Journal of Environmental Monitoring

Cite this: *J. Environ. Monit.*, 2012, **14**, 951

www.rsc.org/jem

PAPER

Penetration patterns of monomeric and polymeric 1,6-hexamethylene diisocyanate monomer in human skin

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Received 6th July 2011, Accepted 5th January 2012
DOI: 10.1039/c2em10546b

We investigated penetration patterns of monomeric and polymeric 1,6-hexamethylene diisocyanate (HDI), experimentally and as part of commercial products, in excised full-thickness human skin at 5, 10, 30, or 60 min after exposure. We observed that both monomeric and polymeric HDI were readily absorbed into the skin and that the clearcoat composition affects the penetration rate of the individual isocyanates. The short-term absorption rates for HDI monomer, biuret, and isocyanurate were determined and used to estimate the exposure time required to reach a body burden equal to the American Conference of Governmental Industrial Hygienists (ACGIH) inhalation threshold limit value (TLV) or Oregon State occupational exposure limit (OEL). Oregon is the only government entity in the United States to promulgate a short-term exposure limit (STEL) for HDI-based polyisocyanates biuret and isocyanurate. Based on these absorption rates for a slow-drying clearcoat after 10 min (1.33 μg cm^{-2} h^{-1}) or 60 min (0.219 μg cm^{-2} h^{-1}), we calculated that 6.5 and 40 min dermal exposure, respectively, is required to achieve a dose of HDI equivalent to the ACGIH TLV. For biuret, the time to achieve a dose equivalent to the Oregon OEL for slow-drying clearcoat was much shorter (<31 min) than that for fast-drying clearcoat (618 min). Isocyanurate had the shortest skin absorption times regardless of clearcoat formulation (14 s–1.7 min). These results indicate that the dose received through dermal exposure to HDI-containing clearcoats has a significant potential to exceed the dose equivalent to that received through inhalation exposure at established regulatory limits. A critical need exists to monitor dermal exposure quantitatively in exposed workers, to use proper protective equipment to reduce dermal exposure, and to re-evaluate regulatory exposure limits for isocyanates.

Introduction

In the automotive refinishing industry, polyurethane paints used typically contain monomeric (usually <0.5%) and polymeric species (*i.e.*, uretdione dimer and biuret and isocyanurate trimers; 2.5–20%) 1,6-hexamethylene diisocyanate (HDI).[1] Isophorone diisocyanate (IPDI)-based polyisocyanates may also be used in automotive coatings but are typically present at lower levels than HDI-based polyisocyanates.[2] Although airborne isocyanate exposures have been reduced through improved controls and use of less-volatile isocyanates (*i.e.*, polyisocyanates), asthma due to sensitization to isocyanates continues to occur, and it is often observed in work settings where measured isocyanate respiratory exposures are very low and/or below the levels detectable by commonly used methodologies.[3] This observation has prompted a concerted investigation of dermal exposures.[3,4] Dermal exposure to monomeric and

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Environmental impact

Occupational exposure is a leading cause for sensitization to inhaled isocyanates and occupational asthma. Dermal exposure has also been linked to isocyanate sensitization and asthma. However, the skin penetration patterns and absorption rates of isocyanates are not well understood. We were able to demonstrate that monomeric and polymeric 1,6-hexamethylene diisocyanates present in clearcoat products used in automotive paints penetrate readily into human skin and that isocyanate species have different dermal penetration patterns depending on the product. A critical need exists to quantitatively monitor dermal exposure to all isocyanate species, determine the exposure patterns leading to occupational asthma, and to re-evaluate regulatory exposure limits for isocyanates.

polymeric HDI in the automotive refinishing industry may occur *via* deposition of HDI-containing paint onto the skin during mixing and/or spraying or by direct contact with the paint, freshly painted products, and/or contaminated surfaces.[5] Although risk for dermal exposure is evident, skin protective equipment is not always worn or the misuse/failure of personal protective equipment, such as gloves or coveralls, may occur.

Previously, we measured dermal exposures to monomeric and polymeric HDI in automotive spray painters using a tape-strip method.[6–8] Our results indicated that the product of analyte-specific breathing-zone concentration and paint time was the most significant variable in all dermal exposure models. For painters not wearing protective clothing, when the same product of analyte-specific breathing-zone concentration and paint time were considered, the models predicted ~2, 10, and 17 times higher dermal concentrations of uretdione, biuret, and isocyanurate than HDI monomer, respectively.[8] Polymeric isocyanates (*i.e.*, uretdione, biuret, and isocyanurate) potentially may have longer residence time on the skin due to their lower volatility compared to monomers and, thus, they may elicit skin and systemic effects different from that of the monomer. These differences are likely due to different skin absorption rates or chemical reactivities of HDI-polyisocyanates.[6,8]

We have also compared dermal tape-strips with impregnated dermal patch samplers.[9] We observed that the tape-strip sampling underestimated monomeric and polymeric HDI levels, which was likely due to the penetration of these compounds into the deeper layers of the skin.[9] Bello *et al.* investigated the dermal penetration of isocyanates using cadaver guinea pig skin.[10] They observed that polymeric HDI may remain on the skin as unreacted species for many hours, with only 15–20% of the total isocyanate amount disappearing within one hour, while lower molecular weight isocyanates rapidly disappear from the skin surface (>80% in 30 min). They postulated that isocyanates most likely leave the skin predominantly by diffusion, with minimal reaction with skin surface proteins.

Although dermal sampling methods have been developed, differences in the penetration patterns and absorption rates of monomeric and polymeric HDI into human skin are not well understood. Knowledge of these differences is required to further our understanding of the contribution of dermal exposure to the internal dose received and any related toxicity and associated health effects. The main objectives of this study were to investigate the penetration patterns of monomeric and polymeric HDI as well as slow- and fast-drying clearcoats containing these compounds in human skin, and to evaluate the efficiency of the tape-strip method to measure dermal exposure to monomeric and polymeric HDI.

Materials and methods

Excised human full-thickness skin from 8 donors (5 for HDI experiments and 3 for clearcoat experiments) was obtained from the tissue bank at the Pathology and Laboratory Medicine Department, University of North Carolina at Chapel Hill (UNC-CH). Skin was stored in a refrigerator at ~4 °C and used for the experiments described within 48 h of surgical removal. Excess fat from the skin was removed and the skin was cleaned with deionized (DI) water and blotted with gauze to remove any iodine or blood. Following cleanup, the skin was sectioned into ~3.5 × 5 cm pieces. After the skin was cut into sections, the individual pieces were again cleaned with DI water and patted dry with gauze. The skin was then pinned to a Styrofoam™ board that was covered with foil and a piece of thin dental wax. An additional piece of foil was placed on top of the wax to account for any breakthrough of the test agents. The experimental setup is shown in Fig. 1.

