Quantitative monitoring of dermal and inhalation exposure to 1,6-hexamethylene diisocyanate monomer and oligomers

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Respiratory sensitization and occupational asthma are associated with exposure to 1,6-hexamethylene diisocyanate (HDI) in both monomeric and oligomeric forms. The monomer and polymers of diisocyanates differ significantly in their rates of absorption into tissue and their toxicity, and hence may differ in their contribution to sensitization. We have developed and evaluated a liquid chromatography/mass spectrometry (LC-MS) method capable of quantifying HDI and its oligomers (uretidone, biuret, and isocyanurate) in air, tape-stripped skin, and paint samples collected in the automotive refinishing industry. To generate analytical standards, urea derivatives of HDI, biuret, and isocyanurate were synthesized by reaction with 1-(2-methoxyphenyl)piperazine and purified. The urea derivatives were shown to degrade on average by less than 2% per week at −20 °C over a 2 month period in occupational samples. The average recovery of HDI and its oligomers from tape was 100% and the limits of detection were 2 and 8 fmol μl^{-1}, respectively. Exposure assessments were performed on 13 automotive spray painters to evaluate the LC-MS method and the sampling methods under field conditions. Isocyanurate was the most abundant component measured in paint tasks, with median air and skin concentrations of 2.4 mg m^{-3} and 4.6 μg mm^{-3}, respectively. Log-transformed concentrations of HDI ($r = 0.79$, $p < 0.0001$) and of isocyanurate ($r = 0.71$, $p < 0.0001$) in the skin of workers were correlated with the log-transformed product of air concentration and painting time. The other polyisocyanates were detected on skin for less than 25% of the paint tasks. This LC-MS method provides a valuable tool to investigate inhalation and dermal exposures to specific polyisocyanates and to explore relative differences in the exposure pathways.

Introduction

Exposure to the monomeric and polymeric forms of 1,6-hexamethylene diisocyanate (HDI) may cause adverse health effects, specifically to the respiratory tract and skin. A number of studies describe occupational asthma[1–3] and allergic contact dermatitis[4,5] associated with HDI exposure. Although the mechanistic pathway is unknown, there is increasing toxicological and epidemiological evidence that dermal exposure to diisocyanates play a role in the development of respiratory sensitization and occupational asthma.[6] Automotive paints based on HDI commonly include, in addition to the monomer, the HDI oligomers: uretidone, biuret, and isocyanurate (Fig. 1).

Dermal[7–10] and inhalation[11–15] exposures to isocyanates have been characterized in spray painters in the automotive repair industry. Methods have been published that use liquid chromatography-mass spectrometry (LC-MS) to quantify exposure to HDI oligomers (*i.e.*, biuret and isocyanurate).[16,17] However, these methods lack pure analytical standards for the oligomers. The most recent method[17] uses chemiluminescence nitrogen detection to characterize reference solutions for use as analytical standards. Although preparation of pure analytical standards is not trivial, once generated, such pure analytical standards provide a simpler, more efficient way of quantifying specific HDI oligomers.

Recently, investigators have reported inhalation exposure to isocyanates as total reactive isocyanate groups (TRIG),[18–20] rather than specific isocyanate species. Measuring TRIG, which

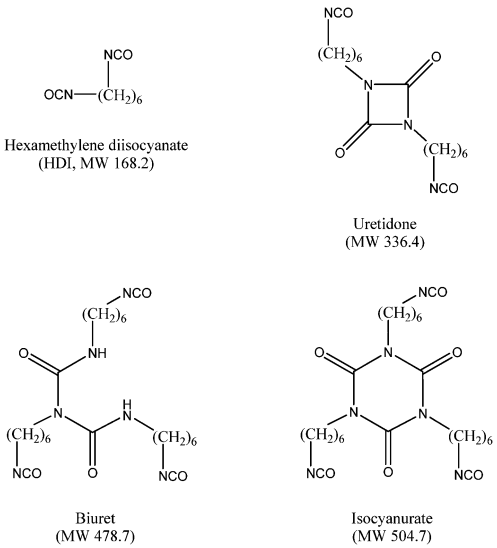

Fig. 1 Molecular structures of 1,6-hexamethylene diisocyanate monomer and its oligomers.

