Biomarkers of exposure, antibodies, and respiratory symptoms in workers heating polyurethane glue

G Skarping, M Dalene, B-G Svensson*, M Littorin, B Åkesson, H Welinder, S Skerfving

Abstract

Objectives—The pathogenic basis of respiratory disorders associated with isocyanates are still obscure. One reason for this is the lack of good estimates of human exposure. In this study exposure was estimated by measurement of isocyanate metabolites in biological samples. **Methods**—In a factory using isocyanate based polyurethane (PUR) glue, isocyanate concentrations in air were measured by liquid chromatography. Samples from 174 employees were analysed for metabolites of 4,4'-methylene diphenyl diisocyanate (MDI) in plasma (P-MDX) and urine (U-MDX). After hydrolysis, 4,4'-methylenedianiline was measured by gas chromatography-mass spectrometry (GC-MS). The employees were screened for work related respiratory symptoms and tested for specific immunoglobulin E (IgE) and IgG antibodies directed against isocyanate conjugated to human serum albumin.

Results—The time weighted isocyanate concentrations in air were low (MDI < 0.2–7; hexamethylene diisocyanate (HDI) < 0.1–0.7; 2,6-toluene diisocyanate (TDI) < 0.1 µg/m³). All subjects had detectable P-MDX and U-MDX. There were significant associations between the estimates of exposure to thermal degradation products of an MDI based glue and P-MDX (range ≤ 0.10–5.5 µg/l), and U-MDX (≤ 0.04–5.0 µg/g creatinine); in cases of heavy exposure. P-MDX and U-MDX were associated with each other ($r = 0.64$; $P = 0.0001$), work related symptoms (P-MDX: $P = 0.03$; Mann-Whitney U test), and serum concentrations of MDI specific IgG antibodies ($r = 0.26$; $P = 0.0007$). Unexpectedly, high P-MDX and U-MDX concentrations were also encountered in workers cutting textile (P-MDX 2.4–4.5 µg/l; U-MDX 0.81–3.8 µg/g creatinine); the reason is still unknown. Equally unexpected, there were significant negative associations between P-MDX and liver function tests.

Conclusions—The results clearly show the value of biomarkers for isocyanate exposure; in particular, P-MDX is useful. Further, these results show the risk connected with thermal degradation of PUR.

Keywords: biomarkers; polyurethane glue

Isocyanates are used in the production of polyurethane (PUR) polymers, which are widely used in industry. Their main applications are as flexible and rigid foams, lacquers, and glues. 4,4'-Methylene diphenyl diisocyanate (MDI), hexamethylene diisocyanates (HDI), and 2,4- and 2,6-toluene diisocyanate (TDI) are the most frequently used isocyanates. Isocyanate exposure is associated with occupational hazards.^{1–4}

The first step in the manufacturing of MDI is the reaction of aniline with formaldehyde. The main product is generally the 4,4'-methylene dianiline (MDA), but 2,2'- and 2,4'-MDA are also formed. These products then react further with formaldehyde and aniline at free *o* and *p* positions, whereupon larger molecules are formed. Depending on the process several other components are formed. Isocyanates are then formed by the reaction of the amine group with phosgene. Thus, the technical product MDI is a mixture of a variety of isocyanates with different chemical structure.

The HDI and TDI have rather high vapour pressures, and exposure to the vaporous compounds may easily occur in an occupational setting. Conversely, the vapour pressure of MDI is low. However, an aerosol of MDI can result in exposure to MDI. This is the case during spraying operations. Moreover, high exposure to isocyanates and amines occurs when PUR is thermally decomposed.^{5–8} Then more complex and new isocyanate compounds are formed. For example, at thermal decomposition of TDI based PUR, the 2-toluene-isocyanate-6-amine and 2,6-toluenediamine (TDA) have been detected.^{5,6} Exposure to isocyanates is often intermittent, with peaks, which occur both during production of PURs, and, in particular, at thermal decomposition. The complicated exposure pattern is one factor behind the lack of understanding of the pathomechanism behind isocyanate associated disease. Biological monitoring of exposure may add information.

Methods have been presented for the biological monitoring of exposure to HDI⁹ and TDI^{10,11} by measurement of the corresponding amines in plasma or urine. Also, we have monitored the exposure in a worker performing thermal decomposition of an MDI based PUR, by measurement of MDA in hydrolysed

Department of Occupational and Environmental Medicine, University Hospital, S-221 85 Lund, Sweden
G Skarping
M Dalene
B-G Svensson
M Littorin
B Åkesson
H Welinder
S Skerfving

Correspondence to:
Dr Gunnar Skarping,
Department of Occupational
and Environmental
Medicine, University
Hospital, S-221 85 Lund,
Sweden.