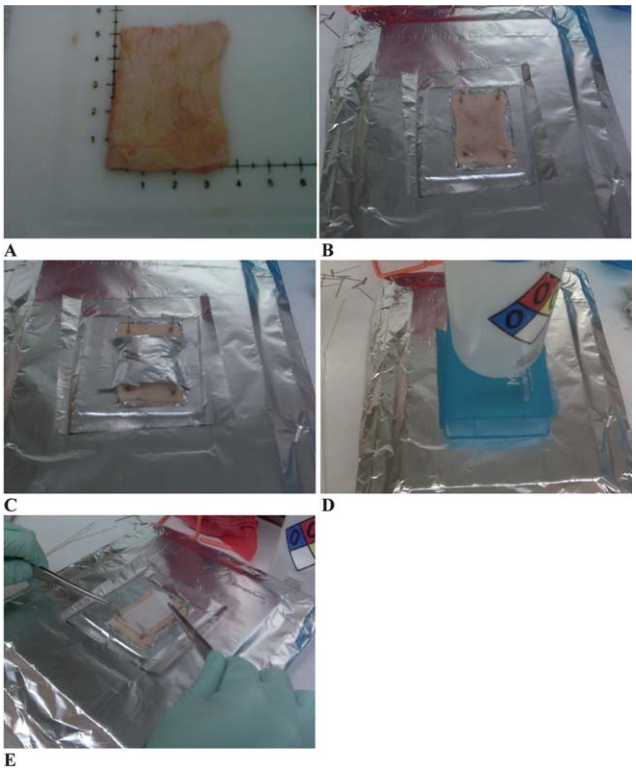

Fig. 1 Experimental set-up: (A) skin is sectioned into 3.5 × 5 cm pieces, (B) skin is pinned to a Styrofoam™ board that is covered with foil and a piece of wax, (C) isocyanate is applied to the skin and occluded with foil, (D) an additional cover is placed over occluded skin, (E) skin is tape-stripped.

Experiments with HDI monomer

Sample collection. HDI neat (10 μl) or a solution of HDI in ethyl acetate (HDI/EA; 50 μl) was applied to skin tissues from 5 individuals. The HDI/EA mixture was a concentration of 0.3 g HDI/l of EA, which corresponds to the average concentration of HDI monomer measured in bulk paint samples collected during our previous field study.[11] EA was used because it is a common solvent for isocyanates, it evaporates quickly, it is non-reactive towards isocyanates, and has low toxicity.[10] Following the epicutaneous application of HDI, the skin was occluded with a piece of foil and covered with a plastic lid to minimize evaporation (Fig. 1). Skin tissues were exposed for 5, 10, 30, or 60 min. Following the exposure period, the foil that was used for occlusion was removed and placed in a vial containing 5 ml of derivatizing solution [2 g of 1-(2-methoxyphenyl)-piperazine (MPP) in 1 l of 30% v/v solution of *N*,*N*-dimethylformamide (DMF, 73.09 g mol^{-1}) in acetonitrile (ACN, 41.05 g mol^{-1})]. The

surface of the skin was then patted with gauze to remove any leftover HDI not absorbed into the skin, and gauze placed in 5 ml of derivatizing solution. Next, the skin tissue was tape-stripped 30 times at the same location and each tape-strip (2.5 × 4 cm, Cover-Roll® adhesive tape Beiersdorf AG, Hamburg, Germany), placed in 5 ml of derivatizing solution. In order to prevent cross contamination, forceps cleaned with acetone were used to apply and remove the tape-strip and place them in the vials. After tape-stripping was complete, the skin tissue was placed in 5 ml of derivatizing solution for extraction. The foil underneath the skin was placed in 5 ml of derivatizing solution to investigate potential breakthrough. A bulk sample of the HDI neat or HDI/EA (equal amount of what was applied) was injected into a vial containing 20 ml of derivatizing solution. All samples were stored at −40 °C until processing and analysis of HDI monomer by LC-MS.[6,7]

Sample processing and analysis

HDI neat. For analysis, skin samples were returned to room temperature. A white precipitate was observed in some of the samples. All samples were heated at 80 °C to dissolve precipitate (~20 min) and then vortexed. Samples were diluted by removing 100 μl of sample and placing it in 5 ml of derivatizing solution and then vortexed. The occluded foil, gauze, first 5 tape-strips collected from each skin tissue, and bulk samples were further diluted by removing 100 μl aliquot from the first dilution and placing it into a vial with 5 ml of derivatizing solution. The final dilutions were vortexed and 100 μl of acetic anhydride was added to acetylate residual MPP. After 15 min, internal standard (53 pmol μl^{-1} urea derivative of 1,8-octamethylene diisocyanate; ODIU) was added (100 μl) to give an internal standard concentration of 1 pmol μl^{-1}. Samples were analyzed for HDI monomer using LC-MS.[6]

HDI/EA. For analysis, skin samples were returned to room temperature and acetic anhydride was added (200 μl bulk, 100 μl for tape-strips, foils and gauze) to acetylate residual MPP. After 15 min, internal standard (2 pmol μl^{-1} ODIU) was combined (1 : 1 v/v ratio) with aliquots of each bulk paint sample to give an internal standard concentration of 1 pmol μl^{-1}. For all other samples (tape-strips, gauze, foil) after 15 min, internal standard (52 pmol μl^{-1} ODIU) was added (100 μl) to give an internal standard concentration of 1 pmol μl^{-1}. Samples were analyzed for HDI monomer using LC-MS.[6]

Skin tissues. For analysis, skin tissue samples were thawed to room temperature and 1 ml aliquot removed and placed into a vial (whole skin extract). The remaining skin tissue was chopped up with scalpel and surgical scissors, agitated, and vortexed inside a conical tube containing 4 ml of derivatizing solution. The pieces in the conical were extracted overnight at ~4 °C. The following day, 1 ml aliquot was taken from the conical and processed (minced skin extract). The whole and minced skin extract samples applied with HDI neat were diluted by taking a 50 μl aliquot of the sample and bringing it up to 5 ml with derivatizing solution after which acetic anhydride (100 μl) was added to acetylate residual MPP. For the whole and minced skin extract samples applied with HDI/EA, 20 μl of acetic anhydride was added to acetylate residual MPP. After 15 min, internal standard (52 pmol μl^{-1} ODIU; 100 μl for samples applied with HDI neat and 20 μl for HDI/EA) was added to give an internal standard concentration of 1 pmol μl^{-1}. A 200 μl aliquot of sample was then centrifuged for 30 min at 15,000 RFC to remove any large particulate. Samples were analyzed for HDI monomer using LC-MS and whole and minced skin extract results compared.[6]

