

Urinary excretion of toluene diisocyanates in rats following dermal exposure

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Received 13 February 2007; Revised 10 April 2007; Accepted 10 April 2007

ABSTRACT: Toluene diisocyanates (TDI) are commonly used in polyurethane (PU)-related products. TDIs have been documented as the leading cause of occupational asthma. Skin exposure to TDI in the workplace is common. However, no studies in the literature have investigated the exact biomarker concentration profile for skin TDI absorption through any *in vivo* animal studies. In this study a rat model was used to evaluate the TDI skin absorption to explore the dose-response pattern and to determine the kinetic characteristics of urinary toluene diamine (U-TDA) during skin exposure. TDIs were topically exposed on the dorsum of rat skin at 0.2%, 1% and 5%. Consecutive urine samples were collected for 6 days and U-TDA were analysed using GC/ECD. It was demonstrated in this rat study that absorption of 2,4- and 2,6-TDI through skin contact is possible. A clear dose-dependent skin absorption relationship for 2,4- and 2,6-TDI was demonstrated by the AUC, C_{max} findings and accumulative amounts ($r \geq 0.968$). U-TDA concentration profiles in 6-day consecutive urine samples fit well in the first-order kinetics, although higher order kinetics could not be excluded for the high dose. The apparent half-lives for excretory urinary TDA were about 20 h consistent at various skin exposures. It is concluded that skin absorption of TDI was confirmed in a rat model and a clear dose-dependent skin absorption relationship for 2,4- and 2,6-TDI was demonstrated. Excretory U-TDA concentrations in 6-day consecutive urine samples via skin exposure reveal the first-order kinetics and the half-lives were about 20 h. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS: toluene diisocyanate; skin absorption; kinetics; toluene diamine; dermal exposure

Introduction

Toluene diisocyanates (TDI) are commonly used in polyurethane (PU)-related manufacturing processes, including PU foam, elastomers, coatings, paints and adhesives. Most of the TDI commercial forms in industry are a mixture of 80%:20% (w:w) 2,4- and 2,6-TDI (Timchalk *et al.*, 1994; Yeh *et al.*, 2002). Five to ten percent of the workers exposed to TDI will suffer from occupational asthma, which makes TDIs the leading cause (Baur, 1996; Bernstein, 1996; Deschamps *et al.*, 1998; Mapp *et al.*, 1988; Ott *et al.*, 2003; Wang *et al.*, 1998). Overexposure to TDI is also reportedly associated with elevated hypersensitivity pneumonitis and contact dermatitis (Wang *et al.*, 1998).

After exposure, TDIs will convert into toluene diamine (TDA) in humans and in animals (Brorson *et al.*, 1991; Dalene *et al.*, 1997; Kaaria *et al.*, 2001; Lind *et al.*, 1996; Maitre *et al.*, 1993; Persson *et al.*, 1993; Rosenberg and Savolainen, 1985; Saikai *et al.*, 2005; Skarping *et al.*, 1991; Tinnerberg *et al.*, 1997). Urinary TDAs (U-TDA) have been demonstrated as a good biomarker for TDI exposure. In the literature, humans were exposed to TDI, mainly through the respiratory tract. Quite a few studies have shown a satisfactory correlation between the concentrations of respiratory TDI and U-TDA (Brorson *et al.*, 1991; Dalene *et al.*, 1997; Kaaria *et al.*, 2001; Lind *et al.*, 1996; Maitre *et al.*, 1993; Persson *et al.*, 1993; Saikai *et al.*, 2005; Skarping *et al.*, 1991). However, several studies conducted in occupational settings were unable to locate any significant association between the airborne TDI exposure and the urinary TDA (Tinnerberg *et al.*, 1997). Exposure other than the respiratory route, e.g. skin exposure, therefore, could not be excluded.