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is the sum of free isocyanate (NCO) groups found on all isocyanate species in a sample, would be appropriate if all isocyanates behaved the same. However, rates of absorption into tissue and toxicity may vary between monomeric and polymeric isocyanates because of differences in their physical and chemical properties, including molecular weight, lipid solubility, and reactivity.

Despite evidence suggesting that isocyanates may differ in absorption into tissue and toxicity, to our knowledge, quantitative analysis has not been used for identification of isocyanate species for both inhalation and dermal exposures. Our objective for this study was to develop an analytical method capable of quantifying HDI and its oligomers in air-filter, dermal tape-strip, and paint samples collected in occupational exposure settings. To meet this objective, our previously published tape-strip-LC-MS method for quantitation of dermal exposure to HDI[9] was modified to also quantify dermal exposure to the most common HDI oligomers in hardener (using purified analytical standards) and was adapted for the analysis of air and paint samples. The specificity of the analytical method we describe provides investigators with a tool to quantify exposure to individual monomeric and polymeric isocyanates and to explore quantitative relationships between the different routes of exposure for characterization of toxicity and adverse health effects.

Methods

Synthesis of standards

All chemicals used in this study were obtained from Sigma Aldrich (St. Louis, MO, USA), unless otherwise specified. Desmodur N 3200 and N 3300 A (Bayer Material Science, Pittsburgh, PA, USA) were used as the sources of biuret and isocyanurate, respectively. It is important to note that the Desmodur products (Bayer) are not pure (*i.e.*, <85%) and often contain significant amounts of other isocyanates.[21]

All synthesized standards were characterized by ^{1}H nuclear magnetic resonance (^{1}H-NMR) on an Inova spectrometer (Varian Inc., Palo Alto, CA, USA) at 500 MHz in dimethyl sulfoxide (DMSO) and by mass spectra on a Surveyor LC-MS system (Thermo, Austin, TX, USA) in methanol.

The urea derivatives of HDI (HDIU) and 1,8-octamethylene diisocyanate (ODIU) were synthesized according to the National Institute for Occupational Safety and Health (NIOSH) Method 5521.[22] As reported previously,[9] synthesized HDIU and ODIU were >98% pure based on their total ion (*m*/*z* 100 to 2000) chromatograms. The urea derivatives of biuret (BU) and isocyanurate (IU) were synthesized as described below.

1-(2-Methoxyphenyl)piperazine derivative of biuret (BU). In a 100 ml flask, 1-(2-methoxyphenyl)piperazine (MPP, 782 mg, 4.07 mmol) was stirred into 25 ml of DMSO under argon at 60 °C. A solution of N 3200A (Bayer) in DMSO (26 g l^{-1}) was added (25 ml) slowly over 6 min at 64–70 °C, stirring vigorously. This clear solution was poured onto 200 ml of ice water. Voluminous white crystals precipitated, which were then filtered and lyophilized to obtain 1.29 g of dry product as a white powder.

1-(2-Methoxyphenyl)piperazine derivative of isocyanurate (IU). In a 100 ml flask, MPP (626 mg, 3.260 mmol) was stirred into 25 ml of DMSO under argon at 64 °C. A solution of N 3300A (Bayer) in DMSO (21 g l^{-1}) was added (25 ml) slowly over 6 min at 64–68 °C and then stirred continuously for 35 min. This clear solution was added slowly with vigorous stirring to 150 ml of cold water. The reaction was frozen at −80 °C, then lyophilized to obtain 796 mg of dry product as a white powder.

BU and IU were purified by high performance liquid chromatography (HPLC) using a Varian Vista series HPLC and an Alltech C-8 column (22 × 250 mm, 10 μm particle size) (Nicholasville, KY, USA) with an octadecylsilica packed precolumn (37–53 μm particles) eluted with methanol (A) and water (B) at 3 ml min^{-1}. Solvent composition was 85% A during the first 3 min, increasing to 100% A at 50 min. The UV absorbance of the eluate was monitored at 254 nm (PerkinElmer LC-85B spectrophotometer, Waltham, MA, USA). BU and IU were each dissolved in methanol to 10 mg ml^{-1} and injected manually, 1 ml at a time. The most intense peaks, corresponding to BU and IU, were collected in separate vials. After collection, methanol was evaporated by heating to 50 °C under a gentle stream of nitrogen. The remaining water was lyophilized to obtain ~10 mg each of BU and IU as a dry white powder. LC-MS analysis of the HPLC-purified BU and IU dissolved in methanol (20 pmol μl^{-1}) showed that the products were >97% pure (Fig. 2).