Experiments with slow-drying and fast-drying clearcoat

Sample collection. Skin tissues from 3 individuals were applied with 50 μl of either a slow- or fast-drying clearcoat containing monomeric or polymeric HDI and exposed for 10, 30, or 60 min as described above for HDI monomer. The clearcoat used was ChromaClear® 7900S™ Multi-Use Clear with either ChromaClear® 7995S™ Slow Activator-Reducer or ChromaClear® 7975S™ Fast Activator-Reducer (3 : 1 clear to activator by volume; DuPont™, Wilmington, DE). The clearcoat mixture was mixed in the laboratory on the same day experiments were conducted. Following the exposure period, the sample collection and processing were conducted as described for HDI monomer. Control samples in which the skin tissue was not spiked were collected following the same procedure. In addition, a bulk sample of the clearcoat was collected (equal amount of what was applied) and injected into a vial containing 20 ml of derivatizing solution. All samples were stored at −40 °C until processing and analysis of HDI monomer, biuret, and isocyanurate by LC-MS.[6]

Sample processing and analysis. For analysis, skin samples were returned to room temperature and acetic anhydride was added (200 μl bulk, 100 μl for tape-strips, foils, and gauze) to acetylate residual MPP. After 15 min, all the samples were processed as described below. Bulk paint, occluded foils, and gauze samples were diluted by combining 500 μl of sample with 1,500 μl of acetonitrile. Internal standard (2 pmol μl^{-1} ODIU) was combined (1 : 1 v/v ratio) with the diluted aliquots of each bulk paint sample, occlusion foil, gauze, to give an internal standard concentration of 1 pmol μl^{-1}. For the samples applied with the slow-drying clearcoat, the first 5 tape-strips collected were diluted by combining 500 μl of sample with 500 μl of acetonitrile and then internal standard (2 pmol μl^{-1} ODIU) was combined (1 : 1 v/v ratio) with the diluted aliquot to give an internal standard concentration of 1 pmol μl^{-1}. For the samples applied with the fast-drying clearcoat, the first 5 tape-strips were diluted by combining with internal standard (2 pmol μl^{-1} ODIU; 1 : 1 v/v ratio) to give an internal standard concentration of 1 pmol μl^{-1}. For the remaining tape-strip and foil samples from the experiments with both the slow- and fast-drying clearcoat, internal standard (52 pmol μl^{-1} ODIU; 100 μl) was added to give an internal standard concentration of 1 pmol μl^{-1}. Samples were analyzed for HDI monomer, biuret, and isocyanurate using LC-MS.[6]

Skin tissues. For analysis, skin tissue samples were returned to room temperature and 1 ml aliquot removed and placed in a vial. Acetic anhydride was added (20 μl) to acetylate residual MPP. After 15 min, internal standard (2 pmol μl^{-1} ODIU) was combined (1 : 1 v/v ratio) with the sample to give an internal

standard concentration of 1 pmol μl^{-1}. Samples were analyzed for HDI monomer, biuret, and isocyanurate using LC-MS.[6]

Data analysis

A mass balance approach was used to calculate percent recoveries based on the bulk sample analysis. We calculated percent recovery for the following compartments. The tape-strips represent the amount penetrated into each cell layer while the gauze and occlusion foil represent the amount not penetrated into the skin. The remaining skin tissue represents the amount that has penetrated beyond 30 cell layers and remains in the skin while the foil underneath the skin tissue represents breakthrough through the full-thickness human skin. Paired *t*-test ($\alpha = 0.05$) was used to compare the whole and minced skin extract samples.

Short-term absorption rates for HDI, biuret, and isocyanurate were calculated using the data from the slow- and fast-drying clearcoat experiments. The short-term absorption rate ($\mu g\ cm^{-2}\ h^{-1}$) for 10 and 60 min exposures were calculated by dividing the sum of the total amount of isocyanate (HDI, biuret, or isocyanurate) measured in the breakthrough foil (receptor foil) and the skin (skin tissue and the sum of 30 tape-strips) by the exposed area (tape-strip area; 10 cm^2) and exposure time. Because the first tape-strips may include potential residual contamination from the dose applied to the skin,[12–14] we also calculated the short-term absorption rates by excluding the amount measured in the first tape-strips. This allowed us to determine the effect of the potential residual contamination on the short-term absorption rates.

Results

Experiments with HDI monomer

The average recoveries for each compartment [excess (*i.e.*, foil for occlusion and gauze), tape-strips, skin, and breakthrough (*i.e.*, foil under skin)] compared to the reference sample for HDI neat and HDI/EA exposures are presented in Table 1. The total recovery was much higher (>82%) in the skins applied with HDI neat than the skins applied with HDI/EA (21–46%). The percentage of HDI neat that did not penetrate the skin (~58%), as indicated by the amount in the excess compartment (*i.e.*, occlusion foil and gauze), was greater than skins applied with HDI/EA (~16%).

HDI was detected in 100 and 91% of the tape-strips collected from the skins applied with HDI neat and HDI/EA, respectively (Fig. 2). The average HDI amount ($\mu g\ cm^{-2}$) collected with the 30 tape-strips from the skins after epicutaneous application of HDI neat (Fig. 2A) or HDI/EA (Fig. 2B) for all time points indicated rapid penetration into and beyond the stratum corneum. The majority of HDI was measured with the first 5 tape-strips and a decreasing trend in HDI concentration was observed with the successive tape-strips. Overall, the amount of HDI measured with all 30 tape-strips was 20–30% of the total HDI applied regardless of whether the skin was exposed to HDI neat or HDI/EA.

The average amount of HDI extracted from the skins after epicutaneous application of HDI neat was 1.7% of the total HDI applied and 4.2% for those of HDI/EA. Paired *t*-test indicated no significant difference in HDI concentration between whole and minced skin extract samples ($N = 17$; $p = 0.43$). Breakthrough of HDI, although negligible (<1%) relative to the total amount of HDI applied, was detected in 90 and 85% of the skins applied with HDI neat and HDI/EA, respectively. Considering the large standard deviation for the breakthrough in the 5 min exposure experiment compared to the other time points, the largest breakthrough observed at 5 min is likely due to an experimental artifact.

Experiments with slow-drying and fast-drying clearcoat

The average recoveries for each compartment [excess (*i.e.*, foil for occlusion and gauze), tape-strips, skin, and breakthrough (*i.e.*, foil under skin)] compared to the reference samples for slow- and fast-drying clearcoat are presented in Table 2. The results of the short-term absorption rates are presented in Table 3.

