous primary diamines in urine. It is also well suited for other samples of minimal sizes because of the low detection limit.

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REFERENCES

1 R. A. Anderson, I. Thomson and W. A. Harland, *Fire Mater.*, 3 (1979) 91.
2 W. D. Woolley and P. J. Fardell, *Fire Res.*, 1 (1977) 11.
3 F. D. Hileman, K. J. Voorhees, L. H. Wojcik, M. M. Birky, P. W. Ryan and I. N. Einhorn, *J. Polym. Sci.*, 13 (1975) 571.
4 C. J. Hilado and P. A. Huttlinger, *J. Thermal Insul.*, 4 (1981) 276.
5 D. A. Purser and W. D. Woolley, *J. Fire Sci.*, 2 (1983) 110.
6 D. M. Russo, P. Sgro and H. J. Schneider, *Neurobehav. Toxicol. Teratol.*, 3 (1981) 265.
7 J. Chambers, J. Jiricny and C. B. Reese, *Fire Mater.*, 5 (1981) 133.
8 A. Zitting, C. Rosenberg, S. Vainiotalo and H. Savolainen, *Fire Mater.*, 6 (1982) 96.
9 C. Rosenberg and H. Savolainen, *J. Chromatogr.*, 323 (1985) 429.
10 C. Rosenberg, *Analyst (London)*, 109 (1984) 859.
11 U.S. Department of Health, Education, and Welfare, *NIOSH Manual of Analytical Methods*, Vol. 1, Washington, DC, 1977, Method 116.
12 B. Masiulanis, *J. Appl. Polym. Sci.*, 29 (1984) 681.
13 J. Drozd, *J. Chromatogr.*, 113 (1975) 303.
14 G. Skarping, L. Renman and B. E. F. Smith, *J. Chromatogr.*, 267 (1983) 315.
15 I. B. Glowinski and W. W. Weber, *J. Biol. Chem.*, 257 (1982) 1424.