Preparation of standard curves

The urea derivatives were dissolved in methanol to make stock solutions of each derivative (1 nmol μl^{-1}). Preliminary standards of each stock solution, except for ODIU, were prepared by diluting the stock solutions to 400, 200, 100, 20, 10, 4, 2, 0.4, 0.08, or 0.02 pmol μl^{-1}. Internal standard solution was made by diluting ODIU stock to 4 pmol μl^{-1}. The final standards were created by combining 200 μl of the internal standard solution with 200 μl each of the three preliminary standards for a total volume of 800 μl. Thus, the ten final standards were $^{1}/_{4}$ as concentrated as the preliminary standards, each with an internal standard concentration of 1 pmol μl^{-1}. Standard curves were generated by regressing the nominal concentration on the response ratio (*i.e.*, ratio of integrated analyte and internal standard peak) for each standard. The standards were analyzed in triplicate to account for instrument variability. The standard curve generated with the BU data was used to estimate concentrations of the urea derivative of uretidone (UU).

Sample collection

Exposure monitoring in automotive spray painters. Air monitoring, dermal tape-stripping, and bulk sampling of the paint product were performed on 13 automobile repair spray painters who applied clearcoat inside ventilated booths. None of the painters wore protective clothing or gloves; all wore a half-face respirator equipped with organic vapor cartridges.

Derivatizing solution was made by dissolving 2 g of MPP in 1 l of 30% v/v solution of *N*,*N*-dimethylformamide (DMF) in acetonitrile. The derivatizing solution (2 g l^{-1} MPP in 30% DMF) was then delivered to glass vials to be used for sample collection.

Bulk samples of the clearcoat being sprayed by the painter were collected before each task. Samples (10 μl) of the mixed

