BIOLOGICAL MONITORING OF EXPOSURE TO 1,5-NAPHTHALENE DIISOCYANATE AND 4,4'-METHYLENEDIPHENYL DIISOCYANATE

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ABSTRACT

Objectives. Biological monitoring of occupational sensitizers, such as 1,5-naphthalene diisocyanate (NDI) and 4,4’-methylenediphenyl diisocyanate (MDI) is of high importance. In this study, 1,5-naphthalenediamine (NDA) and 4,4’-methylenedianiline (MDA) in hydrolysed urine and plasma were evaluated as biomarkers of exposure to NDI and MDI, respectively.

Methods. The air exposure to NDI and MDI was monitored for 30 exposed workers at four different plants. In parallel, urinary as well as blood plasma samples were collected. Biomarker levels were determined in hydrolysed urine and plasma by means of gas chromatography–mass spectrometry.

Results. Air exposure to both MDI and NDI was correlated to their corresponding urinary and plasma biomarkers. The correlation coefficients for the associations between air and biomarker levels were in the range of 0.51-0.65 and 0.53-0.96 for MDI and NDI, respectively. For NDI, but not for MDI, the significance and correlation coefficients were increased by adjusting the urinary biomarker levels for creatinine content or density.

Conclusions. Biomarker and air levels of MDI and NDI were correlated, but there was a large individual variation.

Key Words: biomarker, occupational, polyurethane, protein adducts, NDI, MDI.

INTRODUCTION

Aromatic diisocyanates such as 1,5-naphthalene diisocyanate (NDI) and 4,4'-methylenediphenyl diisocyanate (MDI) are used in the manufacture of polyurethane. These compounds may induce airway disorders (Harries et al 1979, Vandenplas et al 1993, Baur et al 2001, Merget et al 2002) and therefore it is essential to monitor the occupational exposure for workers. This has mainly been performed by air monitoring. However, biological monitoring may be an alternative methodology in exposure assessment because it holds several advantages as compared to air monitoring. The sampling is less time-consuming and biomarkers with long half-lives, such as protein adducts, may be used to monitor long-term exposure, which is time-consuming to perform with air monitoring. Furthermore, biomarkers reflect the absorbed dose which integrates such factors as lung ventilation, alternative routes of exposure and genetic and acquired metabolic characteristics. Thus, biomarker levels, rather than air levels, may better correlate to systemic adverse health effects, such as sensitisation.

Absorbed NDI and MDI form protein adducts in blood (Sepai et al 1995, Sennbro et al 2003), preferentially with serum albumin in plasma (Johannesson et al 2004). MDI metabolites are excreted in urine as low-molecular weight conjugates (Lind et al 1996a). By hydrolysis of biological samples from exposed subjects, the NDI- and MDI-related metabolites are released as 1,5-naphthalenediamine (NDA) and 4,4'-methylenedianiline (MDA), respectively. Methods for the quantification of MDA in hydrolysed biological fluids have been described (Brunmark et al 1992, Schütze et al 1995, Sepai et al 1995, Dalene et al 1997, Kääriä et al 2001, Sennbro et al 2003), but few studies have concerned biological monitoring of exposure to MDI (Skarping et al 1996, Kääriä et al 2001). For NDA, one method for determination in hydrolysed urine and plasma has been described (Sennbro et al 2003), and no studies on biological monitoring of NDI have been reported. In the present

study, the objective was to evaluate urinary as well as plasma biomarkers of exposure to NDI and MDI, for a group of workers from various work environments.

MATERIALS AND METHODS

Plants and workers

The present study included 30 workers, exposed to NDI and/or MDI. The workers were selected from three moulding plants (plant A-C) and one plant with low heating process (plant D) in southern Sweden. Plant A, B and D have previously been described as M1, M2 and LH2, respectively (Sennbro et al 2004a).

Plant A manufactured NDI- and MDI-based rigid PUR products by moulding and centrifugal moulding. The dosing, mixing and moulding of the PUR were performed manually, partly in ventilated hoods. After hardening of the products overnight, the PUR material was refined by grinding and turning. Respiratory personal protection equipment (PPE) were not used. All five monitored workers were exposed to airborne MDI and three of these workers were also exposed to NDI.

