La Medicina del Lavoro

Med Lav 2012; *103*, 5: 361-371

# Biological monitoring as a valid tool to assess occupational exposure to mixtures of 2,4-:2,6-toluene diisocyanate

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**Key words**

2,4-:2,6-toluene diisocyanate mixtures; 2,4- and 2,6-toluenediamine; air monitoring; biological monitoring

**Parole chiave**

Miscele di 2,4-:2,6-toluenediisocianato; 2,4- e 2,6-toluendiamina; monitoraggio ambientale; monitoraggio biologico

**Summary**

**Background and Objectives:** *Despite its advantages over environmental monitoring, biological monitoring of exposure to 2,4-:2,6-toluene diisocyanate (TDI) mixtures is still underused. The present study was designed in order to evaluate the feasibility and reliability of biological monitoring in a factory producing polyurethane foam blocks.* **Methods:** *Airborne TDI isomers were sampled by both static and personal pumps and determined by HPLC with fluorimetric detection. Specific metabolites 2,4- and 2,6-toluenediamine (TDA) were determined by gas chromatography-mass spectrometry on hydrolysed urine samples collected from 16 workers at the beginning of the workweek and both before (BS) and at the end (ES) of the 4th workday. Additional samples were collected at the end of the 1st half-shift and at the beginning of the 2nd half-shift in 5 workers.* **Results:** *In the foam production shop, TDI values were on average about 20 μg/m³, with higher levels in the 2nd half-shift and peak levels in workers operating along the polymerization tunnel. Average TDI levels were significantly correlated with ES TDA concentrations (p<0.0001). TDA showed a fast urinary elimination phase leading to progressively higher TDA levels either during the shift (5 workers) and at the end-of-shift. A slower elimination phase with a weekly accumulation was demonstrated by values at the beginning of the workweek (higher than in unexposed subjects) and by their elevation in subsequent BS samples.* **Conclusions:** *The study demonstrates the feasibility and reliability of biological monitoring in workers exposed to 2,4-:2,6-TDI mixtures. This approach can provide information about both the daily and weekly exposure levels.*

**Riassunto**

***«Validità del monitoraggio biologico per la valutazione dell'esposizione professionale a miscele di 2,4-:2,6-toluendiisocianato».*** **Introduzione e Obiettivi:** *Rispetto al monitoraggio ambientale, il monitoraggio biologico dell'e-*

Pervenuto il 4.3.2012 - Accettato il 27.6.2012

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Early results were presented as a Poster at the 74th SIMLII National Congress, Turin, Italy, 16-19 November 2011

*sposizione professionale a miscele di 2,4-:2,6- toluendiisocianato (TDI) è ancora scarsamente utilizzato. Il presente studio è stato condotto in una fabbrica di poliuretano espanso, con lo scopo di verificarne fattibilità e validità.* **Metodi:** *I livelli aerodispersi di TDI campionati con postazioni fisse e con campionatori personali sono stati determinati in HPLC con rivelazione fluorimetrica. I metaboliti specifici 2,4- e 2,6-toluendiamina (TDA) sono stati determinati in gas-cromatografia accoppiata alla spettrometria di massa, dopo idrolisi dei campioni urinari raccolti da 16 lavoratori a inizio turno (IT) inizio settimana lavorativa e ad IT e fine turno (FT) del 4° giorno lavorativo. Su cinque lavoratori, le urine sono state raccolte anche alla fine del 1° emiturno ed all'inizio del 2°.* **Risultati:** *In produzione, i livelli di TDI erano mediamente pari a circa 20 μg/m³, con valori maggiori nel 2° emiturno, rispetto al 1° e picchi di esposizione negli addetti al tunnel di polimerizzazione. I livelli di TDI erano significativamente correlati con le concentrazioni urinarie di TDA FT ($p<0.0001$). L'eliminazione urinaria della TDA era difasica, con una fase rapida dimostrata dai livelli progressivamente crescenti durante il turno e a FT, rispetto ai valori IT. La fase di eliminazione più lenta era dimostrata dai valori di inizio settimana lavorativa, superiori a quelli riscontrati in soggetti non esposti e dall'aumento significativo dei livelli di IT durante la settimana.* **Conclusioni:** *I risultati confermano la validità del monitoraggio biologico dell'esposizione professionale a miscele 2,4-:2,6-TDI, i cui risultati possono fornire informazioni sui livelli di esposizione giornalieri e settimanali.*