Liu and colleagues (2000) conducted an investigation in auto body shops to determine if skin exposure to

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hexamethylene diisocyanate (HDI) is possible. Calorimetric skin pads were applied and attached on the forearms, hands and faces of paint workers and moderate to heavy HDI contamination on the skin surfaces was found. They concluded that skin exposure to isocyanates in the workplace could be very common. Moreover, the US Occupational Safety and Health Administration (OSHA, 1999) recommended skin pads for exposure monitoring in isocyanate-related occupational environments. Therefore, the information with respect to skin exposure in addition to respiratory exposure should not be missed to obtain a complete profile to estimate the total uptake of TDI exposure.

However, the aforementioned findings focused only on skin exposure. To the best of our knowledge, no studies in the literature have ever investigated the internal dose concentration profile for skin TDI absorption through an *in vivo* animal study. The aims of this study were to use a rat model to determine the skin absorption of TDI and to determine whether a dose-response pattern exists. Moreover, the kinetic parameters, including the apparent half-life and area under the curve (AUC), will also be investigated to facilitate an understanding of the uptake and elimination behaviors of TDI skin exposure.

Materials and Methods

Reagents

TDI (2,4-TDI: 2,6-TDI = 80%:20%, m:m, purity of 95%) was obtained from TCI (Tokyo, Japan) and heptafluorobutyric anhydride (HFBA, 98%) from Aldrich Chemical (St Louis, MO, USA), 2,6-TDA (97%) from Lancaster (Morecambe, England), H₂SO₄, NaOH, HCl and K₂HPO₄ from Merck (GR grade, Darmstadt, Germany), olive oil from Kanto (cica-reagent, Tokyo, Japan) and GC-grade toluene from Tedia (Fairfield, OH, USA).

Equipment

Previously published sample preparation and analysis methods were adopted to determine urinary TDA in this study (Yeh *et al.*, 2007). All GC analyses were performed using an Agilent Model 6890 GC equipped with μ -electron capture detectors (μ -ECD, Agilent G2397A). A DB-5MS (J&W Scientific, Folsom, CA, USA) (60 m $\times$ 0.25 mm i.d.) with a film thickness of 0.25 μ m fused silica capillary column was used to perform the separation. Ultra high-purity nitrogen (purity $\geq 99.99\%$) was used as the carrier and make-up gases. Their flow rates were set at 0.8 ml min⁻¹ and 30 ml min⁻¹, respectively. The temperature program was initially set at 150 °C for 1 min, followed by an increase rate of 10 °C min⁻¹ to 250 °C, at which the column temperature was held for

5 min. The injector and detector temperatures were 250 and 290 °C, respectively. The injection volume was 1 μ l in the split mode with a split ratio of 30:1.

Animals and Experimental Conditions

Male Sprague-Dawley (SD) rats aged 10–12 weeks (260–380 g) were purchased from the National Cheng Kung University, Laboratory Animal Center, Taiwan. Rats were housed in a temperature and humidity controlled room with 12 h light-dark cycles for at least 7 days prior to exposure. Food and water provided *ad libitum*.

The hair on the dorsum was clipped 24 h before the TDI was applied to avoid wounds to the skin. TDI was prepared in 1.5 ml olive oil (Karol *et al.*, 1981) for the topical application. Because workers in industry could be exposed to liquid containing about 1% TDI during the manufacturing processes in industry (Streicher *et al.*, 2000), the rats in the experiment were dermally exposed at concentrations of 1%, 0.2% (lower 5-fold end), and 5% (upper 5-fold end), respectively for 5 h. Chemicals were gently spread over a cotton pad approximately 3 $\times$ 5 cm then adhered to the test site. To assure no leakage on the tested skin, the experimental site was first covered with a sponge pad followed by an elastic bandage. Throughout the experiment every part of the covered site on the rats was carefully examined to ensure that no loosened or broken pads were found. At the end of exposure, the exposure site was washed three times to remove the residual TDI on skin using cleanser (D-TAMTM, DOD Technologies, USA). The rats in the control group were treated exactly as the exposure group except they were exposed to pure olive oil only. Triplicates ($n = 3$) were performed for each group. The rats were killed on day 7. Each rat was kept in a separate cage to avoid any fighting.

