Biomarkers, September–October 2007; 12(5): 468–483

informa
healthcare

Determination of isocyanate biomarkers in construction site workers

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Abstract

4,4′-Methylenediphenyl diisocyanate (MDI) is the most important isocyanate in the manufacture of polyurethanes, dyes, pigments and adhesives. High concentrations of isocyanates are a potent respiratory irritant. Therefore, it is important to develop methods to monitor exposure to such compounds. We monitored biological samples from 40 non-exposed and 45 exposed construction site workers. 4,4′-Methylenedianiline (MDA) and *N*-acetyl-4,4′-MDA (AcMDA) were determined from untreated urine (U-MDA, U-AcMDA) and MDA was analysed from acid-treated urine (U-MDA-tot). Haemoglobin (Hb) adducts of MDA (Hb-MDA) were determined in all workers. The levels of biomarkers decreased in the following order: U-MDA-tot > U-AcMDA > U-MDA > Hb-MDA. The same order was found for the percentage of samples, which were found positive in exposed workers: 100%, 91%, 91%, 27%. The urine levels U-MDA-tot correlate with U-MDA, U-AcMDA and Hb-MDA with $r = 0.79$, 0.86 and 0.39, respectively (Spearman rank order, $p < 0.01$). U-AcMDA correlates with U-MDA and Hb-MDA with $r = 0.77$ and 0.47, respectively ($p < 0.01$). U-MDA correlates with Hb-MDA ($r = 0.38$, $p < 0.05$). The levels in the controls were significantly lower than in the exposed workers for all compounds (Mann–Whitney test, $p < 0.01$). The median isocyanate-specific IgE-level was higher in the exposed workers, but the difference was statistically not significant. The change of the biomarker levels was compared in a group of workers ($n = 20$), which were analysed prior to isocyanate exposure and after the exposure for ~4–7 months. All urine MDA metabolites and the Hb-adduct levels increased significantly (Wilcoxon sign test, $p < 0.01$). Total IgE increased significantly after the exposure with isocyanate activity ($p < 0.01$). With the present work it could be shown that outdoor workers are exposed to a similar extent as workers from a MDI factory.

Keywords: *Biomonitoring, haemoglobin adducts, urine metabolites, isocyanates, 4,4′-methylenediphenyl diisocyanate*

(Received 8 September 2006; accepted 12 April 2007)

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ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.1080/13547500701395636

Introduction

Isocyanates are highly reactive compounds that have a variety of commercial applications. Diisocyanates such as 4,4′-methylenediphenyl diisocyanate (MDI), are increasingly used for manufacturing polyurethane foam, elastomers, paints, adhesives, coatings, insecticides and consolidation of loose rock zones in coal mining or tunnelling, and many other products (Brochhagen 1991, EPA 1998, IPCS-WHO 2000, European Commission 2005). The worldwide annual production of diisocyanates is estimated to be more than 6 million tons. The high chemical reactivity of diisocyanates makes them toxic. A number of adverse effects at the cellular and subcellular level have been reported, such as irritative and immunological reactions. Inhalation of diisocyanate vapours is associated with various pulmonary ailments, such as eosinophilic airway inflammation, airway hyper-reactivity, early and late-onset asthma, exogenous allergic alveolitis and direct toxic responses (Karol 1986, Baur 1990, Brochhagen 1991, Kennedy & Brown 1992, Mapp et al. 1994, Redlich & Karol 2002). Diisocyanates are of great concern with regard to environmental and occupational health, being considered one of the main causes of occupational asthma (Karol 1986, Baur et al. 1994, Bernstein 1996). The steady rise in asthma over the past decades points strongly to the potential relationship between isocyanates in consumer products and increasing prevalence of asthma in the general population, especially children (Krone & Klingner 2005). The prevalence and incidence of diisocyanate-induced disorders depend on the degree of exposure. Occupational exposure to diisocyanates may take place during their production and application in the production of polyurethane foam and other products containing monomeric or polymeric diisocyanates. The predominant route of occupational exposure is through inhalation. The alleged animal carcinogenicity of MDI would suggest that occupational exposure to these compounds is a carcinogenic risk (reviewed in Bolognesi et al. 2001). The few epidemiological studies available have not, however, been able to clarify if MDI is an occupational carcinogen. Arylisocyanates (Vock & Lutz 1997, Bolognesi et al. 2001) and arylamines (Beland & Kadlubar 1990, Sabbioni & Jones 2002) can bind with proteins and/or DNA (Figure 1) and lead to cytotoxic and genotoxic effects. Protein adducts are believed to be involved in the aetiology of sensitization reactions (Raulf-Heimsoth & Baur 1998).