Plant B manufactured mainly NDI- but also MDI-based rigid PUR products by moulding. The dosing, mixing and moulding of the NDI-based products were performed automatically in a semi-enclosed ventilated area. Within this area, preparation of the moulding matrices and transfer of the ready-made products into the hardening furnace during the manufacturing process was performed. Outside the semi-enclosed area, preparation and sorting of the moulding matrices was performed. The MDI products were only manufactured as prototypes in a pilot system in a separate area. Respiratory PPE were used occasionally. All twelve monitored workers were exposed to airborne NDI.

Plant C manufactured MDI-based rigid PUR products by moulding. The dosing and mixing were performed in an enclosed system. The moulding was performed either automatically in an enclosed system or manually in an enclosed area. During the manual moulding respiratory PPE were used. After hardening, the PUR material were manually removed from the matrices, refined and moved to a hardening furnace. All ten monitored workers were exposed to airborne MDI.

Plant D manufactured different preparations of TDI- and MDI-based PUR and isocyanate formulations, such as jointing sealing compound. The monitored workers performed either dosing and mixing of the formulation ingredients or packaging of the completed formulation products. Respiratory PPE were not used. The formulations were treated at a temperature less than 40 °C. All three monitored workers were exposed to airborne MDI, and were air monitored on their third work shift of the week.

In total there were 30 workers monitored, of which 12 were exposed to NDI, 15 were exposed to MDI, and three workers were exposed to both MDI and NDI. The air sampling was performed during normal production load at all plants and the workload was considered to be moderate for all exposed workers. The present study was approved by the Ethical Committee at Lund University, Sweden, and was performed with the written informed consent of the workers.

Collection of samples

At plant A, B and D, the air samples were collected using 1-(2-methoxyphenyl)-piperazine (2MP) impregnated filters (Health and Safety Executive 1999) with pump flow rates of 1 L/min. At plant C, the air samples were collected using a modified 2MP method (Henriks-Eckerman et al 2000). Each worker was monitored during one work shift. In principle, two consecutive filters, each sampled for approximately four hours, were used to

monitor the whole day personal exposure to isocyanates. When respiratory PPE were used the air sampling was paused, thus assuming zero exposure during periods when PPE was used. The median time for personal monitoring was 6.7 h. Immediately after sampling the filters were desorbed in toluene or acetonitrile containing 2MP and were stored protected from light. Upon arrival to the laboratory, the samples were stored in a refrigerator until analysis.

At the day of air monitoring, a urinary sample (U1) was collected from each of the 30 exposed workers. In connection with a medical examination performed within two weeks from the air monitoring, a second urinary sample (U2) and a blood sample were collected from 23 and 24 of the exposed workers, respectively. A summary of the collected biological samples are given in table 1. The biomarker levels, previously partly described (Sennbro et al 2003) are presented in table 2.

The urinary samples were collected as one pooled sample for each worker during the last four hours of the work shift. The blood samples were collected by arm vein puncture, in Venoject® blood sampling tubes containing heparin. After arrival to the laboratory, the plasma was separated from the blood cells by centrifugation. The biological samples were then stored at -20°C until the day of analysis.

Analysis

The airborne NDI and MDI, sampled on the 2MP-filters, were quantified by high performance liquid chromatography and tandem mass spectrometry as previously described (Östin et al 2002), with the exception that deuterium labelled internal standards were used. For both NDI and MDI, the limit of detection (LOD) was 2 ng/sample and the limit of quantification (LOQ) was 20 ng/sample. The personal 8 h time-weighted average (TWA) air levels of NDI and MDI were calculated and are referred to as A-NDI and A-MDI,

respectively. The range of the personal air levels in the exposed group was 0.2-15 µg/m^3 (median 3 µg/m^3) for NDI, and 0.03-7.8 µg/m^3 (median 0.2 µg/m^3) for MDI.