## Introduction

Isocyanates are a group of low molecular weight alifatic or aromatic compounds bearing a –N=C=O group that display high reactivity towards either hydroxyl groups, forming urethane linkages, or amine groups giving rise to ureas and polyureas. Polyurethane (PU) polymers, obtained by reaction of isocyanates with polyols, include rigid and flexible PU foams, insulation materials, thermoplastic elastomers, two-pack spray paints, surface coatings, floor mastics, seals, fillers and adhesives (22). Toluene diisocyanate (TDI) is mainly used in the PU industry as an isomeric mixture of 2,4-TDI and 2,6-TDI in ratios of 80:20 and 65:35. Recent estimates demonstrate an annual worldwide consumption of about 1.9 million tons of TDI (18) to produce around 15.9 million tons of PU. Approximately a quarter of a million workers are involved in the global PU manufacturing industry (1). Occupational exposure to TDI may be associated with acute or chronic adverse health effects, that may be irritative-inflammatory on exposed mucosae (respiratory, ocular) and skin but also allergic effects by specific sensitization, leading to asthma, contact dermatitis and hypersensitivity pneumonitis (4). Occupational isocyanate asthma displays a prevalence up to 10% among workers exposed to TDI (12). The toxicological mechanisms of isocyanate sensitization have not yet been fully elucidated, but the haptenization of nucleophile groups (SH, $NH_2$, NH and OH) of biological macromolecules by the highly reactive electrophilic –NCO group is believed to play a key role in this process (21). Among workers exposed to TDI, adducts to plasma albumin have been demonstrated (24). Relying on experimental animal studies, TDI was classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (Group 2B), with inadequate evidence for carcinogenicity in humans and limited evidence for carcinogenicity in experimental animals (19). The European Union classifies the compound as a class 3 carcinogen assigning the risk phrase R40 (13). Occupational exposure occurs mainly by inhalation of monomers during preparation of mixtures, mixture pouring and polymerization but also by percutaneous absorption in cases of skin contamination (3, 14, 15, 27). In 2006, the American Conference of Governmental Industrial Hygienists (ACGIH) proposed a "notice of intended change" of the occupational standards for TDI isomers, with aims to lower both the threshold limit value 8-h time weighted average (TLV-TWA®) and the threshold limit value-short term exposure limit (TLV-STEL®) (values established in 1983) from 36 to 7 μg/m³ and from 140 to 21 μg/m³, re-

spectively and to include the "skin" notation, that underscores the possible contribution of percutaneous absorption to the internal dose (2). Although, in this scenario, biological monitoring would be a necessary complementary tool to assess the internal dose of TDI exposed workers, a biological exposure index (BEI®) has not yet been produced. Biological monitoring of TDI exposure has been available for many years (32, 33) and is based on the determination of the specific metabolites 2,4- and 2,6-toluendiammine (2,4-TDA e 2,6-TDA) released by hydrolysis of protein adducts in plasma or urine, the latter being the most suitable approach for assessing exposure in occupational settings. The Deutsche Forschungsgemeinschaft (DFG) established either a Maximum Allowable Concentration (Maximale Arbeitsplatzkonzentrationen – MAK®) and a corresponding biological limit value (Biologischer Leit Wert, BLW®) for workers exposed to 4-4'-methylene diphenyl diisocyanate (4-4'-MDI) but no airborne or biological limit has been defined for 2,4- and 2,6-TDI (11). The UK Health and Safety Executive (HSE) established for all isocyanates, as -NCO groups, a workplace exposure limit (WEL) of 20 μg/m$^3$ as 8h-TWA and 70 μg/m$^3$ as 15'-STEL (16) and a biological monitoring guidance value (BMGV) of 1 μmol/mol creatinine (creat.) (17). The -NCO mass approach directly measures the -NCO content of a sample, expressed as the mass of total -NCO groups (as μg NCO/m$^3$). In the case of TDI, the given figures correspond to about 41 (8h-TWA) and 145 μg/m$^3$ (15'-STEL) and to 1.08 μg/g creat (BMGV), respectively (5, 6).

The present study was performed with the main aim of evaluating biological monitoring as a complementary valuable tool for exposure assessment in workers exposed to 2,4-:2,6-TDI mixtures. To that end, we designed a cross-sectional study and collected urine samples at the beginning of the workweek (WWB) and at both the beginning (BS) and the end-of-shift (ES) of the fourth workday. On the same day, air monitoring was performed and two additional urine samples were collected at the end of the first half-shift (EFHS) and at the beginning of the second half-shift (BSHS) from a subgroup of workers.

## Methods

### Production cycle and subjects

The study took place in January 2011 in a plant producing flexible PU blocks. Production was programmed on 5 working days, of 8 hours each, with a daily production of two main foam blocks, respectively in the morning (1$^{st}$ half-shift) and in the afternoon (2$^{nd}$ half-shift). On the day of environmental sampling (4$^{th}$ workday), both classical 2,4-:2,6-TDI mixtures were used (80:20 and 65:35), the relative proportions being modified during production according to the characteristics of the desired final product.