An established method for biomonitoring exposed people is the identification of adducts with blood proteins (van Welie et al. 1992). Blood protein adducts have been widely used as surrogate for modifications of macromolecules in the target organs where the disease develops. Reactions with biomolecules can occur directly with MDI, with a metabolite of the corresponding arylamine, 4,4′-methylenedianiline (MDA) or with the MDI adduct with glutathione (Figure 1). Arylamines are metabolized to highly reactive *N*-hydroxy arylamines (Delclos & Kadlubar 1997) by mixed function monooxygenases. *N*-Hydroxy arylamines can be further metabolized to reactive conjugates, which are responsible for the genotoxic and cytotoxic effects of this class of compounds. In exposed animals, arylamines such as 4-aminobiphenyl (Kadlubar et al. 1989), a human bladder carcinogen, are known to form adducts with DNA, with tissue proteins, and with the blood proteins albumin and haemoglobin in a dose-dependent manner. The methods of identification of such adducts are well established (Skipper & Tannenbaum 1994, Sabbioni & Sepai 1995, Sabbioni & Jones 2002). Arylamine-specific adducts are of the sulfinamide type (Ringe et al. 1988, Kazanis &

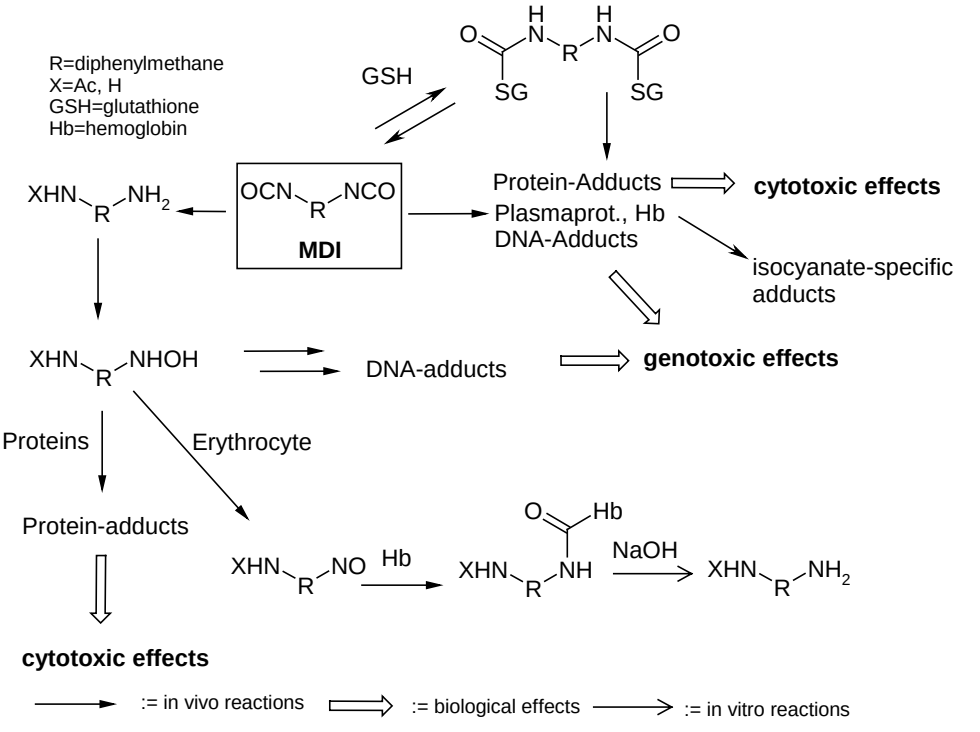

Figure 1. Possible reactions of 4,4′-methylenediphenyl diisocyanate (MDI) and of the corresponding arylamine 4,4′-methylenedianiline (MDA) with biomolecules.

McClelland 1992). In contrast, isocyanates do not need any further activation to react with biomolecules (Figure 1).

Important vehicles for isocyanates are their reaction products with glutathione (Pearson et al. 1991, Slatter et al. 1991). The glutathione adducts release the isocyanate moiety to react with other nucleophiles, e.g. proteins. Therefore, glutathione adducts are thought to be responsible for the transport of isocyanate to reactive sites away from the site of isocyanate uptake. Isocyanates can react with the following amino acids under physiological conditions (Brown et al. 1987): the α-amino group of the *N*-terminal amino acids, the sulfhydryl group of cysteine, the hydroxyl groups of tyrosine and especially serine, the ε-amino group of lysine, and the imidazole ring of histidine. Carbamoylation products with cysteine and tyrosine can be easily hydrolysed with base under mild conditions (Sabbioni et al. 1997). For the products with the other amino acids boiling with acid or base is needed to cleave the products. In guinea pigs, toluene diisocyanate (TDI) formed adducts with the α- and β-chains of haemoglobin in which one of the two original isocyanate groups had been hydrolysed to the amine (Day et al. 1996). In addition, Day et al. (1996) found an amine-nitroso adduct on the α-chain in the *in vivo* sample. These results indicate, that at least one of the isocyanate moieties (or a masked derivative) of 2,4-TDI survived passage through the lung, into the serum, and through the erythrocyte membrane to form adducts with haemoglobin, that were stable to dialysis, gel filtration, and reversed phase HPLC separation under acidic conditions.