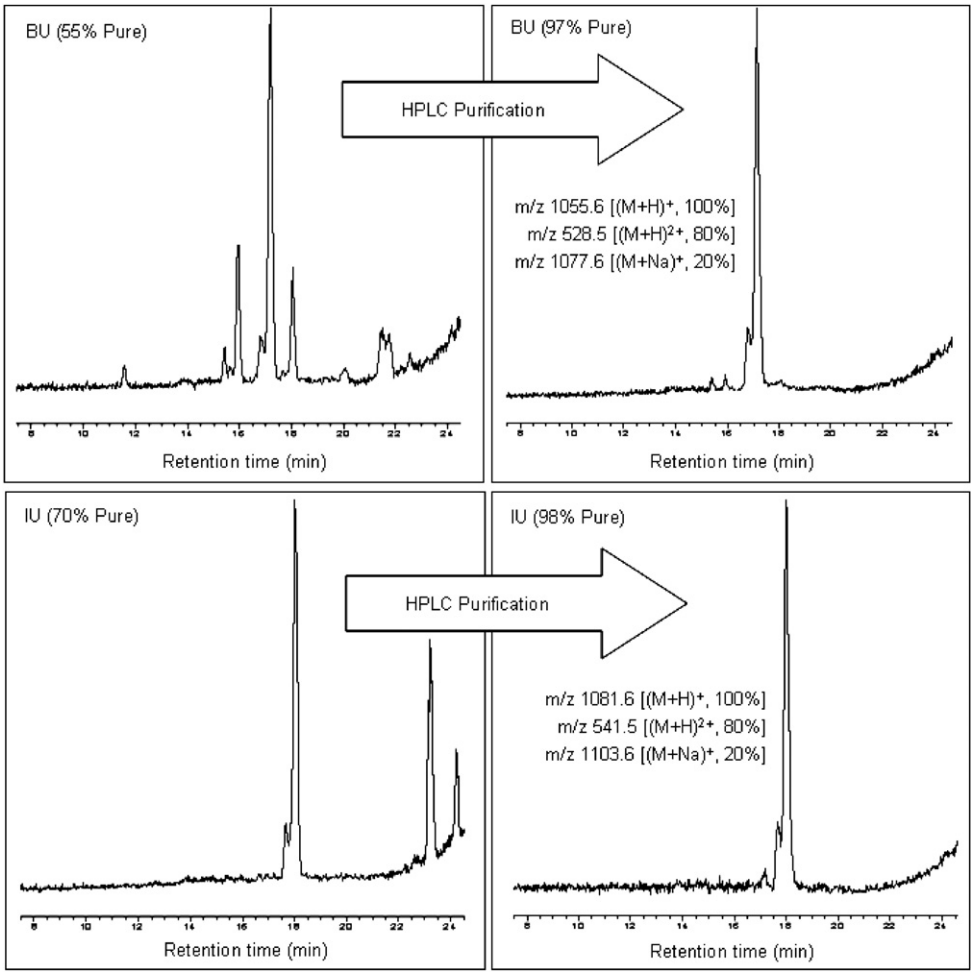

Fig. 2 Total ion chromatograms (*m/z* 100–2000) from LC-MS analysis of 20 pmol μl^{-1} solutions of the synthesized urea derivatives of biuret (BU) and isocyanurate (IU) before and after HPLC purification.

clearcoat were drawn into a 20 µl pipette and delivered to glass vials (I-Chem, New Castle, DE, USA) filled with 15 ml of derivatizing solution. The pipette tip was also ejected into the solution to eliminate side-wall losses due to the viscosity of the clearcoat.

Personal air samples were collected in the worker's breathing zone during each task using a two-stage filter sampling system housed in 37 mm polystyrene cassette (SKC Inc., Eighty Four, PA, USA), which is similar to the ISO-CHEK® sampler (Omega Specialty Instrument Co., Houston, TX, USA), and a high-flow pump at 1 l min^{-1} (SKC). The first stage held a polytetrafluoroethylene filter (PTFE; Millipore Corp., Billerica, MA, USA) with 5 µm pore size designed to collect aerosols. The second stage held a glass-fiber filter (GFF; SKC) with 1 µm pore size designed to collect vapor. The GFF was impregnated with derivatizing agent by adding 400 µl of 43 mg l^{-1} MPP in toluene to the filter and allowing toluene to evaporate before placing the filter in the cassette. A 37 mm cellulose pad (Millipore) was used to support the GFF. The pumps were calibrated before and after sampling using a DryCal® primary flow meter (BIOS Corp., Butler, NJ, USA). For quality control, air sample blanks were collected by opening and closing prepared cassettes in the occupational setting. Immediately after sampling, both the PTFE and GFF were placed into 20 ml glass vials (I-Chem) containing 5 ml of derivatizing solution to minimize the time for any competing reactions, such as isocyanate polymerization.

Tape-strip sampling was performed immediately after each task using a Cover-Roll® adhesive tape (Beiersdorf AG, Hamburg, Germany) cut into 4 × 2.5 cm^2 strips. Three successive tape-strips were collected on the dorsal side of each hand and on the dorsal and volar side of each arm, as described elsewhere.[9] In order to prevent cross contamination, forceps cleaned with acetone were used to apply and remove the tape-strips and place them in 8 ml glass vials (Kimble, Vineland, NJ, USA) containing 5 ml of derivatizing solution. For quality control, tape blanks were collected prior to paint application, which included tape-strip samples of each worker's arm (sample blanks) and samples of unused tape (field blanks).

Recovery of polyisocyanates from tape samples. Recovery of polyisocyanates from tape (Cover-Roll® adhesive tape cut into 4 × 2.5 cm^2 strips) was evaluated using clearcoat prepared at an automobile repair shop. Clearcoat was chosen because it represents the chemical matrix likely to be deposited on worker skin. The clearcoat was a 3 : 1 mixture of Deltron® DC4000 clearcoat and DCH3095 high temperature hardener (PPG Industries, Strongsville, OH, USA).