The biomarker levels in the urinary and plasma samples were analysed according to Sennbro et al (2003). In brief, the biological samples were hydrolysed for 24 h in 0.3 M NaOH in order to release the isocyanate-related diamines NDA and MDA. The diamines were extracted with toluene and after derivatisation with pentafluoropropionic acid anhydride, the derivatives were quantified by gas chromatography and mass spectrometry, using tri-deuterated 2,4-toluenediamine as internal standard. Each sample was analysed in duplicate and the precision was 7-19%. The LOD was 0.1 µg/L for NDA and 0.05 µg/L for MDA. The LOQ was 0.6 µg/L for NDA and 0.5 µg/L for MDA. The levels of NDA and MDA in hydrolysed samples are referred to with prefixes U1- or U2- for urinary samples and with P- for plasma samples. It has been observed that NDA has limited stability in the standard solutions, especially at low concentrations. Therefore, standard stock solutions should be prepared every month and working standard solutions should be prepared on a daily basis. The density of the urinary samples was determined by a hand refractometer (Atago URC-PN) and the urinary creatinine concentration according to Mazzachi et al (2000).

Calculations and statistics

Correlations between air exposure and biomarkers

For the exposed workers, the associations between biomarkers, on the one hand, and personal 8 h TWA air exposure, on the other, were analysed by calculations of Pearson correlation coefficients (r), and Spearman's rank correlation coefficients (r_s). For the urinary biomarkers, the influence on the correlation coefficients by density and creatinine content adjustments, respectively, was studied. Density adjustment was performed by using the

formula $C(corr) = \frac{C(obs) \times (1.016 - 1)}{(\rho - 1)}$, where C(corr) is the density adjusted urinary biomarker level, C(obs) is the observed urinary biomarker level, ρ is the specific density and 1.016 is an assumed average density as presented by Boeniger et al (1993).

Individual inter-day variation of urinary biomarker levels

The individual inter-day variation in urinary biomarkers as determined in the U1 and U2 samples were calculated as the relative deviation; $\frac{|X_1 - X_2|}{X_{mean}}$, where X_1 and X_2 are the biomarker levels in U1 and U2, respectively and X_{mean} equals the mean of X_1 and X_2. The systematic differences in biomarker levels in U1 and U2 were analyzed by a paired t-test and by Wilcoxon signed ranks test.

RESULTS

Correlations between air exposure and biomarkers

The calculated correlation coefficients r and r_s for the correlation between air and biomarker levels of NDI and MDI are presented in table 3. A-MDI was significantly correlated to both U1- and P-MDA, but not to U2-MDA. Contrary, A-NDI was less strongly correlated to U1-NDA, than to U2- and P-NDA. The biomarker levels in urine and plasma, respectively, are plotted against the personal 8 h TWA air exposure levels in figure 1 for NDI and in figure 2 for MDI. For MDI, there was no clear indication that either creatinine or density adjustment of U1- or U2-MDA would increase their correlation to A-MDI. For NDI, on the other hand, both creatinine and density adjustments of both U1- and U2-NDA increased their correlation to A-NDI. All these observations were persistent when calculating only on the workers with both U1- and U2-samples (not shown).

Individual inter-day variation of urinary biomarker levels

For U-MDI, the relative deviation was 12-285% (median 56%) for non-adjusted biomarker levels, 7-141% (median 40%) using creatinine content adjustment and 1-255% (median 43%) when using density adjustment. For U-NDA, the relative deviation was 5-573% (median 79%) for non-adjusted biomarker levels, 5-91% (median 49%) using creatinine content adjustment and 7-246% (median 58%) when using density adjustment. With or without adjustments, there were no systematic differences between biomarker levels in U1 compared to U2, neither for NDI or MDI.

DISCUSSION

In our study we have studied the correlations between ambient whole-day air exposure on one hand, and urine and plasma biomarkers on the other, for NDI and MDI. The correlations were weak, but still significant results were obtained. Biological monitoring of NDI has previously not been reported.