Blood protein adducts of MDI have been analysed in humans (Brunmark et al. 1995, Schutze et al. 1995, Sepai et al. 1995a). Haemoglobin (Hb) or albumin of exposed workers have been isolated and treated with base or acid. Mild base hydrolysis of Hb released MDA and *N*-acetyl-4,4′-methylenedianiline (AcMDA) which originated most probably from sulfinamide adducts (arylamine-specific adduct). Hb adducts give an indication of exposure over the lifetime of the erythrocytes and implies the biological availability of the *N*-hydroxy-MDA and *N*-hydroxy-AcMDA. Isocyanate-specific adducts – carbamoylation of the *N*-terminal valine – of Hb with MDI (Sabbioni et al. 2000) and TDI (Sabbioni et al. 2001) have been demonstrated in rats and in humans, respectively. In rats Hb adducts have been found to correlate with the administered dose of either MDA (Sabbioni & Schutze 1998) or MDI (Sepai et al. 1995b). MDA binds to DNA (Schutze et al. 1996), and ~0.044% of the dose binds to Hb (Sabbioni & Schutze 1998). The DNA adducts (Vock et al. 1996) of the nasal epithelial cell and the Hb adducts (Sepai et al. 1995b) correlate with the dose (Bolognesi et al. 2001) in rats chronically exposed to MDI. Arylamine-specific adducts have been found in humans after exposure to MDI and MDA (Schutze et al. 1995, Sepai et al. 1995a).

Several research groups have measured MDA in acid-treated urine of MDI-exposed workers (Brunmark et al. 1995, Schutze et al. 1995, Sepai et al. 1995b, Sennbro et al. 2003). The chemical structure of the adducts prior to cleavage is, however, unknown. Only a few studies exist in which untreated urine was analysed (Schutze et al. 1995, Sepai et al. 1995b). In this case MDA and AcMDA were analysed.

For the present study we investigated the presence of biomarkers in workers exposed to MDI products on construction sites. For this purpose urine metabolites of MDI, Hb adducts, MDI-specific IgE and total IgE-levels were measured and compared with each other. In addition, for a small group of workers ($n = 20$) the lung function tests were followed over a time period of 10 years in order to determine any abnormal decrease.

Material and methods

Worker population

Workers (62 males and 3 females) involved in construction sites were investigated. The median age (range) of the workers was 32.9 (20.1–59.6) years. The median amount of work years was 2.16 (0–31) years. Most workers were Germans ($n = 31$) or Portuguese ($n = 22$). At the beginning of the study 40 workers (controls) had not been exposed to isocyanate products in the last 4 months and 25 workers had been exposed. The biological samples were collected from 65 workers (40 controls + 25 exposed) at the beginning of the study. After 4–7 months of exposure to isocyanate, samples from 20 former controls were obtained. All biological samples were obtained at the end of the work-shift.

Tasks of workers

After the evaluation of the questionnaire, the following work classifications were obtained: mixing, shovel work, preparatory work ($n = 25$), smooth by hand ($n = 19$); spraying of isocyanate mixture ($n = 18$); mix and lay of artificial turf adhesives ($n = 7$); supervision, foreman ($n = 6$); packing of isocyanate-containing material ($n = 5$);

groutings ($n = 4$); serving the manufacturer ($n = 2$); leading and filling the mixing machine ($n = 2$); maintenance and repair work ($n = 1$); material transport ($n = 1$); soil coating ($n = 2$).

Worker protection

The following preventive measures had been used: (i) half mask; (ii) particle mask; (iii) gloves; (iv) skin cream; (v) increased hygiene; and (vi) consideration of the wind direction. Workers – who were spraying and who were close to the freshly applied material – have worn in at least 50% of the cases a respiratory protection, usually a half mask. Measures to the skin protection were more common. In about 55% of the cases a protective glove was worn. In addition, 40% of the workers applied increased hygiene measures when working with isocyanate material.

Typical work procedures

In the following section a typical procedure to fix cracks in buildings is described. A filling mixture containing polyurethane (PU) is injected under pressure (approx. 20–30 bar). The procedure lasts 2 days. On the first day, threaded nipples in the distance from approx. 20 cm are applied to the crack. The threaded part is the pressure-tight connection to the material pump. The crack is sealed with a polyester resin, so that the nipples are the only entrances to the crack. On the second day the PU mixture is pressed through the nipples (grouting). The PU is freshly prepared from two components in small quantities in an approx. 2–3-l container. The container is directly connected with the material pump. The evaporating surface of PU is small, since the open surface of the PU container is small and only small quantities withdraw from the nipples. These workers usually wear cotton gloves.

PU material was used as bonding agents in the application of floor mats for sports facilities. The workers use a mixture, which is made of granulated rubber and MDI and/or occasionally with addition of TDI. The workers mix MDI and/or TDI with the rubber granulates. The prepared mixture is transported in a special container to the construction site and the material is filled into a spraying machine. The material is then applied to the floor using a pressure of 4 bar. The spraying machine is filled all 10–15 min. In 8 h approximately 1200–1400 l of the product are applied. Under employment of a mobile manufacturer the surface is consolidated and smoothed. Level differences or regions not reached by the machine are smoothed by hand.

Determination of haemoglobin adducts and urine metabolites

The methods published previously and described in detail in Schütze et al. (1995), Sepai et al. (1995a,b) and Sabbioni and Beyerbach (2000) have been used. Hb adducts were measured after base hydrolysis in 0.1 M NaOH, extraction with dichloromethane, derivatization with heptafluorobutyric anhydride and GC-MS analysis in the negative chemical ionization mode. The deuterated MDA (d_4-MDA) and Ac-d_4-MDA were used as internal standards. The peaks of MDA and AcMDA were quantified in relation to the peaks of the deuterated standards.

Urine was analysed in two ways: (i) extraction from raw urine at pH 9; and (ii) acid hydrolysis in 3.3M HCl for 0.5 h at 100°C and then extraction at pH > 9. Extraction, work-up and analysis were performed as for the determination of the Hb adducts.