On the pouring platform, the reagents (polyol, the 2,4-:2,6-TDI mixture, catalysts and blowing agents) contained in tanks located under the platform are mixed and poured via a foaming nozzle onto a carrier paper on a moving conveyor, giving rise to a foam slab. The conveyor draws the expanding foam into a long aspirated tunnel, where the polymerization reaction takes place. Along the tunnel, a worker adjusts the height of the metal plates which control the expansion of the polymerizing foam, whereas other workers periodically open the tunnel windows to check the status of the process or to drain samples for quality controls. They also go into the tunnel in case of technical problems or at the end of the process, to clean the tunnel from residues with compressed air. During the survey, four workers leaned out into the tunnel at least once. At the end of the tunnel, when the foam has stabilized and risen to the right height, the foam blocks are sawn into their appropriate lengths. Hereafter, the blocks are stored into a racking system for at least 24 h to cool down and to complete the curing process. Subsequently, blocks are conveyed to a sawing facility to be cut into thinner bars, and then to the warehouse. Next to the foam production area but separate from it, there is a laboratory dedicated to quality control and research and development activities. The enrolled subjects were sixteen workers (15 males), mean age 39.5±7.6 years (range 25-53), whose information about socio-demographic characteristics and lifestyle habits was collected by a questionnaire

**Table 1** - Socio-demographic and lifestyle characteristics of enrolled subjects

| Variables | No. | Mean±SD (range) |
|---|---|---|
| Age | | 39.5±7.6 (25-53) |
| Gender (males/females) | 15/1 | |
| Body Mass Index (Kg/m$^2$) | | 23.7±3.8 (19.7-34.4) |
| Current smokers (Yes/No) | 7/9 | |
| Cigarettes/day | | 19±6 (10-30) |

administered by a physician specialized in occupational health and resumed in table 1. Over the monitoring period, most of the workers (No.=11) were engaged in the foam production shop, distributed at the pouring platform (No.=4), along the tunnel (No.=4), at the exit from the tunnel (No.=1), and at the mixtures (No.=2). The remaining subjects worked in the laboratory (No.=2), the warehouse (No.=1), the sawing facility (No.=1) and in both the office and production (No=1). All subjects worked every day of the week at the same job. All workers wore appropriate work clothing and safety shoes during the shift. Respiratory protection (half-mask with filters A2P2) was used for short periods. Nitrile or vinyl gloves were worn by most of the workers when handling hot blocks of uncured foam. Informed consent to participate in the study was obtained from each subject, who received an explanation of the purpose and procedure of the study.

## Air monitoring

Both sampling and analysis of airborne TDI were performed according to OSHA method No. 42 (29). In the 4$^{th}$ workday, 4 static and 10 personal samplings were undertaken, the latter on workers on the foam block (No.=8) and on workers in both sawing shop and warehouse (No.=1 each). Samples were collected by drawing a known volume of air at a constant flow (1 L/min) through glass fibre filters coated with 1 mg of 1-(2-pyridyl)piperazine (1-2PP) (ORBO-80, 37 mm diameter, Supelco, Bellefonte, PA, USA) as a derivatizing reagent, required to stabilize the highly reactive 2,4- and 2,6-TDI. Filters were mounted into three-piece styrene open-face cassettes. Personal sampling on foam production workers was repeated twice (once per half-shift), about 180 minutes each. Air samples were stored at 4°C until analysis. The filters were transferred into glass tubes and extracted with 4 mL of 90/10 $CH_3CN$/DMSO for 1 hour. The solution was analyzed by HPLC with fluorimetric detector (λ excitation 240 nm; λ emission 370 nm). The chromatographic separation of the TDI urea derivatives was performed on a Supelcosil LC-8 column (250 mm x 4.6 mm x 5 μm), the mobile phase being 38% $CH_3CN$ and 62% 0.01 M ammonium acetate, pH 6.2 regulated by adding acetic acid. The limit of quantification (LOQ) for TDI derivatives was 0.1 μg, corresponding to about 34.8 ng of each TDI isomer on the filter. The coefficient of variation (CV%) of the method was less than 5%.

## Biological monitoring

Three spot urine samples were collected from each subject, respectively before the beginning of the workweek (WWB) and before (BS) and at the end-of-shift (ES) of the 4$^{th}$ workday. On the same day, two additional samples were collected from 5 foam production workers, respectively at the end of the first half-shift (EFHS) and before the beginning of the second half-shift (BSHS). Urine samples were frozen at -20°C until the analysis, that was performed according to the method described by Sakai et al. (34), with little changes. A strong acid hydrolysis was carried out according to a published method (35). For the calibration curve, standard solutions of 2,4-TDA and 2,6-TDA in HCl 0.1 M were added to urine samples drawn from subjects not occupationally exposed to TDI. After thawing at r.t., each urine sample (2 mL) was incubated with HCl 6 M (3 mL) at 100°C, o.n.. After chilling, 2 mL of the hydrolyzed samples were made basic with NaOH 8 M (2 mL) and underwent a liquid-liquid extraction with methylene chloride (4 mL) containing 3,4-TDA as internal standard for 40 minutes. The organic phase was transferred into a new tube and after derivatization with heptafluorobutyric anhydride (50 μL) at 55°C for 1 h, the solution was dried under nitrogen flow