Urine Sample Collection

Since consecutive urine samples were collected for 6 days, urine samples were collected pre-exposure as the background. The collection frequency was from the end of exposure to day 6 every 12 h on the first 3 days and every 24 h on the last 3 days. The urine was collected in a 50 ml polypropylene conical tube and stored at -20 °C until analysis. The exact volume (ml) for each collected urine was measured. The accumulative U-TDA amount (in μ g) for each collected urine sample was estimated using the multiplicative product of 2,4- and 2,6-TDA concentration in urine (μ g ml⁻¹) and its corresponding volume (ml).

Before and at the end of the experiment, skin irritation was evaluated with no skin irritation being found for any exposure or control group.

Preparation and Analysis of Sample

Two calibration curves (low) from 1 to 50 ng ml⁻¹ and (high) from 50 to 500 ng ml⁻¹ concentration ranges for both 2,4- and 2,6-TDA were obtained separately, respectively, in 0.1 M HCl solution. The TDA derivation procedure was then implemented. The retention times for 2,6- and 2,4-TDA derivatives were 10.796 min and 11.057 min in the GC chromatogram, respectively. The correlation coefficient (*r*) of 2,4- and 2,6-TDA calibration curves yielded at least 0.995 for both TDAs at high and low concentrations. The limit of detection for TDAs was 1 ng ml⁻¹. The recoveries were 95.7% (R.S.D 3.0%) and 97.0% (R.S.D 5.6%) for 2,6-TDA (100–500 ng ml⁻¹) and 2,4-TDA (20–100 ng ml⁻¹), respectively.

The urine samples were hydrolysed with 1.5 ml of 3 M H₂SO₄ for 16 h at 100 °C, as recommended by Skarping *et al.* (1991), using a Firefox Dry Bath 6100 (Panttech, Taiwan) in 15 ml test tubes with Teflon screw caps. After hydrolysis, 5 ml of saturated NaOH solution and 2 ml of toluene were added to the samples. The mixtures were shaken for 2 min and then centrifuged for 10 min at 1500 rpm. One point five milliliters of the organic supernatant was transferred to a new test tube and 25 µl HFBA was added (Rosenberg and Savolainen, 1985). The samples were immediately shaken vigorously for 2 min and allowed to stand for 10 min. Excess reagent was removed by extraction with 1 ml of 1 M phosphate buffer solution (pH 7.2). The toluene layer, containing the amide derivatives, was transferred into 1.5 ml auto sampler vials with Teflon seals for further GC analysis. (Yeh *et al.*, 2007)

Determination of U-TDA Kinetics

Kinetic parameters were tested by fitting the first-order kinetics using linear simple regression (Chang *et al.*, 2002; Vera, 1983), which compares the time-series TDA measurements against the time of urine collection since $t_{\max}$:

$$C_t = C_0 \cdot e^{-kt} \quad (1)$$

where C_0 is the concentration at $t_{\max}$ (ng ml⁻¹), $t_{\max}$ is the time at the U-TDA maximum concentration after the end of exposure, k is the elimination rate constant, which indicates the determinant fraction removed from the body per unit time (h⁻¹), t is the time since the end of exposure (h). The first-order kinetic equation presented above can be transformed into a more straightforward linear equation

$$\ln C_t = \ln C_0 - kt \quad (2)$$

In this linear equation, the intercept ($\ln C_0$) stands for the logarithmic U-TDA concentration at $t_{\max}$. From the

elimination-rate constant (k), the apparent half-life ($t_{1/2}$) can be calculated if the washout line fits the first-order kinetics well

$$k = \frac{\ln 2}{t_{1/2}} = \frac{0.693}{t_{1/2}} \quad (3)$$

The total body burden was the total amount of TDA retained from the beginning of the exposure until the end of the experiment (= total uptake + total elimination). They can be determined as the sum of the area under the uptake curve and the area under the elimination curve. The area under the curve is denoted as *AUC* and can be calculated using the linear trapezoidal equation