Determination of immunological parameters

The radio-allergo-sorbent test (RAST) procedure published by Grunewalder and Karol (1986) was applied with modifications.

The hapten conjugate with human serum albumin (HSA) was synthesized in the following manner. To a 1% human serum albumin solution (HSA) in 0.05 M boric acid buffer (0.05 M KCl, pH 9.4), a 1 to 20 molar excess of hapten (MDI, TDI, phenylisocyanate (PI)) dissolved in tetrahydrofuran or acetone was added under vigorous stirring. After 30 min the reaction mixture was centrifuged to eliminate the precipitate. The supernatant was transferred into dialysis tubes (exclusion size 8000–10 000 Da) and dialysed for 24 h against 0.9% saline solution, and then for 72 h against water. The hapten conjugate was obtained after freeze-drying. This method was used and performed in the laboratory of Dr Lewalter on a routine basis. The ratio of the antigen bound to albumin was not determined.

The RAST-nitrocellulose discs were produced according the following procedure. A batch of 25 nitrocellulose discs (5 mm diameter) were coated each time. Two millilitres of a 0.05% solution of the freeze-dried hapten–HSA conjugate in Tris buffer (0.01 M Tris base, 0.9% NaCl, pH 7.6) were added to a container with 25 discs and were shaken slowly. After 2 h the supernatant was removed. The discs were then incubated for 1 h with a 3% bovine serum albumin (BSA) solution in Tris-buffer. The supernatant was removed and the discs were incubated 1 h with a mixture of 1% BSA and 1% rabbit serum in Tris-buffer. The supernatant was then eliminated and the discs were dried.

RAST test. Two determinations were performed for each serum with the hapten–HSA-coated discs and with the HSA-coated control discs. The discs (which were pre-wetted with 0.1% BSA solutions in Tris-buffer) were added to serum (50 µl) in plastic tubes (3.5 ml). After 2 h at 37°C the supernatant was removed and the discs were washed three times with 1 ml of 1% BSA in Tris buffer. Each disc was then incubated for 16 h at room temperature with 50 µl I-125 labelled IgE-antibody (Sanofi). The supernatant was eliminated and the discs were washed three times with 1 ml 1% BSA solutions in Tris buffer. The radioactive counts of the tubes with the discs were determined in a gamma counter for 2 min each. The relative unit (RU = antigen disc/HSA disc) results from the radioactivity counts of the discs.

Total IgE levels in sera were determined using the paper radioimmunosorbent test (PRIST) kit from Pharmacia and values were expressed in PRIST U ml^{-1}.

Clinical parameters

Routine blood and urine tests, and liver function tests were performed: alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyltranspeptidase (GGT), alkaline phosphatase, total protein and total bilirubin.

Lung function tests were performed with spirometry. The following lung function values were measured: forced expiratory volume in the first second (FEV1), forced expiratory vital capacity (FVC), the peak expiratory flow (PEF) and vital capacity (VC).

In the first year of the present study, the lung function tests of 22 workers were performed. For 13 of the 22 workers, the tests were performed before taking up their seasonal activity with isocyanates. For nine of the 22 workers lung test were performed

after the workers had started their isocyanate activity. Follow-up tests were performed for 20 of these 22 workers. The last health check was performed after 9–10 years for five workers (60% Germans), after 4–6 years for 14 workers (71% Germans), and after 1.5 years for one worker (foreigner).

Statistical analyses

The statistical analyses were performed with SPSS 10.0. The urine metabolites, the Hb adducts and the IgE-tot data were not normally distributed (one-sample Kolmogorov–Smirnov test, $p < 0.05$). Therefore, non-parametric tests were used for the comparison of groups (Mann–Whitney test) and for the comparison of paired samples (Wilcoxon sign test).

Results

Blood and urine samples of construction site workers exposed to isocyanates were analysed in order to test the preventive measures taken. Most workers were employed in the construction of sports facilities, which included the production of floor mats with PU resins and grouting work with isocyanates. The exposure history and the work place description were gathered from the questionnaires. The controls were workers whose isocyanate exposure was more than 4 months ago. The questionnaire, the health checks and the collection of the samples were performed all in the same week. The collected urine samples and the blood samples were transported overnight on ice to the analytical laboratory. The urine samples were analysed following two protocols: (i) extraction from raw urine at pH > 9 and (ii) acid hydrolysis at 100°C. The acid hydrolysis splits many possible conjugates which might be present in urine (mercupturic acids, glucuronic acids, AcMDA, 4,4′-diacetyl-MDA) to MDA (U-MDA-tot). MDA and AcMDA were determined from raw urine (U-MDA, U-AcMDA). The obtained values are listed in Table I. The largest values were obtained for MDA released after acid hydrolysis (U-MDA-tot) (Figure 2). The smallest values were obtained for U-MDA extracted from raw urine. The levels in the controls were significantly lower than in the exposed workers for all compounds (Mann–Whitney test, $p < 0.01$). U-MDA-tot was found in all exposed workers and in all controls. After base hydrolysis, U-MDA and U-AcMDA were found in 91% of the exposed workers and in 17.5% and 21% of the controls.

The Hb adducts were measured in all workers (Table I). MDA was found in 27% of the exposed workers and in 0% of the control workers. In addition, AcMDA was found in two exposed workers.