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on 28 May 1995.

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urine.⁴ Recently, we encountered a worker with clear cut work related asthma, who was employed in a factory where workers handled isocyanate based glues. We decided to study the exposure, mainly to MDI and MDA, by the use of biological markers, in the workers of the factory, and to relate that to immunological sensitisation and symptoms. As MDA is hepatotoxic,¹² we also screened the liver function.

Materials and methods

FACTORY AND EXPOSURE

The company was a subcontractor to the Swedish car industry manufacturing parts for the passenger room inside the car. The parts were made of textile, PUR, and special high performance fibre board. The work essentially took place in two large workshops with open doors in between.

Most of the workers were divided into small groups, three to six workers in each, manufacturing one single part or a few parts only. Inside the group the workers circulated between the different work operations. One of these work operations was spraying blue coloured glue of either MDI or HDI polymeric type on to the fibre board and the textiles.

During spraying the workers wore personal respiratory protection devices and rubber gloves. After the glue solvents had evaporated, the parts were heated to increase the reactivity of the glue, and then put together. The heating procedure was mainly performed by placing the parts under infrared heaters for 10–20 seconds and partly by air from heat guns. The temperature of the air ejected from these heat guns could be as high as 500°C.

Other workers were permanently occupied with work tasks not involving MDI glue, for example cutting, sewing, electromagnetic welding, transport, repair work, assembling, and office work.

The technical grade MDI contains besides the 4,4'-MDI also 2,4-MDI, 2,2'-MDI, and polymeric isocyanates of various structures.¹³ Traces of phenyl isocyanate were also present. A small amount of an glue based on a PUR (HDI based) and an acrylicpolymer water dispersion was also used at the factory. The textile used was mainly coated with a layer of a TDI based flexible foam.

During this study the work usually took place in one shift. A few of the workers worked in two shifts.

SUBJECTS

The entire work force was 228 subjects. No former employees were included. In total, 174 employees were studied (mean age 42, range 19–63). Of these 143 were women (mean age 42, range 21–63), and 31 were men (mean age 41, range 19–59). Another nine subjects (five men) refused to participate. Out of the total work force 18 people were on long leave to take care of children at the time of the study, and 27 (13%) people were on sick leave; those were not examined. Eighteen people (10

women) of those investigated did administrative work in an office, separated from the production rooms. They visited these rooms daily.

Mean duration of employment of the examined subjects was 9.4 (range 1–42) years, and 35% of the subjects were smokers. Atopy, defined as a history of hay fever, asthma, atopic eczema, or urticaria, was present in 29 subjects (17%).

Twenty non-exposed subjects from another factory were used as controls for the antibody measurements.

HEALTH EXAMINATIONS

For each person a medical and an occupational history was obtained through a self administered questionnaire (modified from Nielsen *et al.*)¹⁴ Questions were asked about allergic diseases (hay fever, asthma, atopic eczema, or urticaria) during childhood or adolescence, and smoking habits. Symptoms, both general, like fever and shivering, and local, from the eyes, nose, pharynx, or lower respiratory tract, were recorded. The completed questionnaires were scrutinised by a physician (BGS), who did a complementary interview. Asthma was defined as attacks of wheezing, dyspnoea, and cough. Work related symptoms were defined as those associated with work—that is, recovery during weekends or holidays. The physician was not aware of the exposure status of the employees.

Each subject gave a sample of venous blood; serum was analysed for γ -glutamyl transpeptidase, and aspartate and alanine aminotransferases, by routine clinical methods. The rest of the blood sample was used for the immunological investigations, and for analysis of MDX in blood plasma. Urine was collected from each subject at the end of a work shift.

Skin prick tests with conjugates between human serum albumin (HSA) and MDI, HDI, and TDI were performed on each subject.

MEASUREMENTS OF ISOCYANATES IN AIR

In workers handling isocyanate glue the exposure to monomeric isocyanates was measured in their personal breathing zone, during periods of 30–60 minutes by use of personal sampling pumps (1 l/min). The exposure was measured in nine work groups, six used MDI glue and three HDI glue. The isocyanate concentration in air was also measured in four different work areas in the two workshops.