Table 1 Average percent recovery in each compartment after epicutaneous application of HDI neat (10 μl) or HDI/EA (50 μl of 0.3 g l^{-1})

		Percent Recovery (%)							
		5 min (N = 3)[b]		10 min (N = 7)[c]		30 min (N = 5)[d]		60 min (N = 5)[e]	
Spiking Agent	Compartment[a]	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation
HDI neat	Excess[f]	69.9	13.2	59.8	17.6	53.2	15.4	56.0	9.97
	Tape-Strips	19.6	4.89	21.7	10.4	29.9	10.7	26.2	4.62
	Skin	1.01	1.07	1.05	1.02	3.09	0.67	1.79	0.58
	Breakthrough[g]	0.62	1.07	0.04	0.05	0.06	0.07	0.23	0.29
	TOTAL	**91.1%**		**82.6%**		**86.3%**		**84.2%**	
HDI/EA	Excess[f]	13.6	2.83	14.9	6.30	12.4	4.41	7.50	4.57
	Tape-Strips	22.4	2.33	24.0	5.28	15.4	1.69	11.1	5.41
	Skin	4.7	2.28	6.94	1.50	2.75	0.61	2.46	0.95
	Breakthrough[g]	0.5	0.72	0.07	0.04	0.06	0.01	0.03	0.02
	TOTAL	**41.2%**		**46.0%**		**30.6%**		**21.0%**	

[a] Compartment for mass balance. [b] Donor 1–79-year old female Caucasian lower leg. [c] Donor 2 (N = 2) – 37-year old female African-American pannus; Donor 3 (N = 2) – 68-year old female Caucasian pannus; Donor 4 (N = 3) – 48-year old male Caucasian pannus. [d] Donor 4 (N = 3); Donor 5 (N = 2) – 46-year old female African-American pannus. [e] Donor 4 (N = 3); Donor 5 (N = 2). [f] Excess is the sum of HDI in the foil used for occlusion and gauze sample used to remove excess HDI. [g] Breakthrough is the amount of HDI measured on the foil underneath the skin.

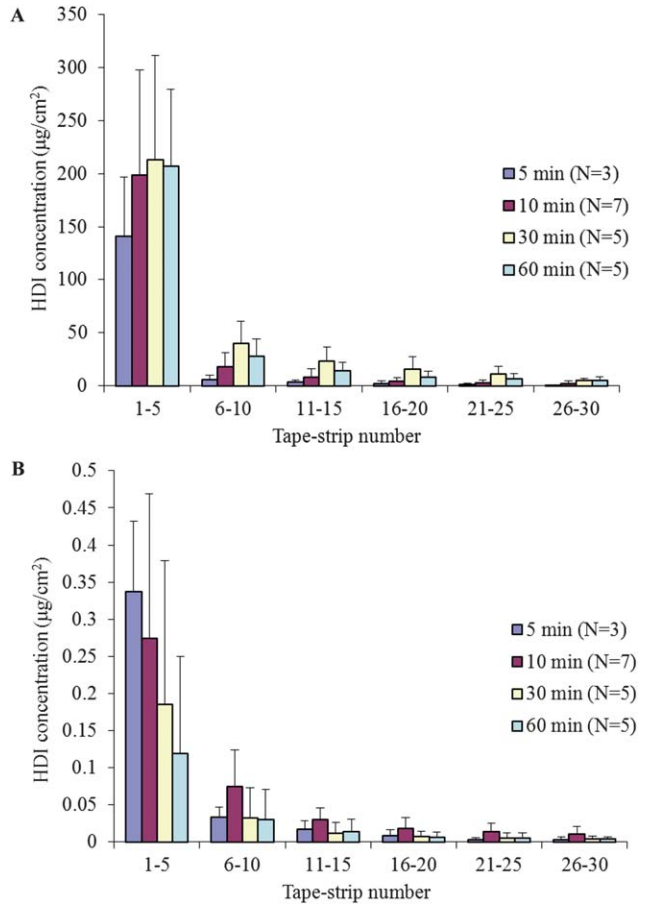

Fig. 2 Amount of HDI measured in 30 sequential tape strips collected after epicutaneous application of (A) with 10 μl of HDI neat or (B) with 50 μl of 0.3 g l^{-1} HDI in ethyl acetate.

Significantly more HDI and biuret ($p < 0.001$) was present in the slow-drying clearcoat (10.6 and 178 μg, respectively) compared with the fast-drying clearcoat (5.9 and 6.3 μg, respectively). No significant difference ($p = 0.25$) in the amount of isocyanurate for the slow- (3,464 μg) and fast-drying (4,118 μg) clearcoat was observed. All control samples were below limits of detection indicating the skin tissue samples did not contain isocyanates.

Slow-drying clearcoat. The amount of HDI, biuret, and isocyanurate that did not penetrate the skin (excess) was greater at 10 and 30 min (74–82%, 66–73%, and 69–70%, respectively) compared to 60 min exposure (54%, 50%, and 56%, respectively). HDI was detected in 83, 86, and 83% of the tape-strips collected at 10, 30, and 60 min, respectively, while biuret was detected in 89, 99, and 100% of the tape-strips collected at these time points, respectively. Isocyanurate was detected in 100% of all samples collected. The average amount of HDI, biuret, and isocyanurate amount (μg cm^{-2}) collected with the 30 tape-strips from the skin tissues applied with the slow-drying clearcoat for all time points indicated rapid penetration of monomeric and polymeric HDI into and beyond the stratum corneum (Fig. 3). The majority of the HDI, biuret, and isocyanurate was measured in the first 5 tape-strips and a decreasing trend in their concentrations was observed with the successive tape-strips.

The average amount of HDI extracted from the skin tissues after epicutaneous application of the slow-drying clearcoat was 3.1% of the total HDI, 3.7% of the total biuret, and 2.9% of the total isocyanurate applied. The amount of breakthrough measured in all experiments was negligible (<0.03% for HDI, <0.2% for biuret, and <0.08% for isocyanurate). However, breakthrough of HDI was detected in 67, 33, and 33% of the samples at 10, 30, and 60 min, respectively, while breakthrough of biuret was detected in all samples at 10 and 60 min exposure. Breakthrough of isocyanurate was detected in all of the samples at all time points.

Fast-drying clearcoat. The amount that did not penetrate the skin (excess) was similar for HDI and isocyanurate. At 10 and 30 min, less HDI (74 and 76%, respectively) and isocyanurate (75 and 72%, respectively) penetrated the skin compared to 60 min exposure (61% and 60%, respectively). The excess of biuret measured at 10, 30, and 60 min was 73, 82, and 70%, respectively.