$$AUC_{t_{\infty}} = AUC_{t_0-t_n} + \frac{C_n}{k} \quad (4)$$

$$AUC_{0 \rightarrow t_n} = \sum_{n=1}^n \frac{1}{2} (t_n - t_{n-1}) (C_{n-1} + C_n) \quad (5)$$

$$AUC_{t_n \rightarrow \infty} = \frac{C_n}{k} \quad (6)$$

where t_0 is the time at the end of exposure, t_n is the interval between two urine samples (h), t_{∞} is the infinity, C_n the U-TDA concentration at t_n (ng ml⁻¹), C_{n-1} is the U-TDA concentration at t_{n-1} (ng ml⁻¹) and k the elimination rate constant.

Statistical Analysis

Linear regression was performed to establish the relationship between the urine-collection time and the logarithm-transform of U-TDA. Kruskal-Wallis ANOVA was used to examine whether there was any difference in $t_{1/2}$ among the various exposure groups. Pearson correlation (*r*) tests were used for testing the association of TDI-dosing and *AUC*, $C_{\max}$ and accumulative amounts. Statistica Software (Release 6.0; StatSoft, OK, USA) was used for statistical analysis. $P < 0.05$ was accepted as significant.

Results and Discussion

Urinary TDA Concentrations over Time Course

No detectable TDA was found in the control group urine at any collection time. Therefore, the control group was excluded from further data analysis. Figure 1a shows 2,4-TDA concentrations over the time course after TDI skin exposure from three doses of TDI. The peak urinary excretion of TDA ($C_{\max}$) occurred during the first 12 h collection interval among three doses of TDI. The $C_{\max}$ of 2,4-TDA was found to be 0.062 ± 0.009 , 0.238 ± 0.060

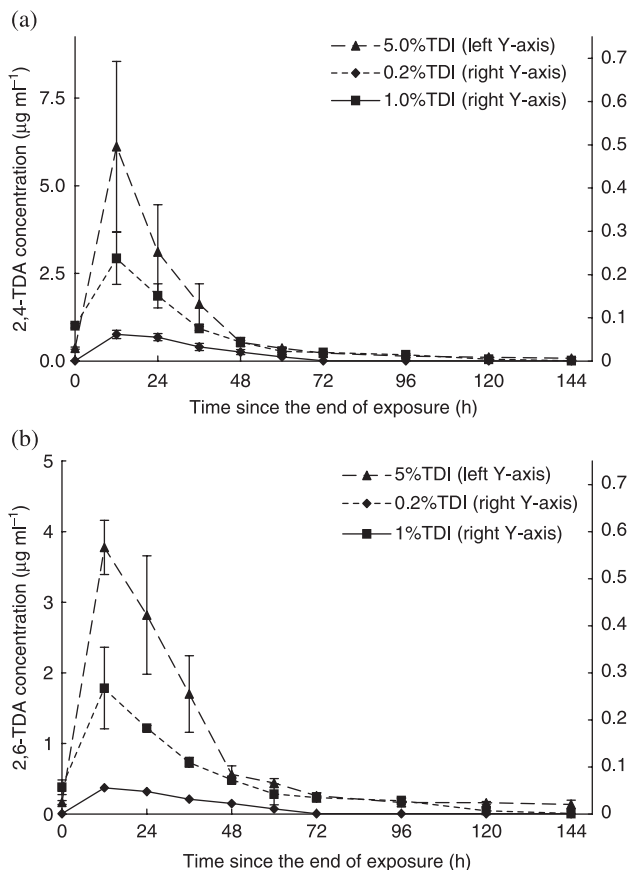


Figure 1. Concentrations profile of TDA in urine after TDI skin exposure over 6 days. (a) 2,4-TDA and (b) 2,6-TDA. Values represent mean $\pm$ SE for each time point and each application dose

and $6.116 \pm 0.429 \mu\text{g ml}^{-1}$ for low (0.2%), moderate (1%) and high (5%) TDI dose group, respectively (Fig. 1a, Table 1). Skin-absorbed 2,4-TDA was not completely eliminated by urinary excretion over 6 days in the high exposure group.