and the residue solubilized with toluene (80 μL) for chromatographic injection. Amide derivatives were determined by an Agilent Technologies GC 6890N gas-chromatograph interfaced with a mass detector 5973, with a capillary column DB-1 ms (30 m x 0.25 mm ID, film thickness 0.25 μm). One μL of sample was injected in the splitless mode at 280°C, under helium at a flow rate of 1.3 mL/min. The other temperatures were: interface 280°C; oven 100°C, with a ramp of 20°C/min up to 280°C (5 min). The specific ions m/z (mass/charge) 514.0 e 345.0 were chosen respectively as *quantifier* and *qualifier*. For interpretation of the data, the peak area of individual analyzed amines were divided by the peak areas of individual standards. Using this quotient, the amine concentrations were estimated with standard curves for each individual isocyanate-amine run in parallel. The limit of quantification (LOQ), calculated as the concentration corresponding to a peak with signal/noise ratio of 10, was 0.1 μg/L. The coefficients of intra-series daily variation (n = 6) of 2,6-TDA (10.8 μg/L) e 2,4-TDA (2.5 μg/L) were 2.0% and 1.6%, respectively. The medium percent recovery, calculated analyzing 6 aliquots of urine from an unexposed subject with additions of 5 μg/L for each isomer, was 102.3% for 2,6-TDA and 95.1% for 2,4-TDA. Concentrations of urinary metabolites were expressed as a function of creatinine concentration (μg/g creat.), that was measured by the Jaffe method (23). We adopted the exclusion criteria of the American Conference of Governmental Industrial Hygienists recommendation (2) for very diluted (creat. concentrations lower than 0.3 g/L) or very concentrated (creat. concentration higher than 3.0 g/L) urine samples (42).

### Statistical Analysis

Statistical analysis was performed using the PASW Statistics 18.0 for Windows® (IBM SPSS Inc; Chicago, IL, USA) statistical package. A value corresponding to half of the LOQ was attributed to undetectable environmental and biological samples. Both environmental and biological variables followed a normal distribution, as assessed by the Kolmogorov-Smirnov test. The paired sample *t* test was performed to evaluate differences of metabolites at different sampling times. Relationships between variables were investigated by both Pearson's correlation and the simple linear regression analysis.

## Results

The results of environmental monitoring are presented in table 2. The levels of both TDI isomers were higher in the foam production shop, compared to other areas, and higher in the 2nd, compared to the 1st half-shift, both in the static sampling at the pouring platform (median values of 20.15 μg/m$^3$ *vs.* 6.05 μg/m$^3$ for the isomer sum) and the personal samplings (median values of 30.89 *vs* 8.67 μg/m$^3$ for the isomer sum). In both cases, the airborne composition of the mixture was constant throughout the working day, the average proportion of 2,6-TDI being about 63% in the personal samplings of both the half-shifts and about 64% and 58%, respectively, in the static samplings of the 1st and the 2nd half-shift. Higher proportions (about 69%) were observed outside the tunnel, whereas in the laboratory the 2,4-TDI isomer represented about 60% of the total.

On average, subjects working along the tunnel were exposed to the highest values, in particular two workers (daily exposure of 46.67 and 64.96 μg/m$^3$) that entered the tunnel for several minutes at the end of each half-shift. Much lower exposure values were determined in the laboratory, whereas for subjects working in the warehouse and in the sawing facility exposure levels were below the LOQ, as in the external environment.

The results of biological monitoring are shown in table 3. No sample was discarded according to creatinine concentration. The 2,4- and 2,6-TDA metabolites resulted undetectable in WWB samples from 3 workers (19%) engaged outside the foam production shop. A worker in the sawing facility showed undetectable levels also in subsequent BS and ES samples. In agreement with environmental monitoring data, the highest TDA concentrations were observed in workers in the foam production shop, in particular workers whose task in-