Air samples were taken with SKC Model HFS 113 pumps (SKC, Eighty Four, USA) and 30 ml midjet impingers containing 10 ml toluene with 1×10^{-4} 9-(*N*-methyl amino-methyl)-anthracene hydrochloride (MAMA) amine reagent.¹⁵ The analyses were performed by liquid chromatography (LC) with a UV detector ($\lambda = 254$ nm). The LC column was a 150×3.2 mm Millipore Waters Bond Elut. The limits for measurement of a 60 minute sampling period were for MDI, HDI, and TDI 0.2, 0.1, and 0.1 $\mu\text{g}/\text{m}^3$, respectively. A Millipore Waters (Millipore-Waters, Milford, MA, USA) 600 multisolvent delivery system

with a Waters 712 WISP with variable injection volume and a Waters 490 programmable multiwavelength detector. The mobile phase (1 ml/min) consisted of 80%/20% acetonitrile/water with 1% triethylamine and pH adjusted to 3 with phosphoric acid.

MEASUREMENT OF MDI METABOLITES IN PLASMA AND URINE

Apparatus

A VG Trio-1000 single quadrupole mass spectrometer (Fission Instruments, VG-Mass-Lab, Manchester, Cheshire, England) connected to a Carlo-Erba GC-8000 gas chromatograph equipped with an A200S autosampler (Fisons Instruments, Milan, Italy) was used. Chemical ionisation by ammonia with selected ion monitoring was used. Columns: fused silica columns with chemically bounded stationary phases, DB-5 (J and W Scientific, Folsom, CA, USA) 25 m × 0.25 mm internal diameter with a film thickness of 0.25 µm were used.

Chemicals

4,4-MDA (MDA) was from Fluka (Buchs, Switzerland), HCl, NaOH and K₂HPO₄, from E Merck (Darmstadt, FRG); pentafluoropropionic anhydride (PFPA), from Pierce (Rockford, IL, USA); acetonitrile and toluene, from Lab-Scan (Dublin, Ireland); and dideuterated 4,4'-MDA (CD₂[C₆H₄NH₂]₂, MDDA) and MAMA from Synthelec (Lund, Sweden).

Procedure

Preparation of standard solutions: standard solutions of MDA were prepared by dissolving accurately weighted amounts in 0.1 M hydrochloric acid. The solutions were further diluted to the appropriate concentrations.

Sampling, handling, and storing of biological samples: the urine samples were collected in polyethylene bottles. Density, creatinine (Department of Clinical Chemistry, University Hospital, Lund by use of KODAK EKTACHEM clinical chemistry slides (CREA) and a Kodak Ektachem 700 XR-C analyser), and pH (Neutralit, Merck; Darmstadt, Germany) were measured in each urine sample. The detection limit was about 0.04 µg/l. The precision was about 6%. Venous blood (20 ml) was taken on each occasion. The urine and blood samples were stored in a freezer, at -18°C, until analysis. All analyses were done after acidic hydrolysis of the urine and plasma samples. The amounts given as MDA in figures therefore reflects the sum of free and hydrolysable compounds.

Samples (n = 174) for measurement of urinary MDA (U-MDA) and plasma MDA (P-MDA) were obtained during a period of three weeks just before the summer vacation. The urine samples were taken immediately after the work shift and the plasma samples were taken during the day.

Work up procedure for biological samples: to a 1 ml urine or plasma sample 100 µl of a 1 M HCl solution containing the internal standard MDDA (6 ng/ml) and 1.5 ml of 3 M

H₂SO₄ were added. The sample was then hydrolysed at 100°C for 16 hours. A 5 ml volume of saturated NaOH and 2 ml of toluene were then added. The mixture was shaken for about 10 minutes and then centrifuged at 1500 g for five minutes. A 1.5 ml volume of the organic layer was transferred to a new test tube and 20 µl of PFPA was added. The mixture was immediately shaken vigorously for about 10 minutes. The excess 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 to a 1.5 ml autosampler vial with a Teflon seal and was then ready to be injected into the gas chromatography-mass spectrometry (GC-MS) system.

Measurements: GC-MS monitored the negative ions m/z = 470 and 472, (m/z = M-20, M = molecular weight) fragments of the MDA-PFPA and the [²H₂]MDA-PFPA derivative. Urine and plasma from the workers exposed to MDI were analysed. The samples were analysed on the GC-MS in batches of about 100 samples. Each batch of samples included two sets of calibration samples and each set of calibration samples included five concentrations of MDA in urine in the range of 0–4 µg/l. The average correlation coefficient for eight GC-MS batches was 0.98 with the relative SD of 2%. Area measurements were made, and for each plasma and urine sample two measurements, with duplicate injections, were made. The limit of detection was 0.05 µg/l (0.3 nmol/l) in plasma, about 0.1 µg/g creatinine (0.06 µmol/mol creatinine) in urine.