We assessed the whole-day air exposure concomitant to collection of biological samples. The strategy for urinary sampling was based on previous knowledge of the elimination pattern for urinary metabolites of toluene diisocyanates (TDI), assuming similar toxicokinetics for NDI and MDI metabolites. For urinary biomarkers of TDI, a large within-, as well as between-day variation has been observed for individual workers (Persson et al 1993, Lind et al 1996b), reflecting variation in the daily exposure. Also, the urinary elimination half-life of biomarkers derived from recent exposure to TDI is about 2-8 h (Skarping et al 1991, Brorson et al 1991, Lind et al 1996b). According to Droz et al (1991), such biomarkers should mainly reflect the exposure during the day of sampling. Thus, by pooling the sampling during the last four hours of a work shift, the within-day variation of biomarker levels should be smoothed out and mainly reflect the recent exposure. Also, by sampling of urine on two different days, the relative deviation in biomarker levels could be regarded as an estimation of the between-day variation of exposure. On the other hand, the urinary levels are also believed to be a measure of long-term exposure to isocyanates, as they depend on the elimination of protein adducts. This is supported by a high correlation between urinary and plasma biomarker levels found by Sennbro et al (2003). The strategy for blood sampling was less strict, since the biomarkers in plasma are protein adducts with long elimination half-lives (approximately 20 days).

As presented in table 3 and in figures 1-2, there were weak but significant correlations between the biomarker levels and the air exposure. For MDI, the biomarkers in the U1-samples correlated to a much higher degree to the air levels as compared to the biomarkers in the U2-samples. However, figure 2 shows that there was a large variation and the biomarker levels are not easily interpreted. For MDI, there was no clear indication that any adjustment of the urinary levels would improve the correlation against the air exposure. Rather, the correlation coefficients were decreased (table 3). Hence, we concluded that making any adjustments would only add another source of uncertainty and used the non-adjusted urinary levels in the evaluations. This is in congruence with a similar study of TDI exposed workers (Sennbro et al 2004b). Both our results and a previous study by Kääriä et al (2001) indicate a poor correlation between airborne exposure to MDI and urinary biomarker levels. This observation could be due to significant dermal uptake of MDA originating from exposure to MDI or MDI-related substances (Brunmark et al 1995, Frick et al 2003). Also, occupationally unexposed subjects have been shown to have significant U-MDA levels, indicating non-occupational background exposure (Sennbro et al 2005). Dermal uptake in combination with this unknown source of exposure may obscure the air exposure-biomarker correlation.

The correlations for NDI differed from the corresponding observations for MDI. A-NDI was better correlated to U2-NDA than to U1-NDA. It appears that the correlation coefficients are much depending on the worker with the highest air exposure to NDI, who has rather low U1-NDA but higher U2-NDA. The cause of this is hard to elucidate, but may be due to errors in sampling or analysis, and could perhaps be regarded as an outlier in a small study like ours. Furthermore, the correlations for NDI were increased both by adjustment for creatinine content and urinary density. This was rather surprising, and the explanation is far from obvious. It may be proposed that the NDI metabolites are of different nature having longer elimination half-lives and subjected to different excretion mechanisms. If the NDI biomarkers

have longer elimination half-lives than expected, previous days or even weeks of exposure may influence the biomarker levels, and may lead to obscure correlations in our study design. Also, the number of observations is few, and the finding may be opportunistic. Both A-MDI and A-NDI were also correlated, even though poorly, to their corresponding plasma biomarkers. The plasma biomarkers have previously been shown to be exclusively related to hydrolysed protein adducts (Sennbro et al 2003).

When using the NDI and MDI biomarkers in exposure assessment, our recommendation is to use both urinary and plasma samples and repeated sampling. By using plasma instead of urinary biomarkers, the discussion of using adjustments or not, is avoided. Still, the urinary sampling is more convenient and the plasma biomarkers cannot be used to assess the 8-h TWA exposure, since these biomarkers reflects a longer exposure time scale. Since the correlation between the biomarker levels and the air exposure is poor, the biomarker levels cannot be compared to an occupational exposure limit for air exposure. Rather, they may be used as relative measures of exposure on the individual or group level. In a recent study (Sennbro et al., 2005), upper reference limits were calculated for these biomarkers, i.e. the biomarker level above which a person is classified as exposed. In exposure assessment, it may be useful to compare biomarker levels with these upper reference limits, in order to screen for exposed subjects.