The elimination pattern of 2,6-TDA was similar to 2,4-TDA (Fig. 1b). The C_{max} was reached at 12 h after the end of exposure and found to be 0.056 ± 0.004 , 0.268 ± 0.087 and $3.777 \pm 0.384 \mu\text{g ml}^{-1}$ for low, moderate and high exposure groups, respectively (Fig. 1b, Table 1). The decrease trend slowed after 60 h for the moderate and high dose groups. However, the U-TDA concentration measurements were below the detection limit from 120 h, 72 h for the moderate and low exposure groups, respectively.

The accumulative amount profiles across 144 h for 2,4- and 2,6-TDA were similar. Excretory U-TDA amount increased abruptly within 24 h since the end of exposure, the elimination amounts were becoming slow within 24–60 h. The elimination amounts reached a plateau after 60 h (Fig. 2a and 2b).

Apparent Half-life Estimates and First-order Kinetic Curve Fitting

Apparent half-lives ($t_{1/2}$) of excretory TDA were about 20.1 h (SD = 1.9) and 22.7 h (SD = 3.4) for 2,4- and 2,6-forms among three exposure groups with relatively narrow ranges (Table 1). An increasing $t_{1/2}$ following by an increase of dose was found consistently for both 2,4- and 2,6-forms. The data indicating slower elimination and longer retention could occur at higher doses. Timchalk and colleagues (1994) found the $t_{1/2}$ derived from the urinary ¹⁴C excretion time-course slope following 2,4-[¹⁴C] TDI inhalation exposure was 20 h, similar to the experiments in this study.

When the first-order kinetic linearity was tested, highly satisfactory coefficients of correlation ($r = 0.930\sim 0.959$ $P < 0.05$ for 2,4-TDA; $r = 0.902\sim 0.953$ $P < 0.05$ for 2,6-TDA) were obtained for U-TDA measurements since the exposure termination (time after t_{max}) (Fig. 3). These results suggested the elimination pattern of excretory

Table 1. Kinetic parameters of urinary TDA, mean (SE)

	2,4-TDA			2,6-TDA			Ratio (CV %)		
	0.2% (1)	1% (2)	5% (3)	0.2% (4)	1% (5)	5% (6)	0.2% [=(1)/(4)]	1% [=(2)/(5)]	5% [=(3)/(6)]
T_{max} (h)	12 (0)	12 (0)	12 (0)	12 (0)	12 (0)	12 (0)	1 (0)	1 (0)	1 (0)
C_{max} ($\mu\text{g ml}^{-1}$)	0.062 (0.009)	0.238 (0.060)	6.116 (0.429)	0.056 (0.004)	0.268 (0.087)	3.777 (0.384)	1.1 (8.5)	0.9 (8.0)	1.6 (29.1)
AUC ($\mu\text{g}\cdot\text{h ml}^{-1}$)	2.186 (0.376)	8.395 (0.919)	158.599 (5.517)	2.046 (0.263)	10.558 (0.538)	133.994 (20.350)	1.1 (8.2)	0.8 (5.9)	1.2 (18.5)
Accumulative amounts (μg)	2.682 (0.631)	12.940 (4.224)	83.843 (29.542)	2.622 (0.779)	14.978 (2.628)	69.810 (11.541)			
k (h^{-1}) ^a	0.0376 (0.002)	0.0341 (0.003)	0.0325 (0.003)	0.0329 (0.0020)	0.00339 (0.0027)	0.0264 (0.004)			
$t_{1/2}$ (h) ^a	18.4 (0.8)	20.4 (1.5)	21.5 (2.2)	21.1 (1.3)	20.5 (1.6)	26.6 (3.7)	0.9 (3.0)	1.0 (0.8)	0.8 (13.8)

^a $P > 0.05$ by Kruskal-Wallis ANOVA test.