IMMUNOLOGICAL EXAMINATIONS

The antibodies were measured as previously described.^{16 17}

Antigen preparations

Conjugations of HDI and MDI HSA: HDI and MDI (32 and 21 mol/mol HSA, respectively) were added to a solution of HSA in 0.05 M boric acid (0.05 M KCl, pH 9.4). The conjugate preparations were purified from low molecular weight compounds (< 30 000 daltons) by filtration in an Amicon ultrafiltration cell (8200; Amicon, Danvers, MASS, USA). The proteins were lyophilised and reconstituted in 0.1 M NaHCO₃ when used.

Skin prick tests were performed with the hapten HSA conjugates already mentioned at an antigen concentration of 3.0 mg/ml in 0.1 M NaHCO₃. Reaction sizes were recorded in relation to histamine (1 mg/ml) response.¹⁸ Positive skin prick reaction was scored, when the size of the weal was at least half that induced by histamine.

Radioallergosorbent test (RAST) of specific IgE antibodies

The hapten HSA conjugates were bound to cyanogen bromide activated filter paper discs for use in the RAST. We used the Phadebas RAST system (Pharmacia Diagnostics AB, Uppsala, Sweden), according to the standard procedure. Results were expressed as percent-

Table 1 Isocyanate concentrations in workers' breathing zones and in different work areas in a factory handling polyurethane glues

Work task	30–60 Minutes work groups n	Sampling periods n	Isocyanate concentrations ($\mu\text{g}/\text{m}^3$)	
			MDI median (range)	HDI median (range)
Glueing:*				
MDI glue	6	17	0.8† (< 0.2–6)	< 0.1 (< 0.1–0.5‡)
HDI glue	3	12	< 0.2 (< 0.2–3§)	0.2¶ (< 0.1–0.7)
No glueing	4	11	< 0.2 (< 0.2–7.1)	< 0.1 (< 0.1–0.4§)

*Active glueing for more than half of the shift.

†Significantly different from the other work tasks ($P = 0.01$).‡One period $\geq 0.1 \mu\text{g}/\text{m}^3$.§Three periods $\geq 0.2 \mu\text{g}/\text{m}^3$.¶Significantly different from the other work tasks ($P = 0.0001$).||Two periods $\geq 0.2 \mu\text{g}/\text{m}^3$.TDI was always $< 0.1 \mu\text{g}/\text{m}^3$.**Table 2** Concentrations of metabolites of P-MDX and U-MDX in workers exposed and clerks in a factory handling polyurethane glues

Work task	n	P-MDX ($\mu\text{g}/\text{l}$) median (range)	U-MDX ($\mu\text{g}/\text{g creatinine}$) median (range)
Workers:			
Cutting	5	3.7† (2.4–4.5)	1.1† (0.81–3.8)
Glueing:*			
With heating:			
MDI glue	64	1.8‡ (≤ 0.10 –5.5)	0.37‡ (≤ 0.04 –5.0) (n = 63)
HDI glue	4	0.35 (≤ 0.10 –1.4)	0.12 (≤ 0.06 –0.19)
Without heating	6	0.77 (≤ 0.10 –1.1)	0.14 (≤ 0.05 –0.33) (n = 5)
Other workers:			
Repairmen	6	1.0 (0.43–2.0)	0.13 (≤ 0.05 –1.7)
Other	68	0.76 (≤ 0.10 –5.2)	≤ 0.09 (≤ 0.04 –2.7)
Office	21	≤ 0.10 § (≤ 0.10 –0.46)	≤ 0.08 § (≤ 0.04 –0.63)

*Active glueing for more than half of the work time.

†Significant differences from workers handling heated MDI glue (n = 64; P-MDX: $P = 0.03$; U-MDX: $P = 0.02$).‡Significant difference from workers, who did not cut (n = 84; P-MDX and U-MDX: $P = 0.0001$).§Significant difference from workers, who did not cut or handle heated MDI glue (n = 84; P-MDX: $P = 0.0001$). No significant difference from workers who did not cut, or handle heated MDI glue (n = 84; U-MDX: $P = 0.12$).

age of specific binding (cpm of test disc—cpm of HSA disc) of total added radioactivity. Levels of specific binding $> 0.3\%$ were considered positive (highest control 0.1%).

Enzyme linked immunosorbent assay (ELISA) of specific IgG antibodies

Wells of polystyrene microtitre plates were coated by adding $100 \mu\text{l}$ of the antigen solution ($150 \mu\text{g}/\text{ml}$; 0.005 mol/l PBS, pH 7.2). The wells were washed and stored with blocking solution until use. Analytical procedure:

Figure 1 Concentrations of metabolites of U-MDX and P-MDX in workers and clerks in a factory handling polyurethane glue (n = 174; $r = 0.64$; $P = 0.0001$). The axes are logarithmic.