In this study we have shown that biomarkers of MDI and NDI are correlated to air exposure levels. These results can support further development of methods for biological monitoring of NDI and MDI in occupational exposure assessment. Biological monitoring of NDI has previously not been reported.

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TABLES

Table 1. The biological samples obtained from the exposed workers. U1 = urinary samples obtained at the day of air monitoring. U2 and P denotes the urinary and plasma samples, respectively, obtained in connection with a medical examination performed within two weeks from the day of air monitoring. NDI = 1,5-naphthalene diisocyanate and MDI = 4,4'-methylenediphenyl diisocyanate (MDI).

Workers	Biological samples				
	U1, U2, P	U1, U2	U1, P	U2, P	Only U1
MDI-Exposed (n=15)	14	-	1	-	-
NDI-Exposed (n=12)	5	1	1	-	5
NDI- and MDI-exposed (n=3)	3	-	-	-	-

Table 2. The levels of biomarkers of NDI and MDI in biological samples obtained from the exposed workers. The limit of detection was 0.05 µg/L for MDA and 0.1 µg/L for NDA. U1 = urinary samples obtained at the day of air monitoring. U2 and P denote the urinary and plasma samples, respectively, obtained in connection with a medical examination performed within two weeks from the day of air monitoring. NDA = 1,5-naphthalenediamine, MDA = 4,4'-methylenedianiline.

	Exposed workers		
Biomarker	N	Range (µg/L)	Median (µg/L)
U1-MDA	18	0.4 – 38	2
U2-MDA	17	0.3 – 78	2
P-MDA	18	0.2 –74	0.7
U1-NDA	15	0.7 – 81	8.4
U2-NDA	9	3 –81	8
P-NDA	9	4.7 – 59	20

Table 3. The Pearson correlation coefficients (r) and Spearman's rank coefficients for (r_s) the associations between the personal air and biomarker levels of NDI and MDI, respectively, for the exposed workers. U1 = urinary samples obtained at the day of air monitoring. U2 and P denote the urinary and plasma samples, respectively, obtained in connection with a medical examination performed within two weeks from the day of air monitoring. NDA = 1,5-naphthalenediamine, MDA = 4,4'-methylenedianiline.

Exposure	Biomarker	N	Correlation coefficients [a]					
			Unadjusted		Creatinine adjusted		Density adjusted	
			r	r_s	R	r_s	r	r_s
A-MDI	U1-MDA	18	0.51*	0.65**	0.55*	0.60**	0.57*	0.63**
	U2-MDA	17	0.46	0.63**	0.38	0.60*	0.47	0.62**
	P-MDA	18	0.64**	0.51*				
A-NDI	U1-NDA	15	0.38	0.37	0.82**	0.53*	0.62*	0.56*
	U2-NDA	9	0.89**	0.63	0.96**	0.95**	0.89**	0.87**
	P-NDA	9	0.87**	0.72*				

[a] The correlations without asterisks are not significant ($p > 0.05$).

* The correlation is significant at the 0.05 level ($p < 0.05$).

** The correlation is significant at the 0.01 level ($p < 0.01$).

LEGENDS

Figure 1. The association between biomarker versus air levels for workers exposed to 1,5-naphthalene diisocyanate (NDI). All urinary samples were collected as one pooled sample for each individual during the last four hours of the work shift. A-NDI = the personal 8 h TWA air exposure, U1-NDA = biomarker level in urinary sample collected on the same day as the air monitoring, P-NDA = biomarker level in plasma sample collected within two weeks from the day of air monitoring, U2-NDA = biomarker level in urinary sample collected on the same day as the plasma sampling.