HDI was detected in 60, 43, and 64% of the tape-strips collected at 10, 30, and 60 min, respectively, while biuret was detected in 31, 10, and 23% of the tape-strips collected at these time points, respectively. Isocyanurate was detected in all of the tape-strips collected at all time points. The average HDI, biuret, and isocyanurate amount (μg cm^{-2}) collected with the 30 tape-strips from the skin tissues applied with the fast-drying clearcoat for all time points indicated rapid penetration of monomeric and polymeric HDI into and beyond the stratum corneum (Fig. 4). The majority of the HDI, biuret, and isocyanurate was measured in the first 5 tape-strips and a decreasing trend in their concentrations was observed with the successive tape-strips. However, for the 30 min exposure we only measured detectable levels of biuret in the first 3 tape-strip samples.

The average amount of HDI extracted from the skins after epicutaneous application of the fast-drying clearcoat was 3% of the total HDI, 12% of the total biuret, and 3.7% of the total isocyanurate applied. We observed more HDI in the skin with increasing exposure time. We also observed more biuret and isocyanurate in the skin at 60 min (16.4% and 5.84%, respectively) compared to 10 min (12.3% and 3.69%, respectively) exposure. The amount of breakthrough measured in all experiments was negligible (<0.11% for HDI, <0.7% for biuret, and <0.25% for isocyanurate). However, breakthrough of HDI was only detected at 10 min exposure (in 67% of the samples) while breakthrough of biuret was detected at 10 and 60 min exposure (in 67 and 33% of the samples, respectively). Breakthrough of isocyanurate was detected in all of the samples at all time points.

Discussion

Dermal exposure to monomeric and polymeric HDI comprises a significant route for exposure to spray painters employed in the automotive refinishing industry.[3,5–8,15–19] Although the dermal exposure route has been established to be significant,[8,16,17,20] there are no regulatory limits or standards regarding dermal exposure to isocyanates. Here, we report our investigation on the penetration patterns and rates of monomeric and polymeric HDI in human skin in order to gain insight to the potential contribution of dermal exposure to internal dose received in this worker population.

Table 2 Average percent recovery for each compartment after epicutaneous application of slow- or fast-drying clearcoat

			Percent Recovery (%)					
			10 min (N = 3)[a]		30 min (N = 3)[a]		60 min (N = 3)[a]	
Analyte	Spiking Agent	Compartment	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation
HDI	Slow-drying clearcoat	Excess[b]	74.4	15.9	81.6	23.0	53.9	10.9
		Tape-Strips	16.3	3.76	16.9	5.68	18.0	4.55
		Skin	4.00	2.06	2.08	0.68	3.27	1.13
		Breakthrough[c]	0.03	0.03	0.01	0.00	0.02	0.01
		TOTAL	**94.7%**		**100.6%**		**75.1%**	
	Fast-drying clearcoat	Excess[b]	73.7	16.0	75.7	9.63	60.9	15.07
		Tape-Strips	20.5	7.76	17.6	0.93	19.1	4.95
		Skin	1.97	0.26	2.03	0.98	5.07	2.50
		Breakthrough[c]	0.11	0.12	0.02	0.00	0.02	0.00
		TOTAL	**96.3%**		**95.4%**		**85.1%**	
Biuret	Slow-drying clearcoat	Excess[b]	65.6	12.3	72.5	30.3	50.2	11.42
		Tape-Strips	18.0	6.27	17.5	5.47	20.37	6.02
		Skin	4.40	1.75	2.72	0.80	3.95	1.04
		Breakthrough[c]	0.11	0.09	0.01	0.00	0.02	0.02
		TOTAL	**88.1%**		**92.7%**		**75.5%**	
	Fast-drying clearcoat	Excess[b]	73.1	3.58	81.9	17.7	70.1	9.78
		Tape-Strips	27.8	11.3	21.9	3.66	22.3	8.62
		Skin	16.4	12.0	6.32	1.36	12.3	3.13
		Breakthrough[c]	0.68	0.40	0.24	0.00	0.44	0.36
		TOTAL	**118.0%**		**110.4%**		**105.1%**	
Isocyanurate	Slow-drying clearcoat	Excess[b]	69.4	5.46	70.4	27.3	55.8	10.1
		Tape-Strips	13.5	3.12	13.9	4.44	20.1	6.50
		Skin	3.37	1.55	1.82	0.58	3.56	1.52
		Breakthrough[c]	0.08	0.04	0.00	0.00	0.01	0.01
		TOTAL	**86.4%**		**86.1%**		**79.4%**	
	Fast-drying clearcoat	Excess[b]	74.6	11.4	72.3	13.85	59.6	17.19
		Tape-Strips	21.4	9.12	18.5	2.70	17.1	6.14
		Skin	3.69	3.28	1.69	0.86	5.84	3.77
		Breakthrough[c]	0.25	0.37	0.01	0.00	0.01	0.01
		TOTAL	**99.9%**		**92.5%**		**82.5%**	

[a] Donor 6 (N = 1) – 47-year old female Caucasian pannus; Donor 7 (N = 1) – 72-year old male Caucasian thigh; Donor 8 (N = 1) – 53-year old male Caucasian abdomen. [b] Excess is the sum of HDI, biuret, or isocyanurate in the foil used for occlusion and gauze sample used to remove excess HDI, biuret, or isocyanurate. [c] Breakthrough is the amount of HDI, biuret, or isocyanurate measured on the foil underneath the skin.

Our results show that HDI monomer and its oligomers, biuret and isocyanurate, readily penetrate the human skin, and confirm our previous dermal exposure assessment studies.[6–8] This is clearly demonstrated by the fact that we recovered more HDI monomer in the deeper cell layers of the stratum corneum for experiments with HDI neat at 30 and 60 min exposures compared to the 5 and 10 min exposures. However, we observed the opposite trend for the experiments with HDI in EA, *i.e.*, less HDI was measured in the later tape-strips for the longer exposure periods. We believe that this observation is due to the fact that EA is likely enhancing dermal penetration[21,22] of HDI and driving it faster into the deeper layers of the skin where it was likely retained. The enhancement of dermal penetrations was further confirmed by the fact that at 5 min 81% of the total HDI applied in EA had penetrated the skin (only 13.6% of the dose was recovered on the skin surface). Similar results were also recorded for 10, 30, and 60 min exposures. When the skins were applied with HDI neat, we observed 53–70% of the HDI to remain on the skin surface further providing evidence of the enhancement of penetration into the skin with EA. For HDI neat, exposure time did not appear to influence penetration. This is likely due to the fact that HDI neat was spiked at a large concentration and achieved saturation on the skin surface and/or steady state absorption during the experiments.