(a) addition of a 1:50 dilution (phosphate buffered saline (PBS) merthiolate) of serum, (b) incubation, (c) addition of $100 \mu\text{l}$ of an optimal dilution of alkaline phosphatase conjugated rabbit antihuman IgG, (d) incubation at 20°C , (e) addition of a substrate solution (disodium *p*-nitrophenol phosphate), and (f) incubation in darkness. Each serum sample was read (Titertek-Multiscan, Flow Laboratories, Eflab OY, Helsinki, Finland) at 405 nm (ELISA value). All samples were read against HSA antigen as a control of non-specific binding to the hapten HSA conjugate. Values higher than the highest values in the controls were regarded as positive.

STATISTICAL METHODS

Non-parametric statistics (Mann-Whitney *U* test and Spearman's rank correlation coefficients *r*) were used for comparisons between groups, and for tests of correlations, respectively. Values were considered to be significant at $P \leq 0.05$. All tests were two tailed.

Results

The air concentrations of both MDI and HDI were low in all samples (table 1). As expected, tasks with MDI glue gave measurable low concentrations of MDI in air, and with HDI glue gave low measurable concentrations of HDI in air. Air concentrations of TDI were never detected—that is, they were well below $0.1 \mu\text{g}/\text{m}^3$.

All subjects had detectable MDX concentrations in plasma and urine (table 2). Surprisingly, the highest median P-MDX was found in workers cutting fabric. Among the rest of the workers, those who heated MDI glue had higher concentrations than the others, who had, in turn, higher concentrations than the office clerks. Further, there was a decrease of P-MDX with rising age ($r = -0.14$; $P = 0.05$). Moreover, the women had higher concentrations than the men (medians 1.1 v $0.4 \mu\text{g}/\text{l}$; $P = 0.0009$). There was no significant effect of smoking.

Again the cutters had the highest U-MDX values (table 2). Among workers who performed glueing, those who did not heat the glue had lower U-MDX values than those who heated the glue, among whom those using MDI glue had higher values than those handling HDI. Among subjects who did not glue, the repairmen had values similar to the non-heating MDI glueers and HDI glueers, and the other workers and office clerks had lower values. Further, there was a decay of U-MDX with increasing age ($r = -0.18$; $P = 0.01$). Moreover, the women had higher values than the men (medians 21 v $7 \mu\text{g}/\text{g creatinine}$; $P = 0.0001$). On the other hand, there was no significant difference between smokers and non-smokers.

There was a weak, but significant association between U-MDX and P-MDX, although the U-MDX showed considerable variation at certain values of P-MDX, (fig 1).

The prevalence of work related symptoms was similar between workers with and without

Table 3 Work tasks, work related symptoms, and specific serum antibodies against MDI or HDI in 174 workers and clerks in a factory handling polyurethane glue

Work task	n	Workers with symptoms (n)	Symptoms from		Specific antibodies (n)			
			Eyes or nose or both	Lower airways	IgE-MDI	IgG-MDI	IgE-HDI	IgG-HDI
Workers:								
Cutting	5	1	1	1	—	2	—	—
Glueing:								
With heating:								
MDI glue	64	19	15	10	3	20	—	10
HDI glue	4	2	1	1	—	1	1	1
Without heating	6	2	1	2	—	2	—	—
Other workers:								
Repairmen	6	1	1	—	—	—	—	—
Other	68	14	12	6	—	7	—	1
Office	21	—	—	—	—	3	—	3
Total	174	39	31	20	3	35	1	15

IgE-MDI, IgE-HDI, IgG-MDI, and IgG-HDI = specific antibodies against MDI or HDI.

Table 4 Symptoms, specific serum antibodies against MDA and metabolites of MDI in plasma (P-MDX) and urine (U-MDX) in workers and clerks (n = 174) in a factory handling polyurethane glue

Symptoms and antibodies	n	P-MDX (µg/l) median (range)	U-MDX (µg/g creatinine) median (range)
Symptoms:			
Absent	113	0.82* (≤0.10–5.5)	0.13 (≤0.04–3.8)
Present:			
Not work related	22	0.74 (≤0.10–5.2)	0.16 (≤0.06–2.1)
Work related	39	1.35* (≤0.10–5.2)	0.19 (≤0.04–5.0)
Antibodies:			
IgE-MDI:			
Negative	171	0.9 (≤0.10–5.5)	0.14 (≤0.04–5.0)
Positive	3	1.9 (0.10, 1.9, 1.9)	0.29 (0.28, 0.29, 0.32)
IgG-MDI			
Negative	136	0.9† (≤0.10–4.9)	0.13‡ (≤0.04–5.0)
Positive	35	1.9† (≤0.10–5.5)	0.28‡ (≤0.06–3.1)

*P = 0.03; †P = 0.005; ‡P = 0.02.