The urine levels U-MDA-tot correlates with U-MDA, U-AcMDA and Hb-MDA with $r = 0.79$, 0.86 and 0.39, respectively ($p < 0.01$). U-AcMDA correlates with U-MDA and Hb-MDA with $r = 0.77$ and 0.47, respectively ($p < 0.01$). U-MDA correlates with Hb-MDA ($r = 0.38$, $p < 0.05$).

The immunological parameters IgE-tot and the isocyanate-specific IgE-MDI, IgE-TDI and IgE-PI were determined in the sera of all workers. The data are summarized in Table I. The IgE-tot, IgE-MDI, and IgE-PI were higher in the exposed workers, but the difference was statistically not significant. IgE-TDI increased significantly in the exposed workers (Mann–Whitney test, $p < 0.05$). All the different IgE levels did not correlate with any of the measured urine metabolites or Hb adducts. Three workers

Table I. Urine metabolite levels and haemoglobin (Hb) adduct levels in construction workers, MDI (4,4′-methylenediphenyl diisocyanate) factory workers (Schutze et al. 1995), rubber factory workers (Sepai et al. 1995a) and MDA (4,4′-methylenedianiline) factory workers (Schutze et al. 1995).

	Exposed, construction workers median (25th, 75th, 90th)[i]	Non-exposed, construction workers median (25th, 75th, 90th)	Exposed, MDI factory[c] median (75th, 90th)	Exposed, rubber factory[d] median (75th, 90th)	Exposed, MDA factory[c] median (75th, 90th)
U-MDA-tot (nmol l^{-})	1.340 (0.491, 1.99, 6.29) $n=45$	0.297 (0.184, 0.473, 0.602) $n=37$	1.70 (2.57, 3.54) $n=23$	2.29 (13.5, 37.5) $n=20$	1.89 (5.16, 12.7) $n=35$
U-MDA (nmol l^{-1})	0.033 (0.018, 0.108, 0.219) $n=45$	0 (0, 0, 0.018) $n=40$	0.056 (0.076, 0.09) $n=23$	0.082 (0.229, 0.634) $n=20$	0.071 (0.170, 0.269) $n=33$
U-AcMDA (nmol l^{-1})	0.378 (0.164, 0.680, 1.93) $n=45$	0 (0, 0, 0.196) $n=40$	0.540 (0.911, 1.21) $n=23$	0.710 (1.89, 3.26) $n=20$	0.370 (1.12, 2.63) $n=33$
Hb-MDA (pmol g^{-1} Hb)	0 (0, 0.051, 0.177) $n=44$	n.d.[a] $n=40$	0 (0.075, 0.129) $n=26$	0.115 (0.235, 0.364) $n=20$	0.284 (0.507,0.768) $n=34$
Hb-AcMDA (pmol g^{-1} Hb)	2 positive[g]	n.d.	n.d.	1 positive[h]	0.425 (0.725, 2.26) $n=34$
IgE-tot (PU)[e]	37.1 (12.9, 116, 715) $n=45$	36.9 (14.6, 119, 635) $n=40$	na[b]	27.5 (138, 188) $n=19$	na
IgE-RAST-MDI (RU)[f]	1.00 (0.895, 1.14, 1.29) $n=45$	0.965 (0.883, 1.09, 1.20) $n=40$	na	0.990 (1.12, 1.36) $n=19$	na
IgE-RAST-TDI (RU)	1.10 (0.955, 1.25, 1.43) $n=45$	1.02 (0.912, 1.23, 1.21) $n=40$	na	na	na
IgE-RAST-PI (RU)	1.01 (0.875, 1.21, 1.49) $n=45$	1.19 (0.965, 1.19, 1.45) $n=40$	na	0.89 (1.17, 1.33) $n=19$	na

[a]n.d., not detected. [b]na, not applied. [c]Schutze et al. 1995. [d]Sepai et al. 1995a. [e]Total IgE levels in sera were determined using the Paper radioimmunosorbent test (PRIST) kit from Pharmacia and values were expressed in PRIST U ml^{-1}. [f]The relative unit (RU = antigen disc/HSA-disc) results from the radioactivity counts of the discs. [g]2.3 and 3.7 pmol g^{-1} Hb. [h]20 pmol g^{-1} Hb (Sepai et al. 1995a). [i]Percentiles.

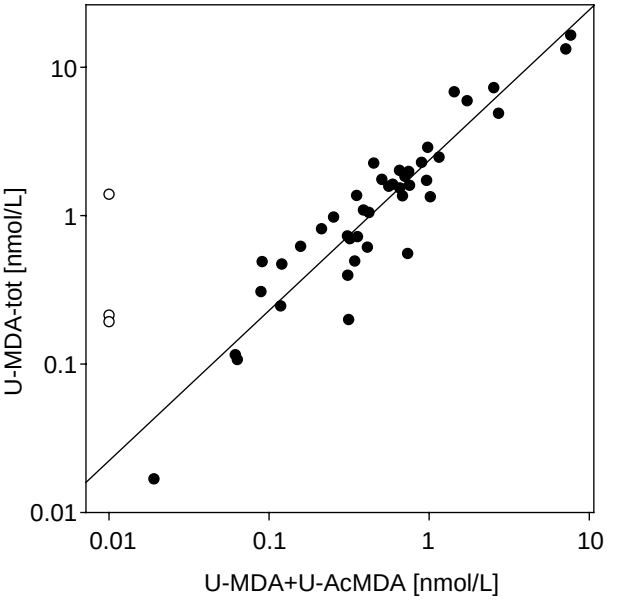

Figure 2. Comparison of urine metabolites of untreated and of acid hydrolysed urine in exposed workers. The three points (white circles) without measurable levels of *N*-acetyl-4,4′-methylenedianiline and 4,4′-methylenedianiline in urine (U-AcMDA + U-MDA) were not included in the regression analysis ($r = 0.93$).

