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Biomonitoring of 2,4'–methylene diphenyldianiline for assessment of exposure to methylene diphenyl diisocyanate aerosol

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ABSTRACT

The exposure to methylene diphenyl diisocyanate (MDI) aerosol was investigated by biomnitoring of 2,4'–methylene diphenyldianiline (MDA) in urines of exposed workers. Biological monitoring was done for its metabolite by the analysis of isocyanate–derived diamines released from protein adducts in urine or plasma by GC–MS. The urine samples, at the end of working shifts of polyurethane factory, were collected in polystyrene bottles containing 10 g citric acid, and stored at 4 °C until analysis. The mean concentration values of MDA in the five selected factories were in the range of 3.01 to 3.58 µmol/mol creatinine for all subjects and the highest mean value of MDI concentration was 99 µg/m³ from indoor air samples analysis. The results show a linear relationship between MDI and MDA concentrations with a value of R^2=0.801 (P<0.05). This study demonstrates that not only urinary MDA is detectable following diisocyanates aerosol exposure but also it is likely to be a useful practical biomarker to monitor diisocyanates exposure in the workplaces or for the epidemiologic studies.

Keywords: *Biomonitoring, isocyanates, exposure, pollution, workers*

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1. Introduction

Metabolites of isocyanate are known as biomarkers of exposure to isocyanates (Brorson et al., 1990; Dalene et al., 1990; Dalene et al., 1994; Brunmark et al., 1995; Skarping and Dalene, 1995; Skarping et al., 1995; Lind et al., 1996; Tinnerberg et al., 1996; Lind et al., 1997; Tinnerberg et al., 1997; Littorin et al., 2000; Littorin et al., 2002). 2.4'–methylene diphenyldianiline (MDA) is an industrial chemical metabolite of diisocyanates that is known not occurring naturally. It is also commonly known as diamino diphenyl methane or MDA. It occurs as a colorless to pale yellow solid and has a faint odor (NIOSH, 2005). Sampling and measuring diisocyanates in urine or blood plasma is called "biomonitoring" (NIOSH, 2005). As a result of well–conducted biomonitoring studies, Brunmark et al. (1995) have obtained a picture of the amount of a MDI actually absorbed into human body. In the current study MDA is reported as "µmol/mol creatinine" in urine. Its concentration in urine sample above 1.0 µmol/mol creatinine indicates that the worker is exposed to MDI in the polyurethane factory (Brown et al., 1984; Williams et al., 1999).

The isocynates can induce cancer risk in animals but the carcinogenic potency is weak. The researchers have reported a kind of adrenal medulla and tumor of pancreas in methyl isocynate exposed animals (Senthilkumar et al., 2012). However, no work is available for its carcinogenicity in human beings.

Rosenberg et al. (2002) evaluated the use of MDA as a biomarker to assess human exposure to MDI aerosol. Those researchers found that urinary MDA can assess acute respiratory exposure to MDI aerosol, but may have limited use as a biomarker of exposure in the workplace. Williams et al. (1999) introduced a method for the measurement of a metabolite of MDI and used it to assess the exposure of sprayers employed in motor vehicle repair shops. They concluded that biological monitoring can provide a useful additional tool to assess diisocyanates exposure and the adequacy of controls for the exposed workers.

A study was conducted by Geens et al. (2012) to evaluate the correlation between the exposure to total TDI (2,4– and 2,6–TDI) in air and the corresponding biomarker concentration of total TDA (2,4– and 2,6–TDA) in hydrolyzed urine. They reported strong correlation between the two (R^2=0.816). Reeb–Whitaker et al. (2012) reported that short–term exposure level (STELs) is harmful for exposed workers and personal respiratory equipments (PRE) are needed to protect the workers.

In the current study, biomonitoring assessment was performed on selected exposed workers out the 500 workers. Fifty exposed workers were selected for this research. The selection criterions in biomonitoring assessment were: (i) workers were exposed to the diisocyanate, (ii) exposure was assessed based on NIOSH (1994) guidelines, and (iii) biological monitoring was included. The aim of this study was to find a relationship between biological sampling and air pollution concentrations of isocynates in polyurethane factories.

2. Materials and Methods

In this work, 50 exposed workers (in an age range of 20–50 years) from 5 factories were selected and they participated in this study. Their urine samples were collected and analyzed for the presence of MDA using the analytical method by Williams et al. (1999). Samples were grouped as M_1, M_2, M_3, M_4 and M_5 on the basis of 5 factories they were collected from. The study period was from November 2011 to September 2012 and was non–continuous. Subjects provided urine samples on the day of working, at the end of the shift day (it repeated three times for accuracy in measurements). They recorded the amount of time spent for working. Due to the short half life (about 1.5–3 h) of MDA in urine, samples were collected at the end of the shift to detect any short–term exposure as well as an estimation of the 8 h time weighted average exposure (Brown et al., 1984). Workers took a shower and changed their clothes and then gave their urine samples in the factory's medical service center. All samples were collected in polystyrene bottles containing 10 g citric acid and stored at 4 °C until analysis.