In the experiments with clearcoats, 74% of HDI remained on the skins surface at 10 min exposure regardless of clearcoat type while 54 and 61% of the HDI remained on the skin surface with slow- and fast-drying clearcoat, respectively, at 60 min exposure. It appears that the clearcoat mixture is not quite as an effective vehicle to enhance penetration of HDI into the skin as EA alone. The clearcoat is a viscous mixture while HDI/EA mixture is fluid and, therefore, the viscosity likely decreases the penetration rate of the isocyanates. However, in the occupational exposure setting, clearcoat is often mixed with reducers (*i.e.*, solvents), which could enhance dermal penetration and, thus, our estimates may be underestimates of the skin penetration rates of these mixtures. We observed the slow-drying clearcoat to penetrate the skin more rapidly than the fast-drying clearcoat. This was evident as we observed less biuret and isocyanurate to remain on the skins surface with slow-drying clearcoat compared to the fast-drying clearcoat. This may be due to the concentration gradient differences between the two clearcoats (slow-drying clearcoat contained significantly more HDI and biuret than fast-drying clearcoat). The data indicated initial rapid flux through

Table 3 The means ± standard deviations of HDI monomer, biuret, and isocyanurate amounts measured in the tape-strips, skin, and receptor foil (RF; *i.e.*, breakthrough), and calculated short-term absorption rates after 10- or 60-min exposure to a finite dose of HDI-containing slow- or fast-drying clearcoat in excised full-thickness human skin (N = 3)

Exposure time (min)	RF (μg)	Skin (μg)	Tape-strips (μg)	Total absorbed amount (μg)	Absorption rate (μg cm^{-2} h^{-1})
Slow-drying clearcoat					
HDI					
10	0.004 ± 0.003	0.437 ± 0.226	1.78 ± 0.411[a] 0.780 ± 0.270[c]	2.22 ± 0.431	1.33 ± 0.258[b] 0.733 ± 0.221[d]
60	0.002 ± 0.001	0.336 ± 0.116	1.85 ± 0.468[a] 0.747 ± 0.132[c]	2.19 ± 0.376	0.219 ± 0.038[b] 0.109 ± 0.004[d]
Biuret					
10	0.198 ± 0.175	8.21 ± 3.26	33.5 ± 11.7[a] 16.2 ± 8.57[c]	41.9 ± 13.3	25.2 ± 7.98[b] 14.8 ± 6.50[d]
60	0.039 ± 0.027	6.77 ± 1.78	34.9 ± 10.3[a] 15.5 ± 4.19[c]	41.7 ± 9.30	4.17 ± 0.930[b] 2.23 ± 0.29[d]
Isocyanurate					
10	3.19 ± 1.60	126.7 ± 58.5	507.8 ± 117.3[a] 238.8 ± 81.2[c]	637.7 ± 87.2	382.6 ± 52.3[b] 221.2 ± 56.0[d]
60	0.445 ± 0.299	108.5 ± 46.3	611.4 ± 198.2[a] 253.4 ± 48.5[c]	720.3 ± 177.6	72.0 ± 17.8[b] 36.2 ± 1.41[d]
Fast-drying clearcoat					
HDI					
10	0.006 ± 0.007	0.112 ± 0.014	1.16 ± 0.439[a] 0.506 ± 0.189[c]	1.28 ± 0.432	0.767 ± 0.259[b] 0.374 ± 0.109[d]
60	0.001 ± 0.0	0.275 ± 0.136	1.04 ± 0.269[a] 0.435 ± 0.131[c]	1.31 ± 0.394	0.131 ± 0.039[b] 0.071 ± 0.026[d]
Biuret					
10	0.040 ± 0.024	0.965 ± 0.705	1.63 ± 0.662[a] 0.888 ± 0.155[c]	2.64 ± 1.37	1.58 ± 0.825[b] 1.14 ± 0.514[d]
60	0.026 ± 0.021	0.727 ± 0.186	1.32 ± 0.510[a] 0.800 ± 0.254[c]	2.07 ± 0.493	0.207 ± 0.049[b] 0.155 ± 0.020[d]
Isocyanurate					
10	8.90 ± 13.3	133.1 ± 118.1	769.7 ± 329.0[a] 296.1 ± 77.3[c]	911.7 ± 426.6	547.0 ± 256.0[b] 262.9 ± 72.7[d]
60	0.611 ± 0.602	246.9 ± 159.4	720.9 ± 259.6[a] 292.8 ± 88.8[c]	968.5 ± 417.2	96.8 ± 41.7[b] 54.0 ± 24.4[d]

[a] Sum of tape-strips 1–30. [b] Calculated with tape-strips 1–30. [c] Sum of tape-strips 2–30; the first tape-strip was not included because of potential residual contamination from the dose applied to the skin. [d] Calculated with tape-strips 2–30; the first tape-strip was not included because of potential residual contamination from the dose applied to the skin.

the skin and it seems likely we achieve steady-state penetration over the short exposure intervals.

Our results are supported by the study of Bello *et al.*[10] They investigated the residence time of model isocyanates [octyl isocyanate, polymeric HDI (pHDI), polymeric isophorone diisocyanate isocyanurate (pIPDI) and methylenediphenyl diisocyanate (MDI)] in EA vehicle on hairless guinea pig skin *in vitro* using attenuated total reflectance-Fourier transform infrared spectrometry. They observed that approximately 85% of the octyl isocyanate, a low molecular weight isocyanate and thus similar to HDI, disappeared from the skin surface within 30 min. We observed that approximately 87.6% of the HDI disappeared from the skin surface within 30 min when exposed to the HDI in EA mixture. Bello *et al.*[10] further observed that polymeric isocyanates (pHDI and pIPDI) remained on the skin as unreacted species for many hours, with only 15–20% of the total isocyanate group disappearing from the skin within one hour.

The total percent recovery of all analytes for all experiments decreased with increasing exposure time. It is likely that with the increased exposure time, more monomeric and polymeric HDI are trapped in the skin tissue. Losses due to evaporation in this study are unlikely as the skins were occluded to minimize evaporation. Bello *et al.*[10] observed evaporative losses to be very small or non-existent in their study and support our findings. For experiments with HDI/EA, where EA enhanced penetration, we recovered less than 46% of the total HDI at all exposure time points and the amount of HDI extracted from the skin tissues was minimal. Our inability to recover all of the applied isocyanates is a limitation of this study. For the experiments with HDI neat and clearcoats, our total percent recoveries of all compounds were much higher than those in the HDI/EA experiments.

Previously, we compared dermal patch samplers with tape-strips.[9] These results indicated that monomeric and polymeric HDI is either reacting with the skin or rapidly penetrating into the deeper layers of the skin. The results of this current study confirm this rapid penetration into deeper layers of the skin.