**Table 2** - Results of environmental sampling of 2,4- and 2,6-toluene diisocyanate (TDI) (μg/m$^3$). For personal samplings, when more measurements were available for homogeneous jobs, values are expressed as medians (with ranges)

| Areas/Jobs (No.) | 2,4-TDI | | | 2,6-TDI | | | ∑ TDI |
|---|---|---|---|---|---|---|---|
| | 1st half-shift | 2nd half-shift | Whole shift | 1st half-shift | 2nd half-shift | Whole shift | Whole shift |
| **Static samplings** | | | | | | | |
| External environment (1) | | | <LOQ | | | <LOQ | <LOQ |
| *Foam production* | | | | | | | |
| • Pouring platform (1) | 2.16 | 8.49 | 5.11 | 3.89 | 11.66 | 7.50 | 12.61 |
| • Along the Tunnel (1) | | | 0.93 | | | 2.09 | 3.02 |
| *Outside production* | | | | | | | |
| •Laboratory (1) | | | 0.32 | | | 0.21 | 0.53 |
| **Personal samplings** | | | | | | | |
| *Foam production* (8) | 3.20 (0.25-13.62) | 11.18 (3.73-31.08) | 7.26 (3.32-18.86) | 5.51 (0.55-22.96) | 19.05 (7.87-72.29) | 12.50 (5.48-47.84) | 20.22 (9.04-64.96) |
| • Pouring platform (3) | 3.22 (3.09-3.43) | 11.50 (5.04-5.70) | 7.46 (5.78-9.63) | 5.32 (5.04-5.70) | 17.56 (11.69-20.54) | 11.60 (8.34-13.40) | 19.06 (14.12-23.03) |
| • Along the tunnel (4) | 4.77 (2.88-13.62) | 15.71 (3.73-31.08) | 12.09 (3.32-18.86) | 7.63 (3.37-22.96) | 34.23 (7.87-72.29) | 21.07 (5.72-47.84) | 34.03 (9.04-64.96) |
| • Mixtures (1) | 0.25 | 8.12 | 4.54 | 0.55 | 8.74 | 9.61 | 10.02 |
| *Outside production* | | | | | | | |
| • Warehouse (1) | | | <LOQ | | | <LOQ | <LOQ |
| • Sawing shop (1) | | | <LOQ | | | <LOQ | <LOQ |

LOQ: limit of quantification, corresponding to 34.8 ng of each TDI isomer on the filter

**Table 3** - Distributions (as means ± standard deviations) of the urinary concentrations of 2,4- and 2,6-toluenediamine (TDA) (μg/g creat.) and of their sum in workers classified according to jobs. Urine samples were collected at the beginning of the workweek (WWB) and at both beginning (BS) and end-of-shift (ES) of the fourth working day. Among workers engaged in "Other jobs", outside the foam production shop, five out of nine samples (56%) showed values below the LOQ, their distribution being 2/3 (66%) in both the WWB and BS samples and 1/3 (33%) in the ES sample, respectively

| Subjects/Jobs (No.) | WWB | | | BS | | | ES | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2,4-TDA | 2,6-TDA | ∑ TDA | 2,4-TDA | 2,6-TDA | ∑ TDA | 2,4-TDA | 2,6-TDA | ∑ TDA |
| **All workers (16)** | 0.58±0.56 | 1.37±1.40 | 1.94±1.94 | 0.88±0.76$^{\S}$ | 2.28±2.39$^{\S}$ | 3.16±3.12$^{\S}$ | 1.81±1.57$^{**\S\S}$ | 4.88±4.78$^{**\S\S}$ | 6.69±6.29$^{**\S\S}$ |
| **Foam production (11)** | 0.75±0.59 | 1.90±1.40 | 2.65±1.96 | 1.17±0.75$^{\S}$ | 3.16±2.42$^{\S}$ | 4.32±3.12$^{\S}$ | 2.39±1.56$^{**\S\S}$ | 6.80±4.60$^{**\S\S}$ | 9.19±6.09$^{**\S\S}$ |
| • Pouring platform (4) | 0.59±0.35 | 1.24±0.61 | 1.83±0.95 | 0.85±0.65 | 1.61±1.05 | 2.45±1.69 | 2.05±1.60 | 4.75±2.98 | 6.80±4.58 |
| • Along the tunnel (4) | 1.13±0.82 | 3.20±1.56 | 4.33±2.37 | 1.80±0.66 | 5.79±1.86 | 7.59±2.50 | 3.42±1.71$^{\S}$ | 14.48±6.52$^{\S}$ | 17.10±4.73$^{\S}$ |
| • [1]Other jobs (3) | 0.47±0.34 | 1.05±0.47 | 1.52±0.81 | 0.76±0.49 | 1.71±0.76 | 2.47±1.25 | 1.49±0.65$^{*\S}$ | 3.86±0.40$^{*\S\S\S}$ | 5.35±1.00$^{**\S\S}$ |
| **Outside production (5)** | 0.18±0.14 | 0.20±0.15 | 0.38±0.29 | 0.25±0.22 | 0.34±0.24 | 0.59±0.45 | 0.52±0.34$^{*\S}$ | 0.65±0.35$^{*\S}$ | 1.17±0.66$^{*\S}$ |
| • Laboratory (2) | 0.28±0.07 | 0.33±0.01 | 0.61±0.08 | 0.43±0.18 | 0.50±0.16 | 0.93±0.34 | 0.72±0.47 | 0.72±0.45 | 1.44±0.92 |
| • [2]Other jobs (3) | 0.12±0.14 | 0.12±0.14 | 0.23±0.28 | 0.12±0.15 | 0.24±0.24 | 0.36±0.39 | 0.38±0.23$^{*\S}$ | 0.61±0.37$^{*}$ | 0.99±0.58$^{*}$ |