2.1. Technical methods

Air samples were collected according to NIOSH 5522 method where six steps were involved: collection, derivatization, sample preparation, separation, identification, and quantification (NIOSH, 1994). A generalized isocyanate sampler (mini personal sampler pump, SIBATA MP302 and midget impinger) was used to collect methylene diphenyl diisocyanate on aerosols and in vapor-phase. Mechanisms of collection of isocyanate vapors include dissolution into a solvent (e.g., impingers) or adsorption onto a sorbent.

The urine samples were collected and dispatched in a similar way, frozen and sent blind to the laboratory for analysis. Based on William's method, subjects provided urine samples on the working day at the end of the working shift. The time of exposure and the personal protective equipment worn were recorded. The samples obtained were labeled with code numbers. All the chemicals were of analytical reagent grade and purchased from Aldrich, UK.

Analytical procedure. Water was purified through a Millipore Milli–Q system. An aliquot of HpDA solution (internal standard) was added to the sample urine (2 mL). Concentrated sulfuric acid (200 µL) was then added. Samples were mixed thoroughly and then hydrolyzed at 100 °C for 90 minutes. Samples were then cooled and 2 mL sodium hydroxide solution (10 M) was added. After hydrolysis, 4 mL diethyl ether was added and the samples were extracted for 20 minutes, and then centrifuged. About 3 mL of the organic layer was transferred and evaporated to dryness under a stream of nitrogen. Samples were then reconstituted in 500 µL toluene and derivatized with 50 µL HFBA at 55 °C for 1 hour. Samples were then cooled, evaporated to dryness, and reconstituted in 100 µl toluene. The samples were analyzed by GC–MS with negative ion chemical ionization (with methane as the reagent gas).

Samples (1 µL, splitless) were injected (at 350 °C) onto a BP–5, fused silica capillary column (1 µm film thickness). The oven temperature was ramped from 150 °C (initially held for 1 minute) to 280 °C at 10 °C/min where it was held for 1.5 minutes. The interface temperature was 280 °C and the source was held at 200 °C. With selected ion monitoring, ion mass/charge ratio was monitored for MDA.

The concentration of MDA is reported as "µmol/mol creatinine". The guidance value of MDA level is 1.0 µmol/mol creatinine and values above this may indicate either no exposure or well–controlled exposure.

2.2. Statistical analysis

The sample size required to produce an estimate of the total number of potentially exposed workers within specified limits, with 95% confidence, was calculated using the formula:

$$n \geq N \left[1 + \frac{1}{N}\left(\frac{d}{1.96\,S}\right)^2\right]^{-1} \quad (1)$$

where n is the required sample size, N is the total number of subjects for sampling or workers and S is the standard deviation across workplaces. The margin of error "d" is estimated with plus or minus. Calculation of sample size for the current study depended on a number of assumptions. The statistical methodology of this study is similar to that of Christensen (1997).

3. Results

The biomonitoring assessment of isocyanates exposed workers, grouped as M_1, M_2, M_3, M_4 and M_5, was done along with assessment of air samples of the five factories. The highest mean value of MDI concentration was 99 µg/m³ from indoor air samples. The maximum concentration of MDA measured from worker's urine in the polyurethane factories was 4.0 µmol/mol creatinine and the mean value of the 5 groups was in the range of 3.01 to 3.58 µmol/mol creatinine for all factories (Table 1). The results of urine analysis indicated high exposure with respect to MDI, because of the lowest concentration of MDA in their urine was 2.0 µmol/mol creatinine. This value was higher than the guideline value (1.0 µmol/mol creatinine). Figure 1 shows the statistical summary of MDA concentrations in different factories.

Table 1. *Concentration of MDA (µmol/mol creatinine) among polyurethane workers (n=50)*

Workers in Factory	Mean	SD	Minimum	Maximum
M_1	3.30	0.67	2.0	4.0
M_2	3.27	0.42	3.0	4.0
M_3	3.23	0.54	2.5	4.0
M_4	3.20	0.54	2.0	4.0
M_5	3.15	0.72	2.0	4.0

Figure 1 shows the box plots of the sampling results for exposed workers (50 selected workers from 5 factories, 10 per factory). There are 2 cases with short tails and 3 cases with long tails; the length of some whiskers are shorter than the length of the box. Three boxes show extremely short tails (with a dip in the middle rather than a hump) and the whiskers are absent. It shows that in three groups the measured samples are close to median of concentration.

Several studies stated that the peak excretion of isocyanates metabolite occurs at the end of exposure and the initial elimination half–life is 2 h for MDI. This suggests that urine sample collection for biological monitoring should be done at the end of exposure (Brorson et al., 1990; Brunmark et al., 1995; Tinnerberg and Sennbro, 2005). Since the urinary half–life is about 2 h, the results reflected the levels after 2–4 h exposure. In order to assess the MDI concentration in the workstation, the air samples were collected from the personal breathing zone.

Figure 2 illustrates the relationship between air MDI concentrations and urinary MDA concentrations. Similar results from 50 samples are overlapped and located into 16 points in the graph. A positive and linear relationship between workplace air pollutant and metabolite urine concentrations was obtained. No background MDA was detected in the workers and the relationship validates MDA as an initial indicator of a preceding exposure of workers to MDI with R^2=0.801.