The mixture was applied (10 μl) to strips of tape in 20 ml glass vials (I-Chem). Vials without tape received 10 μl of clearcoat and were used as blank reference samples. Reference samples were necessary as the concentration of each isocyanate in the clearcoat is unknown. If isocyanates in clearcoat react with tape, then we would expect to see a difference in isocyanate concentrations between the tape samples and reference samples.

The clearcoat added to tape and reference samples was allowed to stand for 3 min, which was determined to be the approximate time required to perform one set of tape-strippings on a worker. After 3 min, derivatizing solution was added (15 ml) to the vials. All the samples were shaken and then stored at 4 °C until returned to the laboratory, and were then stored at −40 °C.

These samples were processed as subsequently described for paint samples and analyzed by LC-MS. The concentration of each isocyanate was calculated and compared between the reference samples ($N = 6$) and tape samples ($N = 6$) to determine the relative recovery of each polyisocyanate from tape.

Sample processing

After sample collection, all samples in 5 ml of derivatizing solution were shaken thoroughly and then stored in a cooler (~4 °C) until return to the laboratory and storage at either −20 or −40 °C. Unless otherwise specified, both the tape and air samples were processed identically. The samples were returned to room temperature, acetic anhydride was added (100 μl) to acetylate residual MPP. After 15 min, internal standard solution (52 pmol μl^{-1}) was added (100 μl) to give an internal standard concentration of 1 pmol μl^{-1}.

The paint samples were processed after thawing to room temperature by addition of acetic anhydride (200 μl), allowing 15 min for the reaction to take place. Internal standard solution (2 pmol μl^{-1}) was then combined (1 : 1 v/v ratio) with aliquots of each paint sample to give an internal standard concentration of 1 pmol μl^{-1}.

LC-MS analysis

After processing, all samples were analyzed by LC-MS. Using a Thermo Surveyor LC, a Thermo Aquasil C18 column (4.6 × 50 mm, 3 μm particle size) with Uniguard® guard column was eluted with acetonitrile (A) and 0.1% acetic acid in water (B) at 1 ml min^{-1}. Water was generated using a NANOpure Diamond™ purifier (Barnstead International, Dubuque, IA). Solvent composition was 20% A during the first minute, increasing to 60% A at 16 to 18.5 min, increasing to 80% A at 24 to 25 min, and returning to the original conditions at 27 to 30 min. The sample tray was maintained at 4 °C and the column at 40 °C. Partial loop 10 μl injections were made by an autosampler. The flow from the LC to the MS was diverted to waste by a 6-position valve (Valco Instruments, Houston, TX, USA) at 0 to 10.5 min and 23 to 30 min.

A Thermo Surveyor quadrupole MS was used in the electrospray mode monitoring for positive ions. Nitrogen sheath gas, regulated at 22 psi, was produced by an NG10LA nitrogen generator (Peak Scientific, Punta Gorda, FL, USA). The probe temperature and cone voltage were maintained at 575 °C and 60 V, respectively. Selective ion monitoring (SIM) was performed for the molecular ions of interest: the $[M + H]^+$ ions for HDIU (*m*/*z* 553.3), UU (*m*/*z* 721.3), BU (*m*/*z* 1055.7), IU (*m*/*z* 1081.7), and the internal standard, ODIU (*m*/*z* 581.3). Each SIM scan covered a *m*/*z* range of 1 mass unit. The corresponding time ranges for the SIM are 10 to 14 min for HDIU, 12 to 16 min for ODIU, 13.5 to 16.5 min for UU, 16 to 20 min for BU, and 16.5 to 21.5 min for IU. Full scan data from *m*/*z* 500 to 650, *m*/*z* 700 to 800, and *m*/*z* 1000 to 1150 were also collected between 10 to 16 min, 13.5 to 17.5 min, and 16.5 to 21.5 min, respectively. Overlapping scans were performed simultaneously with the LC-MS by alternating between the different scans at 1 s intervals.

Storage stability

The stability of derivatized polyisocyanates in occupational samples was evaluated using the first of three successive tape-strips collected from the dorsal side of each hand and the volar side of each arm for worker 3. After adding acetic anhydride (100 μl), the samples were divided evenly (1.0 ml) into three separate vials, which were then stored at −40 °C, −20 °C, and 4 °C. Fresh internal standard solution (2 pmol μl^{-1}) was prepared and combined (1 : 1 v/v) with aliquots of each sample just prior to analysis. The samples were analyzed by LC-MS every two weeks over a two month period. The concentration of each polyisocyanate was determined using a new standard curve and the percent change in concentration over time was monitored.