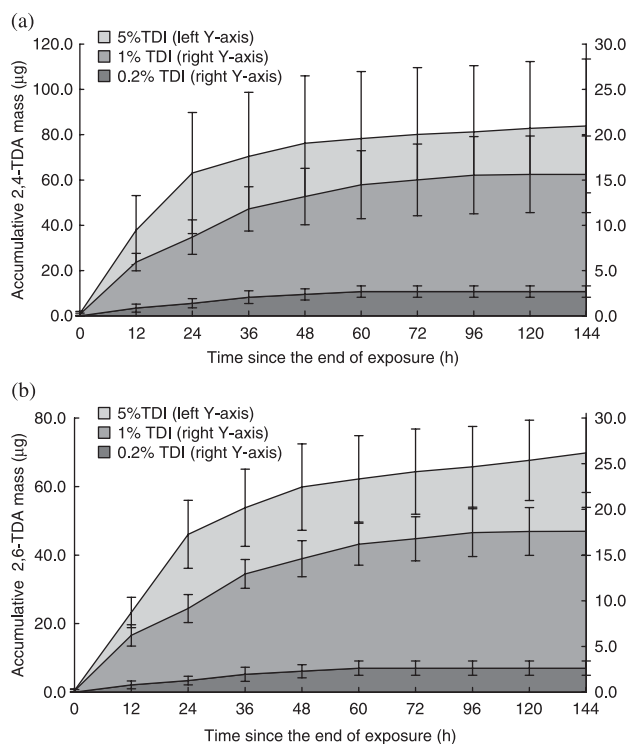


Figure 2. Accumulative TDA amounts across 6-day excretory urine. (a) 2,4-TDA and (b) 2,6-TDA. Mean $\pm$ SE, $n = 3$

TDA concentration profiles in 6-day consecutive urine samples were first-order kinetics. However, a non-linear saturation was found for high exposure at 60 h after $t_{\max}$. The possible explanation for this observation could be: in lower doses, the TDA elimination process in the kidney could connect with the distribution process in highly perfused tissues with hardly any time lag. On the other hand, at a high dose, the TDA elimination process could not be immediately completed because of overwhelming residual TDA following the TDA distribution process in highly perfused tissues. The finding of a non-linear saturation resulting from the higher dose is consistent with other percutaneous toxicokinetic study (Ballantyne *et al.*, 2003).

Comparison of U 2,4-TDA with U 2,6-TDA

The rat skins were originally exposed to a mixture of 2,4- and 2,6-TDI at a ratio of 80%:20% (m:m). The average ratios of 2,4-/2,6-TDA were found, however, to be 1.1, 0.9 and 1.6 in the low, moderate and high exposure groups for $C_{\max}$, respectively. For AUC results, the average ratios were 1.1, 0.8 and 1.2, respectively (Table 1). The overall ratios for both 2,4- and 2,6-form were close to unity, rather than 4:1, as expected from the exposure composition. The discrepancy between skin exposure