had IgE levels above 1000 PU, seven workers had levels with an IgE between 300 and 1000 PU, and 10 workers had IgE levels between 100 and 300 PU. The high IgE levels were spread similarly between exposed and controls. Therefore, the high IgE levels were not related to isocyanate exposure.

We analysed the seasonal variation – spring, summer and autumn – of the IgE-tot and the isocyanate-specific IgE-MDI, IgE-TDI and IgE-PI. The IgE-MDI and IgE-PI levels did not change. The median levels of IgE-tot decreased from spring to autumn (44.5, 21.9 and 24.7 PU), but the changes were statistically not significant. The IgE-TDI changed significantly from season to season (spring = 1.03, summer = 0.93, autumn = 1.11 RU).

The change of the biomarker levels were compared in a group of workers ($n = 20$, controls) which were analysed prior to isocyanate exposure and after 4–7 months isocyanate exposure (Figure 3). All urine MDA metabolites, the Hb adduct levels and the IgE levels (Figure 4) increased significantly (Wilcoxon sign test, $p < 0.01$). Interestingly, the haematocrit and the glucose increased significantly after the take up of the isocyanate activity (Wilcoxon sign test, $p < 0.01$, data not shown). In addition, GGT and ALT decreased significantly (data not shown).

Lung function tests were performed on 22 workers in the first year of the study. For 13 of these 22 workers lung tests were performed before they started their seasonal activity with isocyanates. For 20 of these 22 workers lung tests were performed for up to 10 years. The VC and FEV1 decreased significantly (Wilcoxon sign test, $p < 0.05$) in the follow-up studies. However, after age and body size adjustment of the data (ERS 1993) the differences are not significant anymore. For 12 (75% smokers) of the 20 workers with follow-up examinations, the FEV1 values decreased more than 50 ml per year (median 114, range 52–253 ml per year). For eight workers (63% smokers)

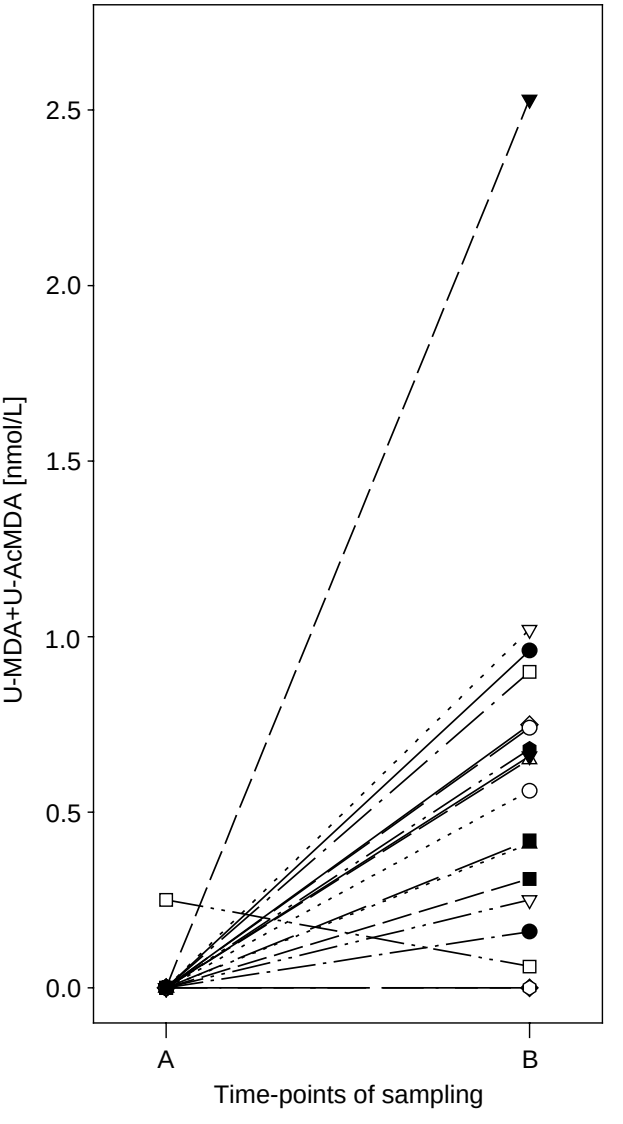

Figure 3. Urine metabolite levels in 20 workers before (A) working with isocyanates and after (B) working with isocyanates in the past 4–7 months.