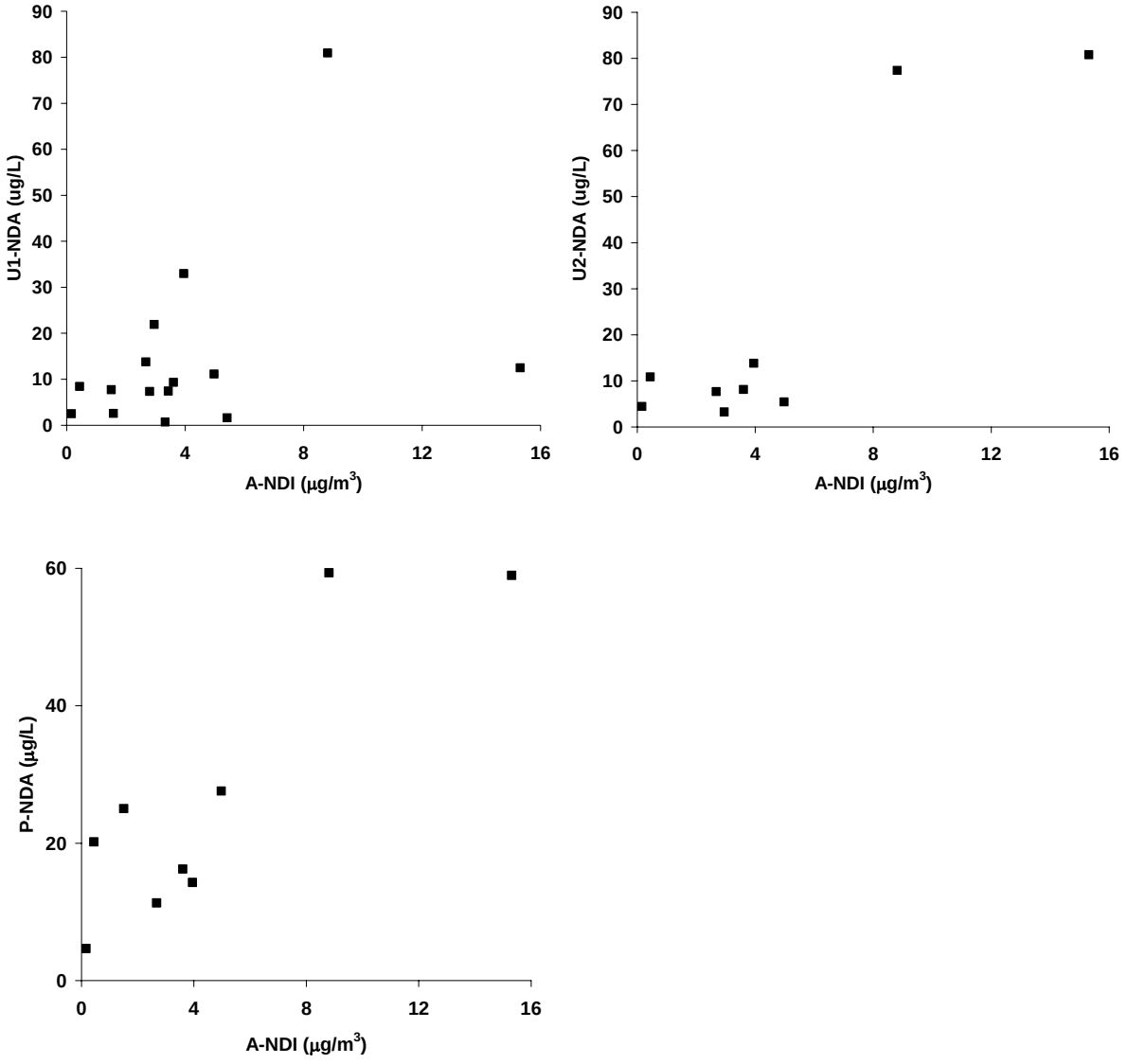

Figure 2. The association between biomarker versus air levels for workers exposed to 4,4'-methylenediphenyl diisocyanate (MDI). All urinary samples were collected as one pooled sample for each individual during the last four hours of the work shift. A-MDI = the personal 8 h TWA air exposure, U1-MDA = biomarker level in urinary sample collected on the same day that the air monitoring, P-MDA = biomarker level in plasma sample collected within two weeks from the day of air monitoring, U2-MDA = biomarker level in urinary sample collected on the same day as the plasma sampling.