We did not observe significant differences in the amount of monomeric and polymeric HDI in the skin for the different exposure time points (Fig. 2–4). This may be associated with the variability of the skin received from the different donors (*i.e.*, age, location) as well as limitations due to small sample size. It is also possible that we achieved steady-state penetration over the short exposure intervals and, thus, these differences did not exist or that saturation occurred, masking these differences.

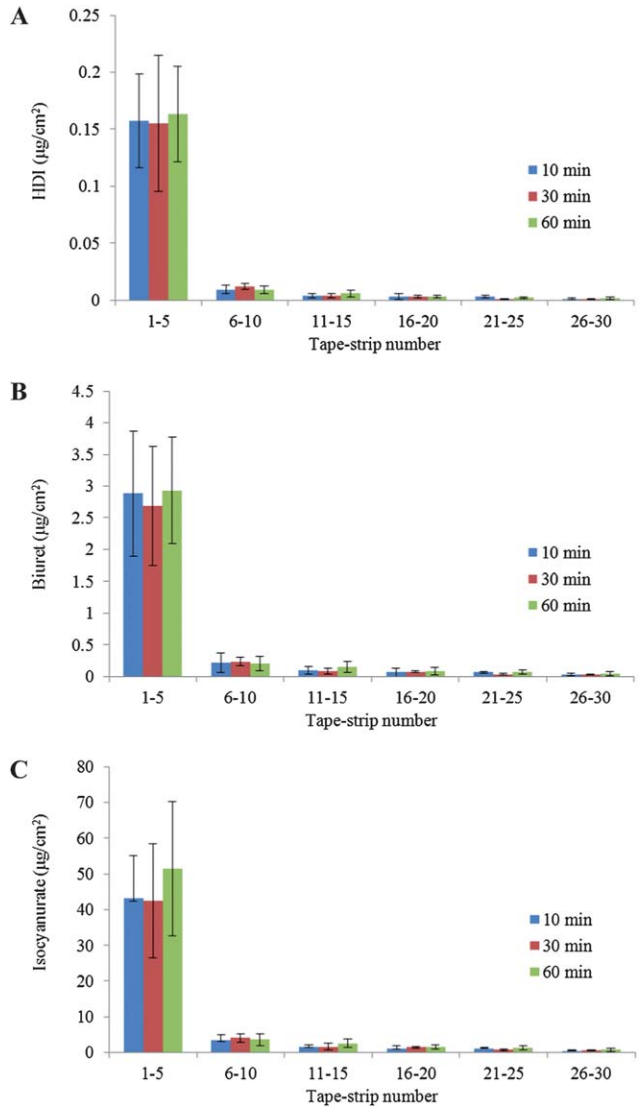

Fig. 3 Amount of (A) HDI, (B) biuret, and (C) isocyanurate measured in 30 sequential tape-strips collected from the human skin after epicutaneous application of 50 μl of slow-drying clearcoat (N = 3) for all time points.

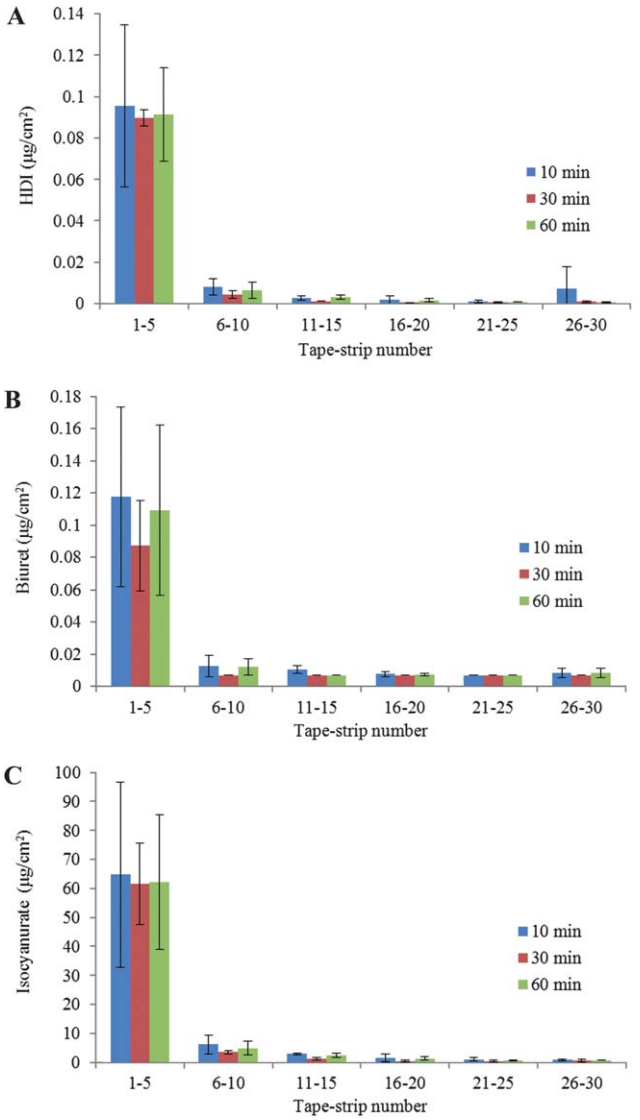

Fig. 4 Amount of (A) HDI, (B) biuret, and (C) isocyanurate measured in 30 sequential tape-strips collected from the human skin after epicutaneous application of 50 μl of fast-drying clearcoat (N = 3 for all time points).

Contribution of dermal exposure to internal dose

Because of the substantial dermal penetration of these compounds, we desired to estimate the duration of dermal exposure, using the short-term absorption rate (μg cm^{-2} h^{-1}) for isocyanates, required to reach the body burden equal to the inhalation threshold limit value (TLV) or occupational exposure limit (OEL). The following equation derived by Walker *et al.*[23] was used to calculate the skin absorption time:

$$Skin\ absorption\ time\ (h) = \frac{Total\ absorption\ at\ TLV\ or\ OEL\ (\mu g)}{Short-term\ absorption\ rate\ (\mu g/cm^2 h)\ \times area\ (cm^2)}$$

For example, if a worker were continuously exposed to HDI-containing clearcoat using a reasonable worst-case scenario, where the worker's lower arms and hands are exposed, the exposed skin area would be 2,320 cm^2.[8] If we assume a standard inhalation volume of 10 m^3 in an 8 h workday and 100% systemic availability of the inhaled dose, the total absorption at the American Conference of Governmental Industrial Hygienist (ACGIH) TLV of 34 μg m^{-3} for HDI monomer[24] would be 340 μg. Based on the short-term absorption rates for the slow-drying clearcoat of 10 min (1.33 μg cm^{-2} h^{-1}) and 60 min (0.219 μg cm^{-2} h^{-1}), it would take approximately 6.5 and 40 min, respectively, to achieve a dose of HDI equivalent to the ACGIH TLV. Using the 10 (0.733 μg cm^{-2} h^{-1}) and 60 min (0.109 μg cm^{-2} h^{-1}) short-term absorption rates that did not include the first tape-strip because of potential residual contamination from the dose applied to the skin, the skin absorption times were 12 and 81 min, respectively. For the fast-drying clearcoat the calculated skin absorption times [11.5 and 67 min for 10 (0.767 μg cm^{-2} h^{-1})

and 60 min (0.131 μg cm^{-2} h^{-1}) short-term absorption rates, respectively] were similar to those of the slow-drying clearcoat.