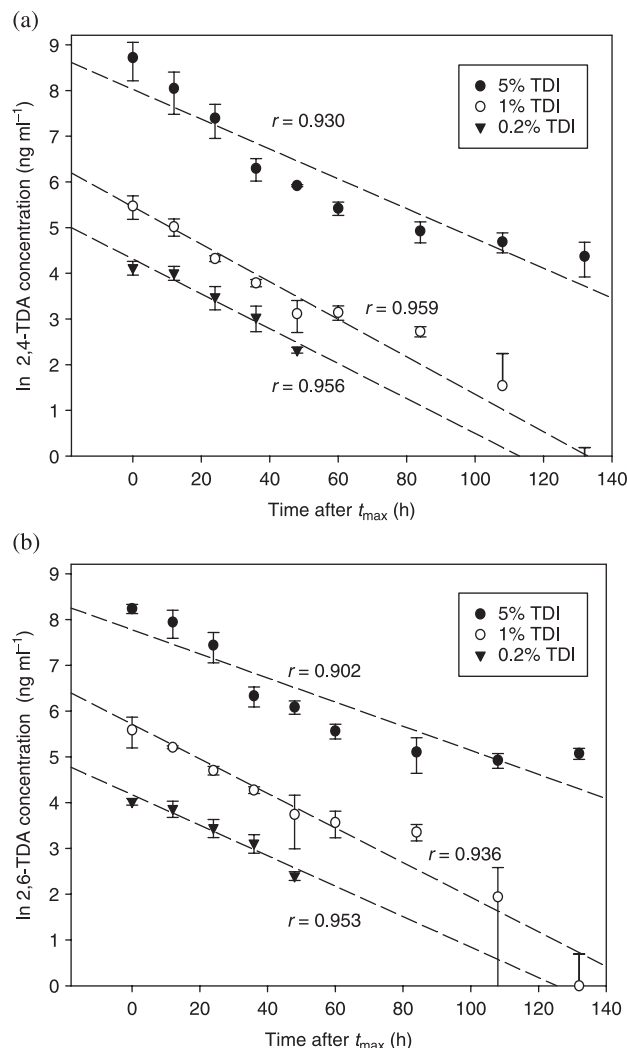


Figure 3. Correlation of the U-TDA concentrations (after log-transformation) with time course. (a) 2,4-TDA and (b) 2,6-TDA. ($P < 0.05$)

application and urinary concentration might be attributed to the greater reactivity of 2,4-TDI, possibly related to higher self-polymerization to form polyurea polymers. The findings of higher reactivity for 2,4- than 2,6-TDI resulting from a faster decay of 2,4-TDI were also consistent with our previous finding (Yeh *et al.*, 2002) and those of others (Rando *et al.*, 1984, Lind *et al.*, 1996).

Bello and colleagues (2006) exposed hairless guinea pig skin *in vitro* to several isocyanate compounds including octyl isocyanate, polymeric hexamethylene diisocyanate (pHDI), polymeric isophorone diisocyanate isocyanurate (pIPDI) and methylenediphenyl diisocyanate (MDI) using attenuated total reflectance Fourier transform infrared spectrometry (ATR-FTIR) to measure the absorbance decrease on skin over 60 min. They found larger isocyanate groups, e.g. pHDI and pIPDI, may remain on

the skin as unreacted species for many hours, with only 15–20% of the total isocyanate group disappearing in 1 h, while smaller isocyanate groups, e.g. octyl isocyanate, and MDI compounds rapidly disappeared from the skin surface (80+% in 30 min). This study, however, was unable to make a distinction as to whether the chemical showing disappearance by FTIR was due to evaporation or skin absorption. On the other hand, in the human field observation study performed by Liu and colleagues (2000), they concluded that skin exposure to isocyanates in the workplace could be very common by using calorimetric skin pads to evaluate HDI exposure. Our previous field investigations on TDI-related industries in a nationwide surveillance found the workers in the manufacturing processes could be easily exposed to TDI via skin contact (Yeh *et al.*, 2002). Up to the present, no absorption and elimination information on kinetics regarding dermal exposure to TDI have ever been presented in the literature. The findings in this study clearly demonstrate the skin absorption capability of topical TDI exposure based on the observation of the internal dose concentration profile of U-TDA across 6 days.

Dose-response Relationship between TDI-exposed and $AUC/C_{\max}$ /Accumulative Amounts

A linear increasing logarithm AUC trend for both forms of U-TDA with increasing TDI exposure was found ($r = 0.968$ for 2,4-TDA; $r = 0.973$ for 2,6-TDA) (Fig. 4a). A similar fashion for $C_{\max}$ ($r = 0.973$ for 2,4-TDA; $r = 0.984$ for 2,6-TDA) and accumulative amounts ($r = 0.998$ for 2,4-TDA; $r = 0.999$ for 2,6-TDA) to AUC was also obtained (Fig. 4b, 4c). The above-mentioned findings suggested a clear dose-dependent fashion of skin absorption for 2,4- and 2,6-TDI.