$^{*}p<0.05$, $^{**}p<0.005$,$^{***}p<0.0001$, comparisons between before-shift and end-of-shift values;
$^{\S}p<0.05$, $^{\S\S}p<0.005$, $^{\S\S\S}p<0.0001$, comparisons between values determined at the beginning of the workweek and either before or at the end-of-shift of the 4$^{th}$ workday
[1] Duties include: tank filling and maintenance (No.=2), foam slab cuting (No.=1)
[2] Workers engaged in: sawing shop (No.=1), laboratory and office and production (No.=1), warehouse (No.=1)

**Table 4** - Pearson's correlation coefficients between environmental concentrations of 2,4- and 2,6-toluene diisocyanates (TDI) (μg/m³) and end-of shift levels of 2,4- and 2,6-toluenediamine (TDA) (μg/L) and creatinine (g/L).

| Variables | 2,4-TDI | 2,6-TDI | ∑ TDI | 2,4-TDA | 2,6-TDA | ∑ TDA |
|---|---|---|---|---|---|---|
| 2,6-TDI | 0.920*** | | | | | |
| ∑ TDI | 0.962*** | 0.992*** | | | | |
| 2,4-TDA | 0.770** | 0.875*** | 0.857*** | | | |
| 2,6-TDA | 0.814** | 0.934*** | 0.912*** | 0.966*** | | |
| ∑ TDA | 0.807** | 0.924*** | 0.903*** | 0.981*** | 0.998*** | |
| Creatinine | - | - | - | 0.593* | 0.511* | 0.535* |

volved entry into the tunnel or in any case the inhalation of air from the tunnel. The four workers opening the tunnel windows at least once during the shift (1 subject working at the pouring platform and 3 engaged in tasks along the tunnel) showed significantly higher ES TDA values compared to the others (means of 14.93±5.81 *vs.* 3.94±3.44 μg/g creat., p<0.0001). Consistent with air isomer proportions, 2,6-TDA was the prominent isomer (on average 74%, range 69%-85%) among workers of the foam production shop, whereas among laboratory workers both isomers showed similar concentrations.

The paired sample analysis in the whole sample and in workers of the foam production shop showed significantly higher values of both TDA isomers at both the sampling times of the 4th workday, compared to levels determined in the WWB sample. A significant rise in ES compared to BS levels was also apparent. Among workers engaged outside the foam production shop, the ES samples were significantly higher than both the BS and the WWB samples.

Table 4 summarizes the results of the Pearson's correlation analysis between environmental and biological determinations. Airborne concentrations of 2,4-and 2,6-TDI isomers and their sum were highly correlated each other and with the corresponding metabolites. TDA levels were also significantly correlated with those of urinary creatinine.

Figure 1 presents the results of linear regression analysis between airborne concentrations of the 2,4-:2,6-TDI mixture, as the sum of both isomers, and the sum of the related urinary metabolites determined in end-of-shift samples ($p$<0.0001). Figure 2 shows the distributions of biological monitoring values including intra-shift samplings in a group of production workers. A progressive increase in both 2,4- and 2,6-TDA concentrations was observed, the end-of-shift values being significantly higher ($p$<0.05) than those measured in previously collected samples. The BSHS TDA values were also significantly higher than the EFHS values ($p$<0.05).

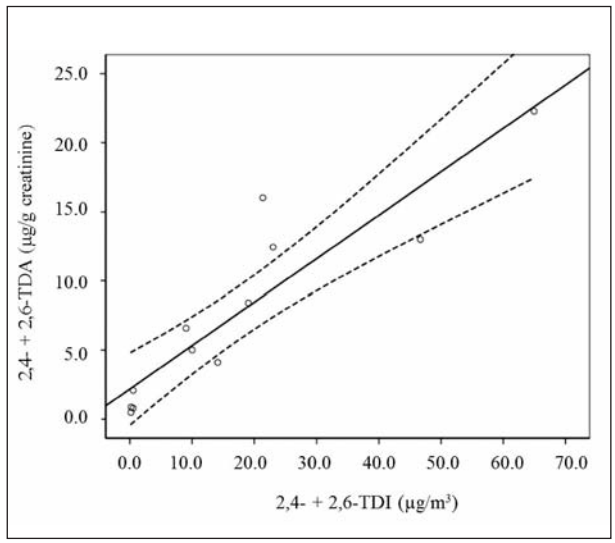


**Figure 1** - Significant positive relationship ($r^2$=0.829, $p$<0.0001) between airborne concentrations of the 2,4-:2,6-toluene diisocyanate (TDI) mixture and end-of-shift urinary levels of the sum of the respective 2,4- and 2,6-toluendiamines (TDA). The equation describing the linear regression line is: 2,4-+2,6-TDA=2.185 + 0.314 (2,4-+2,6-TDI). The 95% confidence intervals of the mean values are shown as dashed lines