the decrease was 30 ml per year (0–42 ml per year). In retrospective, the median urine U-MDA-tot level at the beginning of the study was higher (0.63 vs. 0.21 nmol l^{-1}) in the cases with the abnormal FEV1 decrease, but the difference was not significant. A FEV1 average decrease of 20–40 ml per year is considered normal for a non-smoking adult (age >25 years) (Wu et al. 2002, Dewar & Curry 2006). For 5% of the workers at the first medical examination and for 20% of the workers after the last medical examination, the ratio (FEV1/VC)*100% was below 70%, a value which indicates potential pathological lung effects (Dewar & Curry 2006 and literature cited therein). The odds ratio (OR) for this decrease was 4.8 (95% confidence interval, 0.5–47). In retrospective, the median urine U-MDA-tot level at the beginning of the study was

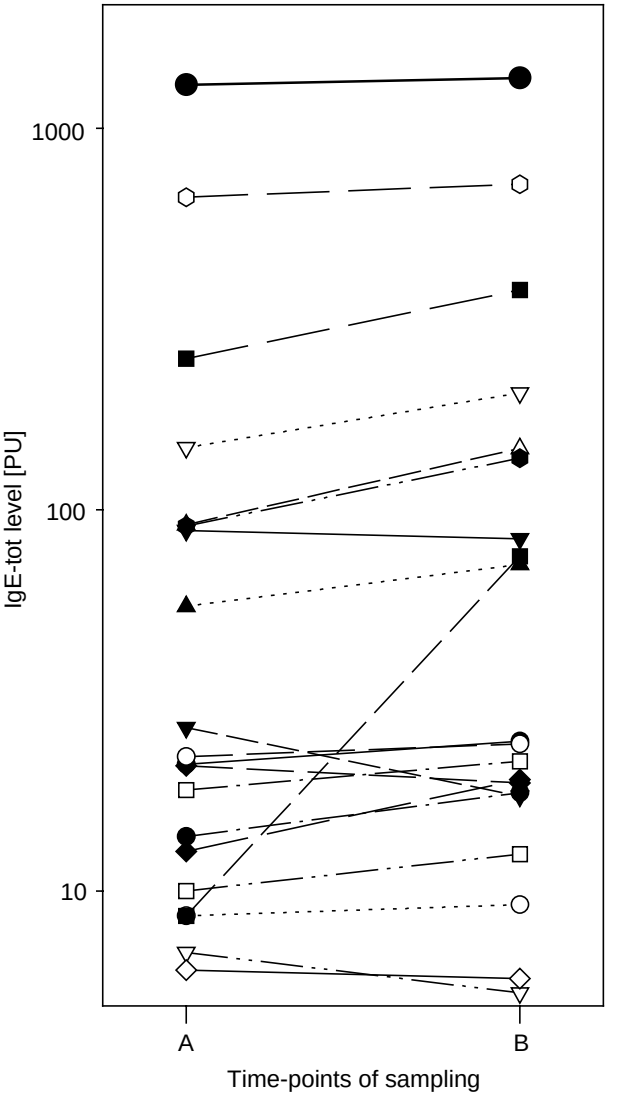

Figure 4. IgE-tot-levels in 20 workers before (A) working with isocyanates and after (B) working with isocyanates in the last 4–7 months.

higher (0.94 vs. 0.21 nmol l^{-1}) in the cases with the abnormal FEV1/VC decrease, but the difference was not significant.

Discussion

The levels found in the present group of workers were compared with other groups of workers, who were investigated in the past. The metabolite and adduct levels are summarized in Table I. The median levels of the urine metabolites or the levels of the Hb adducts in construction workers were lower than in the other workplaces. The differences were statistically significant for U-MDA-tot and Hb-MDA compared to

the rubber factory and the MDA factory. The difference was also significant for U-MDA in comparison to the levels found in MDA workers. The following percentages of the workers were found positive (with detectable concentrations) for biomarkers in the different worker groups: (i) MDI factory with 96%, 96% 78% and 38% of the exposed workers positive for U-MDA-tot, U-MDA, U-AcMDA and Hb-MDA, respectively; (ii) exposed rubber factory workers ($n=20$) with 100%, 95%, 92% and 100% positive for -U-MDA-tot, U-MDA, U-AcMDA and Hb-MDA, respectively; (iii) MDA factory with 97%, 97%, 82%, 97% and 67% positive for U-MDA-tot, U-MDA, U-AcMDA, Hb-MDA and Hb-AcMDA, respectively. In the present group of workers a similar proportion of positive samples was found for the urine metabolites, but not for the Hb adducts. In comparison to the rubber factory approximately four times fewer workers were positive for Hb-MDA. Possibly the exposure of construction site workers is not constant over the working days, so that the Hb adduct cannot accumulate in the same way as from chronic exposure. The Hb adduct levels and urine metabolite levels, and the amount of positive samples were similar to the MDI factory. In the present study, urine samples of controls were found positive for U-MDA-tot.

The ratios of the metabolite means of the four worker groups (Table I) and for rats (Sepai et al. 1995b) exposed for 3 months chronically to monomeric MDI were compared. The ratios of U-MDA-tot/Hb-MDA were 52, 51, 54, 24 ($=9$ including Hb-AcMDA) and 2.9 (1.1 including Hb-AcMDA) for the exposed construction workers, the MDI factory workers, the rubber factory workers ($n=20$), the MDA factory workers and rats, respectively. In contrast, the ratios for U-AcMDA/U-MDA were similar except for rats: 8.5, 11.2, 8.2, 8.6 and 32.3. The ratios for U-MDA-tot/(U-MDA+U-AcMDA) are 2.4, 3.1, 6.7, 6.4 and 7.1. Therefore it appears that approximately 6 and 50 times more Hb adducts relative to urine metabolites were formed in MDA workers and rats, respectively, than in humans exposed to MDI. It is striking that in MDA workers Hb-AcMDA was the major adduct. In the case of MDI-exposed workers Hb-AcMDA was only found in three workers among all MDI worker groups. Therefore it appears that in general AcMDA does not form Hb adducts in MDI workers, although AcMDA was found in urine and the ratio U-AcMDA/U-MDA was similar in all worker groups.