IgE-MDI and IgG-MDI = specific IgE and IgG antibodies, respectively, against MDI.

Table 5 Liver tests and metabolites of MDI in plasma (P-MDX), in workers and clerks (n = 168) in a factory handling polyurethane glue

Liver tests				
P-MDX (µg/l)	n	S-GT (µkat/l)* median (range)	S-ASAT (µkat/l)† median (range)	S-ALAT (µkat/l)‡ median (range)
< 0.26	42	0.20 (0.10–0.90)	0.39 (0.22–0.75)	0.32 (0.04–0.93)
0.26–0.91	43	0.20 (0.0–0.70)	0.36 (0.25–0.69)	0.28 (0.11–1.0)
0.92–1.97	40	0.20 (0.0–0.40)	0.36 (0.25–0.69)	0.20 (0.11–0.87)
> 1.97	43	0.10 (0.10–2.2)	0.32 (0.20–0.68)	0.21 (0.09–1.2)

*r = -0.20, P = 0.008; †r = -0.27, P = 0.0006; ‡r = -0.28; P = 0.0002.

S-GT = γ-glutamyl transpeptidase activity in serum; S-ASAT = aspartate aminotransferase; S-ALAT = alanine aminotransferase. r = Spearman's rank correlation coefficient. Reference limits: S-GT < 0.65 µkat/l; S-ASAT < 0.7 µkat/l; S-ALAT < 0.7 µkat/l.

Figure 2 Concentrations of specific serum IgG antibodies to MDI (S-IgG-MDI) and MDI metabolites in plasma (P-MDX), in workers exposed to MDI and clerks in a factory handling polyurethane glue (n = 174; r = 0.25, P = 0.0008). The axes are logarithmic.

glueing in their work tasks (table 3). Thirty nine subjects experienced work related symptoms of the eyes, nose, lower airways, or a combination of these, at least once a week. Symptoms of the nose (blockage and secretion) dominated, and were present in 28 subjects. Only two subjects had work related asthma. Specific IgE antibodies against MDI were found in three subjects. Only one of these was symptomatic. One of the symptom free workers had IgE antibodies against HDI.

Specific IgG antibodies against MDI or HDI were more prevalent (table 3). However, only a few (11 out of 35) of the subjects with IgG antibodies to MDI had symptoms. Workers exposed to heated MDI glue had a higher prevalence of specific IgG antibodies against MDI than the others. Twelve of the workers with IgG antibodies to HDI also had IgG antibodies to MDI. One of the subjects with IgE antibodies to MDI also had IgG antibodies to MDI. Positive skin prick reaction against MDI conjugated to HSA was recorded for six subjects; three of those also reacted against HDI conjugated to HSA. None of the workers with a positive skin reaction was positive to RAST for specific IgE against HDI or MDI conjugated to HSA. The IgG antibody to MDI increased with rising age (r = 0.26; P = 0.0007). However, there were no associations with sex or smoking.

The workers with work related symptoms had higher concentrations of P-MDX than those without, or those with symptoms without clear association with work (table 4). There were no significant associations between work related symptoms and either sex, age, or smoking. Thus, there was no confounding by these factors.

Workers with IgG antibodies to MDI had significantly higher concentrations of P-MDX and U-MDX than workers without (table 4). Also, there were significant associations between IgG antibodies to MDI and P-MDX (fig 2) and U-MDX (r = 0.21; P = 0.006). The three subjects with IgE antibodies to MDI had higher P-MDX and U-MDX than the others, but the group was too small to be sure of a significant association.

As the P-MDX and U-MDX concentrations were associated with both IgG antibodies to MDI and age, the possibility of confounding was considered by multiple regression

Figure 3 Liver test (S-ALAT = alanine aminotransferase activity in serum) and concentrations of metabolites of MDI in plasma P-MDX, in workers and clerks in a factory handling polyurethane glue ($n = 168$; $r = -0.28$; $P = 0.0002$).

analysis, after logarithmic transformations of the MDX and IgG variables. Then, there were still significant associations between IgG and both P-MDX ($P = 0.0002$) and U-MDX ($P = 0.002$). Hence, there were no indications of confounding from age.