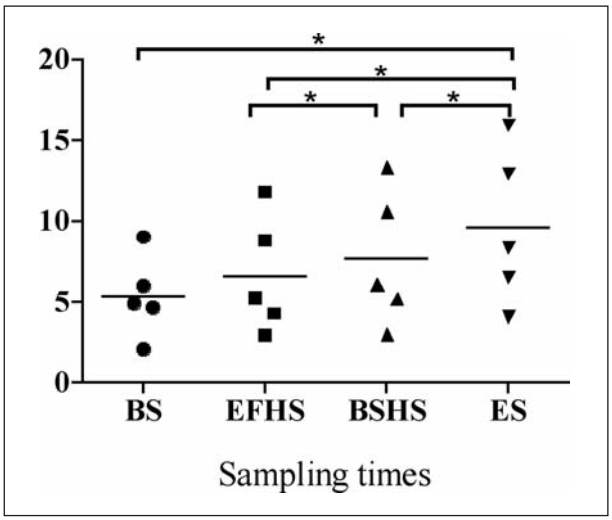


**Figure 2** - Distributions of the sum of the concentrations of 2,4- and 2,6-toluendiamines (∑ TDA) in five workers sampled at the beginning of the work-shift (BS), at the end of the first half-shift (EFHS), at the beginning of the second half-shift (BSHS) and the end of the shift (ES). Horizontal lines represent means of the distributions.
* $p<0.05$, paired sample comparisons between different sampling times.

## Discussion

Although fewer cases of isocyanate asthma are reported in occupational settings with controlled exposure levels (7, 30), the dose-response relationship for allergic outcomes remains poorly defined and the minimum dose necessary for immune sensitization remains unclear. Thus, occupational exposure to isocyanates requires periodical monitoring of exposure levels that should be kept as low as reasonably achievable. The most common way to control exposure for isocyanates is to perform air sampling with subsequent determination of the isocyanates in the laboratory. However, it is widely held that the collection and analysis of air samples for isocyanate monitoring requires considerable expertise and is prone to several drawbacks (28, 38, 41). The exposure levels are typically intermittent and companies using these compounds are often small or medium-sized enterprises, in which the production (and control) processes are only partly developed. Chemical formulations often need to be blended in-house, rather than being brought in. The mixing and pouring tasks together with cleaning operations result in extensive manual activities, creating a significant potential for exposure. In planning the environmental monitoring, we took into account the variability of exposure levels in the foam production area, including low-exposure preparatory phases, mid-exposure during the polymerization process and peaks of exposure if workers entered the tunnel or leaned out into the tunnel windows to check the process. A further variability source was represented by the organization of daily production into two foaming cycles, each lasting about a half-shift and potentially leading to different exposure levels. Thus, in order to achieve an air sampling representative of daily exposure, we planned two samplings in the foam production area, one for each half-shift. Actually, this strategy allowed us to demonstrate higher exposure levels in the 2nd half-shift, compared to the previous one. In the foam production shop, the daily exposure levels resulted on average about half the current TLV-TWA® (2) but looking at the half-shift samplings, four subjects (3 in the 2nd half-shift, 1 in the 1st half-shift) exceeded that limit, being exposed to values up to 92.85 $\mu g/m^3$. Their duties included tasks at the pouring platform (No.=1) or along the tunnel (No.=2). Actually, these subjects reported suffering from occasional ocular irritation. The exposure values in the laboratory were much lower and undetectable levels were recorded in the sawing facility and in the warehouse. Although the proportion of the 2,6-TDI isomer was less represented in the 2,4-:2,6-TDI mixtures, its airborne levels in the foam production shop constantly exceeded those of the 2,4-TDI isomer, with a certain variability in personal samplings (range 55%-74%), because of different tasks performed by workers. This discrepancy, observed also by others (3, 25) could be attributable to the higher reactivity of the 2,4-TDI isomer, leading to a faster decrease in its airborne concentrations, compared to 2,6-TDI. This hypothesis was indeed supported by both environmental and biological results. Both the static and personal sampling in the tunnel area, where polymerization takes place, showed the highest proportions of the 2,6-TDI isomer (about 69%). Biological monitoring showed a consistent gradi-

ent of increasing excretion of ES 2,6-TDA, along the foam production phases. Operatives working on the foam head at front end of the conveyor (pouring platform) excreted a relatively lower ratio of 2,6-TDA (about 70%, on average), than those working further down the conveyor (along the tunnel, about 85%, on average).