Similarly, we calculated the skin absorption time for biuret and isocyanurate using the Oregon OEL (500 μg m^{-3})[25] and the respective short-term absorption rates. Oregon is the only government entity in the United States to promulgate a short-term exposure limit (STEL) for HDI-based polyisocyanates biuret and isocyanurate. For biuret, the skin absorption time was much shorter for the slow-drying clearcoat [5 and 31 min for the 10 (52.2 μg cm^{-2} h^{-1}) and 60 min (4.17 μg cm^{-2} h^{-1}) short-term absorption rates, respectively] compared to the fast-drying clearcoat [81 and 618 min for the 10 (1.58 μg cm^{-2} h^{-1}) and 60 min 0.207 μg cm^{-2} h^{-1}) short-term absorption rates, respectively]. Isocyanurate had the shortest skin absorption times regardless of clearcoat drying time (between 14 s and 1.7 min). The skin absorption times doubled when calculated using short-term absorption rates that did not include the first tape-strip. Although the time to achieve a dose equivalent to that received through inhalation exposure at the TLV or OEL doubled, these times are still very short.

The calculation proposed by Walker *et al.*[23] is a nice approach that incorporates both absorption rate and toxicity data into a time-based number to provide an estimate for skin absorption time for a compound and the potential contribution that dermal exposure can make to the internal dose. This calculation makes a conservative assumption that 100% of the total inhaled dose has contact with the respiratory tract and, therefore, the total dose is systemically available. For this reason, the skin absorption time equation likely overestimates the absorbed amount and, consequently, also the skin absorption times. However, the skin absorption time can facilitate decisions making about the need to limit dermal exposure to protect individuals from systemic effects. The skin absorption time allows industrial hygienists and others to easily determine a time(s) that a body part(s) is/are dermally exposed to a dose necessary to achieve the same internal does as 8 h inhalation exposure at the TLV or PEL in addition to the benefit that the risks related to additive exposures can be evaluated.

Fasano *et al.*[26] also provided an alternate approach to estimating absorbed dose and time to reach the TLV/OEL equivalent. They suggested using the total amount of chemical in the receptor fluid (absorbed, in our case breakthrough) and skin (absorbable, skin, and tape-strips), an exposure area (2,320 cm^2) with an assumption that 100% of the dermal dose is systemically available. For HDI, the absorbed-absorbable dose a 10 and 60 min exposure would yield potential exposure approximately 1.5 times the current TLV for the slow-drying clearcoat and 87% for the fast-drying clearcoat. For biuret, the absorbed-absorbable dose at 10 and 60 min exposure would yield potential exposure approximately 2 times the current OEL for the slow-drying clearcoat and 10% for the fast-drying clearcoat. For isocyanurate, the absorbed-absorbable dose at 10 and 60 min exposures would yield potential exposure approximately 30–45 times the current OEL.

Typically, short-term exposure experiments would be conducted using diffusion cells with a receptor fluid. A finite does (*i.e.*, μl cm^{-2}) would be applied to a donor chamber and the opening occluded. At the end of the exposure interval, the skin surface would be washed and rinsed to remove any chemical on the skin surface and the receptor fluid analyzed. The remaining skin would also be extracted and analyzed for the compound of interest. In our study, we did not use diffusion cells. Isocyanates are highly reactive, reacting with nucleophiles, such as amines, alcohols, water, carboxylic acids, and thiols[10] thus making it difficult to select an acceptable receptor fluid for use of this experimental setup. The finite dose applied in our study was not evenly distributed over a certain area and the area used to calculate the short-term absorption rate was that of the tape-strip. It is likely that we have underestimated our short-term absorption rates due to the overestimation of exposure area, and, therefore, our results may overestimate the skin absorption times.

Here, we have demonstrated that monomeric and polymeric HDI rapidly penetrate into and beyond the stratum corneum of excised human full-thickness skin. We have also shown that the tape-strip is capable of detecting monomeric and polymeric HDI in human skin at least 30 tape-strips deep. We determined that differences exist in penetration patterns between a slow- and fast-drying clearcoat. This is a clear indication that the composition of a clearcoat mixture may affect the penetration rate of the individual isocyanate compounds (both monomeric and polymeric). Further, by relating the absorbed dose to the dose received at equivalent air concentration corresponding to TLV or OEL, we were able to show that the dose received through dermal exposure to isocyanate-containing clearcoats in an occupational setting has a great potential to exceed established regulatory limits for inhalation exposure. Thus, our results indicate that dermal exposure can greatly contribute to the internal dose in workers occupationally exposed to isocyanates. Therefore, a critical need exists for quantitative monitoring of dermal exposure in exposed worker populations and to re-evaluate regulatory exposure limits for both dermal and inhalation exposure of isocyanates. Additionally, the use of proper dermal protective equipment to reduce dermal exposures is necessary when working with these compounds.

Conclusion

Although this study is limited in size, we have demonstrated that monomeric and polymeric HDI rapidly penetrate into and beyond the stratum corneum. We have demonstrated the potentially large contribution that dermal exposure may have on the internal dose and determined the time it would take to reach a body burden equal to inhalation exposures at occupational exposure limits. We have also further validated the tape-strip method for measuring monomeric and polymeric HDI. Future studies, with larger sample sizes, should be conducted to establish protective short-term exposure limits for dermal exposure to isocyanates. In addition, we have also demonstrated the potential use of excised human full-thickness skin and the tape-strip technique as a tool to investigate penetration patterns of industrial chemicals and their mixtures through human skin.

Acknowledgements

This research was approved by the Institutional Review Board in the Office of Human Research Ethics at the University of North Carolina at Chapel Hill and supported by grants from the

National Institute for Occupational Safety and Health (T42 OH008673) as well as NIOSH Training Grant and NC Occupational Safety and Health Education and Research Center Pilot Grant (T42/CCT422952). The authors are grateful to University of North Carolina Pathology and Laboratory Medicine Department for providing skin tissue specimens.

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