Six subjects had liver enzyme activities above the reference concentrations (table 5). Only one of these subjects belonged to the quartile with the highest P-MDX concentrations. Surprisingly, there were significant negative associations between liver enzyme activities and P-MDX (for γ -glutamyl transpeptidase (GT) $r = -0.20$, $p = 0.008$; aspartate aminotransferase (ASAT) $r = -0.27$, $p = 0.0006$; alanine aminotransferase (ALAT) $r = -0.28$, $p = 0.0002$; fig 3), but not U-MDX.

Discussion

The main findings of this study were the higher concentrations of the MDI metabolites P-MDX and U-MDX in workers exposed to thermal degradation products of PUR based on MDI, and the higher prevalence of symptoms, likely to be caused by the isocyanate exposure, as well as specific IgG antibodies against MDI among those workers. This clearly shows the value of biomarkers for evaluation of isocyanate exposure and associated risks. Further, this study shows the risks connected with thermal degradation of PUR.

The handling of MDI based glue was, of course, associated with increased air concentrations of MDI, and HDI based glue with HDI. Concentrations of TDI were never detected. The present concentrations of monomeric isocyanates in air were low, far below the time weighted average (TWA) permissible exposure limits in Sweden for HDI (TWA during a working day was 30, ceiling (for five minutes) was $70 \mu\text{g}/\text{m}^3$), MDI (50

and $100 \mu\text{g}/\text{m}^3$), and TDI (40 and $70 \mu\text{g}/\text{m}^3$).¹⁹

However, the present methods (as well as other chromatographic ones) do certainly not give a full picture of the exposure to isocyanates. Thus, the technical MDI contains several isocyanates other than the 4,4'-MDI. Furthermore, these react with the polyol to give still others. Also, the spraying caused an exposure to aerosols of glues. Hence, there was an inhalation exposure to mainly aerosol MDI, which was not fully accounted for in the impinger sampling of air. Moreover, white smoke was observed when the workers were activating the glues containing isocyanate with the heat gun. The thermal decomposition of PUR is complex; a large variety of thermal degradation products are formed. However, there are no methods available for the complete analysis of these. Some low molecular weight isocyanate compounds are formed during the thermal decomposition of TDI and HDI based PUR.^{5,20} Then, not only the monomeric diisocyanates were found, but also compounds such as aminoisocyanates and amines, which in fact dominated. The toxicity of the aminoisocyanates is unknown. It is probable that MDI will decompose thermally in a similar way. The decomposition products formed are even more complex than in HDI and TDI due to the complex isomeric composition of technical grade MDI. No measurements of compounds in air can be made without reference compounds. Hence, in this study, only TDI, HDI, and 4,4'-MDI could be quantitatively measured. However, the presence of oligomeric compounds has been shown.²¹ This means that our estimates of the exposure may be considerably lower than all the isocyanates present. One important aspect of the thermal degradation is the high possibility of peak exposure, as heat was applied intermittently, and the degree of thermal degradation may differ over time, depending on the equipment and the skill of the worker. During the spraying operations, the workers were wearing personal respiratory protection devices: half mask with a charcoal filter for solvents, a prefilter for aerosols, and rubber gloves. There were, however, spraying periods, of unknown length and frequency, when the workers did not take advantage of the protection devices. Also, dermal exposure must be considered. Thus, the glue was blue in colour and it was easy to see droplets all over the gluing work sites, and after work most of the workers involved had numerous blue spots on the skin. The likelihood of considerable variation in air concentrations associated with thermal degradation over time and the intermittent use of personal protection devices, and the possibility of dermal uptake makes biological monitoring of exposure particularly relevant.

We measured the total 4,4'-MDA in plasma and urine after hydrolysis. The amount of free MDA in urine and plasma is negligible, as MDA is only seen after hydrolysis of the biological samples. In the TDI studies, we have shown that the half life of metabolites of TDA measured after hydrolysis, is about seven

hours in urine, but as long as 20 days in plasma²²⁻²⁴ (Skarping *et al*, personal communication). This indicates the presence of adducts to blood proteins. We do not know the chemical form of the MDI metabolites in urine. To stress that there was no amine in untreated plasma and urine (in fact, we do not know if amine was ever present in the body), we chose the metabolite MDX.

All subjects, even the clerks, who only occasionally entered the production area, had detectable P-MDX and U-MDX.

This is probably not due to exceptionally poor hygiene in the factory. At least, the working conditions are hardly different from similar work places manufacturing similar products. The ubiquitous presence of MDX is also our experience from other studies of subjects without occupational exposure (Skarping *et al*, personal communication). The measurement of 4,4'-MDA is very specific. There is no known endogenous source of MDX. This indicates that all subjects are, to some extent, exposed to MDX precursors. Polyurethane is a very common polymer. Most of the PURs used are based on TDI and MDI. The reason for the background concentration of MDA in unexposed people is possibly the everyday exposure to degradation products of PUR polymers.