Biological monitoring of TDA metabolites in urine poses several advantages over air monitoring, including greater simplicity and lower costs. Biomarkers allow an estimation of the true internal dose resulting from both respiratory and cutaneous absorption routes and integrate inter-individual differences in anthropometric and metabolic characteristics. Moreover, they allow an evaluation of the effectiveness of the control measures implemented at the workplace, included wearing personal protective equipment (39). On the other hand, it is known that analytical results may be affected by the hydrolysis method, since for instance alkaline hydrolysis may release twice as much TDA compared to hydrochloric acid hydrolysis (26). Our method relied on the previous experience of several authors in this field (34, 35, 37, 26, 9).

In agreement with previous volunteer and occupational studies in the field (8, 20, 27, 31, 33), we found good relationships between airborne concentrations of TDI isomers and their hydrolysable adducts in urine. This would demonstrate that the main exposure route was by inhalation. Dermal contact was prevented, apart from wearing gloves, by a polyethylene film wrapping the foam slabs inside the tunnel. Respiratory protection (half-masks with A2P2 filters) were worn for a few minutes per shift by subjects working along the tunnel. Two workers entering the tunnel for cleaning operations at the end of each half-shift wore the protection for about 30 minutes overall. The data obtained are insufficient to evaluate the effectiveness of these devices in the breakthrough of TDI concentrations. The regression of the exposure-dose relationship (figure 1) was not substantially modified after removal of both the subjects from the analysis ($R^2$ changing from 0.829 to 0.835). The study design allowed us to draw some conclusions about the toxicokinetics of TDA. The significantly higher end-of-shift as compared to pre-shift concentrations on the 4$^{th}$ day and the significantly increasing levels during the same shift (additional samplings in a worker subgroup) were in agreement with a fast diphasic ($t_{1/2}$ of 1.6-1.9 h and 5 h) urinary elimination pattern demonstrated in volunteer studies (9, 37). However, the higher TDA values we observed at the beginning of the 4$^{th}$ workday, compared to values recorded at the beginning of the workweek, are indicative of metabolite accumulation that appear to be in agreement with a previous study on chronically exposed workers where half-lives of 6-10 days were demonstrated (25). Such data would support the hypothesis that the fast phase would be related to recent exposure, whereas the slower phase would be consistent with the release of TDA from plasma albumin adducts in the body and would be representative of longer exposure periods. This would also explain the baseline levels that were detectable in most of the workers, as evidenced also by others (40), with values higher than the upper reference limit of 0.1 μg/L (36). At the different sampling times in the foam production shop, TDA values constantly exceeded, on average, the UK HSE BMGV. However, it has to be considered that this limit is not health-based, since the BMGV corresponds to the 90$^{th}$ percentile of biological monitoring data from workplaces with very controlled exposure to isocyanates (17). Thus, it should be considered a sort of quality objective, more than a true limit value. The expected urinary TDA concentration in workers exposed to the UK WEL for TDI is in the range 10-20 μmol/mol creat, i.e. about 11-22 μg/g creat (20, 27). Hence, urinary TDA values below the BMGV are indicative of exposure levels below the WEL and also below the proposed TLV-TWA® of 7 μg/m$^3$.

In our study, the linear regression between daily airborne TDI levels and end-of-shift TDA concentrations, although obtained on a limited sample and assuming a linear trend, leads to estimate an ES TDA average concentration of 13.49 μg/g creat. for workers exposed to the TLV-TWA® (36 μg/m$^3$) of TDI. This estimation is comparable to that reported by Maitre et al. (27), who assessed a TDA excretion of 18 μg/g creat. for an airborne TDI exposure of 38 μg/m$^3$. Both such evaluations

are consistent also with a volunteer study in which subjects eliminated average urinary levels of 5 μg/L (2.8-9.6 μg/L) of 2,4-TDA and 8.6 μg/L (5.6-16.6 μg/L) of 2,6-TDA (37) after controlled exposure to TDI for 7.5 h (2,4-TDI 17-20 μg/m$^3$; 2,6-TDI 20-23 μg/m$^3$). Higher values were proposed by Sakai et al. (34) (33.8 μg/g creat. of 2,6-TDA).

The present study, although limited in size, shows a particular strength in the thorough characterization of airborne TDI concentrations and corresponding biomarkers. The study demonstrates the feasibility and reliability of biological monitoring as a valuable tool to characterize the internal dose of workers occupationally exposed to 2,4-: 2,6-TDI mixtures. The difference between end-of-shift and before-shift values determined at the end of the workweek would be indicative of the daily exposure, whereas the end-of-shift value would be indicative of the exposure over the entire workweek.

No potential conflict of interest relevant to this article was reported

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