Conclusion

It has been demonstrated that the absorptions of 2,4- and 2,6-TDI through skin contact are possible in this rat study. Clear dose-dependent skin absorption for 2,4- and 2,6-TDI was demonstrated by the findings of AUC , $C_{\max}$ and accumulative amounts. Excretory 2,4- and 2,6-TDA concentration profiles in 6-day consecutive urine samples were shown to fit in first-order kinetics, although higher order kinetics could not be excluded for high doses. The apparent half-lives for excretory urinary TDA were about 20 h at various skin exposures, similar to that from the inhalation exposure in the previous animal experiment. The overall yield ratios for 2,4- to 2,6-TDA in urine were found to be close to unity, apparently lower than the expectancy of 4:1, possibly due to the higher self-polymerization reactivity of 2,4- than 2,6-TDI.

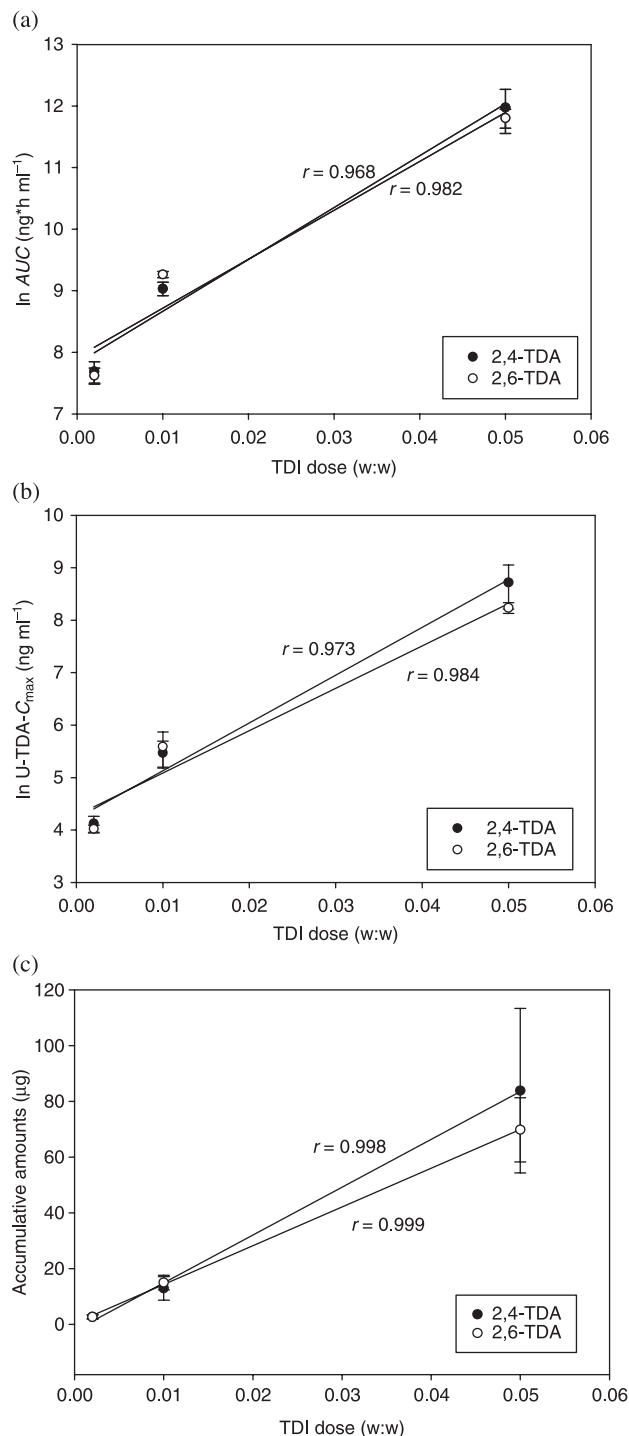


Figure 4. Association between TDI skin exposure dose and U-TDA (a) Area under the curves; (b) $C_{\max}$ and (c) accumulative amounts

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