The Hb adducts found in the present group of workers most likely originates from MDA, which becomes biologically available after exposure to MDI (Sepai et al. 1995a). MDA and AcMDA were released after mild base hydrolysis of Hb. Such mild conditions will release arylamines from the typical sulfinamide adducts formed by arylamines with the cysteine of haemoglobin. However, mild base hydrolysis can also release isocyanate adducts with tyrosine and cysteine (Sabbioni et al. 1997). Carbamylated cysteines are not very stable and the isocyanate moiety is transferred easily to other functional groups in amino acids, e.g. amino groups – which form much more stable products (Pearson et al. 1991, Slatter et al. 1991, Day et al. 1997). The presence of AcMDA after mild base hydrolysis of Hb is a strong indication that these adducts are generated from MDA. We consider it unlikely that only one isocyanate group of MDI is hydrolysed to the amine and then acetylated, while the other isocyanate group could reach the erythrocytes intact and form a covalent bond with Hb. Acetylation is also possible in erythrocytes (Risch et al. 1996). However, then we would expect that most MDA adducts would be acetylated. In addition AcMDA was also found in urine of MDI-exposed workers.

The IgE-tot, IgE-MDI and IgE-TDI levels were also measured in the rubber company (Table I). The isocyanate-specific IgE levels were at the same level as the levels found in the present group of workers. The IgE-tot levels were lower. For the rubber factory workers, the Hb adduct levels, the urine metabolite levels, the albumin-MDA adduct levels and the plasma protein adduct levels did not correlate with the IgE and isocyanate-specific IgE. However, the ratio of Hb adducts/albumin adducts correlates with the MDI-specific IgE (Pearson correlation, normally distributed data, $r=0.63$, $p<0.05$). The relationship between Hb adducts and immunological parameters was reported for one worker exposed accidentally to MDI in a MDI factory (Lewalter 1998). The IgE-tot, IgE-MDI and Hb-MDA were monitored over a 4-month period. Hb-MDA and IgE-MDI peaked after 2 months and the IgE-tot after 4 months. It is surprising that the Hb-MDA adduct levels were raised for 2 months. It appears that MDI, which was ingested, is released over a longer time period in the body. This could explain the presence of U-MDA-tot in construction workers without declared isocyanate exposure. On the other hand MDI is used in a large amount of consumer products (European Commission 2005). U-MDA-tot was also found in non-exposed workers in Sweden (Sennbro et al. 2003).

The biological work tolerance value for MDI-exposed workers was set by the German research commission at 50 nmol ($=10$ μg) MDA l^{-1} in acid hydrolysed urine (DFG 2006). These values were obtained after acid hydrolysis of urine in 2M HCl for 2 h at 100°C. For the present work the hydrolysis was performed in 3.3M HCl for 0.5 h at 100°C. Therefore the conditions are comparable. The urine values of all construction workers were below 10 nmol l^{-1}, except for two subjects who had levels in the same order of magnitude (13.3 and 16.4 nmol l^{-1}) as the BAT value. The Health and Safety Executive of the UK proposes a 'Biological Monitoring Guidance' value of 1 μmol isocyanate per mol creatinine obtained as U-MDA-tot ($\sim$8 nmol l^{-1} urine) by boiling urine (2 ml) with concentrated sulfuric acid (200 μl) at 100°C for 90 min. Taking this value, two workers in the present study were above this level. We compared the values issued by the authorities with U-MDA-tot levels in rats exposed to MDI for a carcinogenicity study. The rats were exposed to monomeric MDI aerosol at three doses (0, 0.23, 0.7 and 2.03 mg m^{-3}) for 17 h per day, 5 days per week for up to 24 months (Hoymann et al. 1995, Sepai 1995b). First effects were seen in the lowest dose group in the lung. Interstitial fibrosis was found in 79% of the lowest dose group and 10% bronchiolar type hyperplasia was found in the lowest dose in comparison to 0% in the control group. The urine metabolite levels in the rats of the lowest dose group (lowest observed adverse effect level, LOAEL) were 70 nmol l^{-1} for U-MDA-tot and 8 nmol l^{-1} for U-MDA+U-AcMDA (Sepai et al. 1995b). Taking these levels as guidance values, two workers (the same workers with the highest U-MDA-tot-level, see above) with 7.1 and 7.7 nmol l^{-1} U-MDA+U-AcMDA were close to the LOAEL values. Based on the rat experiment it appears that the threshold values chosen by the authorities are too high.

In summary, we showed that the MDI exposure of construction site workers is similar to that found in workers in a MDI factory. Follow-up studies could be performed only for $\sim$30% of the workers. The rest of the workers left these small enterprises. This makes a determination of long-term consequences from isocyanate exposures in small companies very difficult.

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