In accordance with these findings in subjects exposed to TDI,²²⁻²⁴ U-MDX most likely reflects recent MDI exposure (during the past few days), P-MDX the exposure for several weeks before sampling. This fact may also be the reason why the variation was larger for P-MDX than for U-MDX and for air concentrations of isocyanates; there may have been occasional, very high exposure peaks, which may also be crucial for airways sensitisation, as proposed earlier.²⁵ Thus, P-MDX may be a valuable tool to track the association between MDI exposure and respiratory disease.

Although most of the variation of P-MDX and U-MDX should be due to long and short term variations of the exposure, there might also be an effect of variation in metabolism between subjects, as found earlier for TDI²² and MDA.²⁶

As expected, increased concentrations were found in workers handling MDI based glue, in particular in those heating the glue. However, subjects who cut fabric, unexpectedly, had the highest median P-MDX and U-MDX values. The reason for this is unknown. Some fabrics were laminated with TDI based PUR, but we are not aware of any association with MDI. Possibly the explanation is due to bystander exposure caused by thermal degradation in work operations a few metres away in combination with insufficient ventilation. This problem will be studied further.

The study is a cross sectional one, and a certain selection of healthy workers must be anticipated. Workers with symptoms may have left their employment. Also, the sick leave rate was high; six employees were on sick leave because of bronchial symptoms at the time of the study. Thus, the risk may have been underestimated. Further, several of the workers who experienced symptoms, had been

moved to tasks with less exposure to glue. These had low MDX values. The symptoms were eyes and upper airway irritation, as well as lower airway irritation, including asthma, but not of hypersensitivity pneumonitis type. Of course, the present data do not allow firm conclusions on the medical nature of the complaints. It was not possible to perform lung function tests. However, spirometry and methacholine testing have later showed that many of the subjects with respiratory symptoms had lung function disturbances (Littorin *et al* personal communication). Symptoms were associated with P-MDX and U-MDX. The frequent symptoms, despite the low air concentrations of isocyanates found, may reflect sensitisation to isocyanates. However, there is a possibility of short peaks of isocyanates derived from thermal decomposition and not detected by available monitoring methods. The irritant effect of such is not known. Of course, we cannot be sure that the present symptoms were casually related to the isocyanate exposure. Thus, the thermal decomposition of PUR may give rise to a variety of other thermal decomposition products, for example, from the polyol ingredient. Some of these are irritants, for example, aldehydes. Also, the glueing workers were exposed to organic solvents such as acetone, ethyl acetate, methylethylketone, and toluene. However, the mean eight hour TWAs in their breathing zones were low: 32 (range 7-63), 12 (3-20), 11 (3-18), and < 1 mg/m³, respectively. Workers not handling glue were exposed to one tenth or less of these air concentrations.

The pathogenesis of respiratory disease associated with isocyanates has not been clarified and the role of specific antibodies is unclear.^{3,27} A low prevalence of specific IgE¹ and IgG antibodies^{28,25} have been found in exposed workers. There may be an IgE-mediated mechanism in a subgroup of workers with diseases induced by isocyanate. Thus, Baur *et al* found a strong correlation between symptoms and positive findings of specific RAST and skin tests.²⁹ However, Cartier *et al*, in specific provocation tests, found a better correlation with specific IgG than with IgE.³⁰ Thus, the present findings are not surprising. Specific IgE was rarely encountered, neither in subjects with high exposure, nor in those with various symptoms. It is not known whether the lack of antibodies is because the complaints are not mediated through such a mechanism, or because other isocyanates in the technical grade or heated MDI PUR are responsible. There are at present no methods to measure antibodies to other components.

Interestingly, the findings for specific IgG antibodies were associated with P-MDX and U-MDX. Our results support the opinion that specific IgG is an index of exposure to isocyanates.^{16,25} This is in accordance with the corresponding findings in workers exposed to organic acid anhydride.^{31,32} However, the association between IgG and P-MDX was fairly weak. Further, the exposure may vary over time, and the kinetics of the MDI metabolites and the antibodies may be quite different.

Our hypothesis was, at the start of the study, that any MDA exposure from the technical grade or heated MDI glue, possibly in combination with MDA formed during biotransformation of absorbed MDI, might affect the liver. However, unexpectedly, there were negative associations between several tests of liver function and P-MDX. (We are not aware of any earlier publications on this.) The reason for this is not known. It is probably not due to confounding. Possibly, it may be due to the enzyme inhibition; such as is caused by several isocyanates.²

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