Data analysis

The data were analyzed using SAS 9.1 statistical software (Cary, NC, USA). Air, tape-strip, and paint samples containing levels of polyisocyanates below the limits of quantitation (LOQ) and detection (LOD) were assigned values determined by dividing the respective limits by the square root of two. Polyisocyanates collected with three successive tape-strips were summed together to estimate the dermal exposure to each sampled site of skin. However, subsequent tape-strips were excluded if the previous tape-strip collected levels below the LOD. These site-specific levels were averaged to determine the mean dermal exposure level for each task. Each tape-strip removes approximately one layer of corneocytes and any chemicals in that cell layer.[23] According to Marks *et al.*,[24] corneocytes average 0.66 μm in thickness. Given the uncertainty and variability associated with tape-stripping, we assumed that triplicate tape-stripping would collect approximately 1 mm^3 of skin (10 cm^2 area × 1 μm thickness). Thus, dermal exposure was reported as a concentration in the skin (ng mm^{-3}). Shapiro–Wilks tests for normality indicated that the dermal concentration data and the product of air concentration and paint time data were approximately log-normal for HDI ($W = 0.81$, 0.97, respectively) and isocyanurate ($W = 0.95$, 0.93, respectively). Therefore, regression analysis was performed on the natural log-transformed data.

Results

Performance of the analytical method

Different weighting factors ($w = x^{-1}, x^{-2}, y^{-1}, y^{-2}$) were evaluated for fitting standard curves. As specified in the literature,[25] the

weighting factor that gave the smallest sum of the absolute relative error as a percentage of the nominal concentration was used for fitting the standard curve. The linear range of the standard curve was 0.005 to 1 pmol μl^{-1} for HDIU ($w = x^{-2}$, $R^2 = 0.968$) and 0.02 to 5 pmol μl^{-1} for BU ($w = y^{-2}$, $R^2 = 0.999$) and IU ($w = y^{-2}$, $R^2 = 0.992$). However, after analyzing samples, it was found that 47% of air samples and 94% of paint samples contained levels of isocyanurate greater than 5 pmol μl^{-1}. Levels of the other polyisocyanates in those samples were well within the dynamic range. To extend the upper limit of quantitation to 100 pmol μl^{-1}, the IU data were fit using a third order polynomial equation ($w = y^{-2}$, $R^2 = 0.997$). Polynomial fitting has proven useful for analyzing mixtures of highly variable compounds.[26] Thus, the polynomial curve was used to quantify isocyanurate in all occupational samples. None of the samples contained concentrations of isocyanurate exceeding 100 pmol μl^{-1}.

All the standard curves predicted values within 20% of the nominal concentrations for the dynamic range. The LOD was 2 and 8 fmol μl^{-1} for HDI and the oligomers, respectively, as determined using the average of six peak areas with a signal to noise ratio $\geq$3. The LOQ was 5 and 20 fmol μl^{-1} for HDI and the oligomers, respectively, as determined using the average of six peak areas with a signal to noise ratio $\geq$10.

Analyzing three sets of quality control standards representing the low, middle, and high concentrations of the linear calibration curves allowed the evaluation of the precision and accuracy of the LC-MS assay. A set of isocyanurate standards representing the upper limit of the polynomial calibration curve was also used for the evaluation. Each set of quality control standards contained three replicates. In addition to the intra-day variation, analysis was performed one week later to evaluate the inter-day variation. The results are given in Table 1. The average quantified levels were within $\pm$13% of the nominal concentrations for all the analyzed standards, each with a relative standard deviation less than 7%.

The LC-MS method was able to separate each of the HDI-based polyisocyanates in occupational samples. Tape samples are expected to provide the most complex matrix for analysis due to the presence of dissolved tape adhesive and skin components. Fig. 3 presents chromatograms from the LC-MS analysis of a tape sample, showing symmetrical peaks for each of the isocyanates of interest with no interfering peaks.

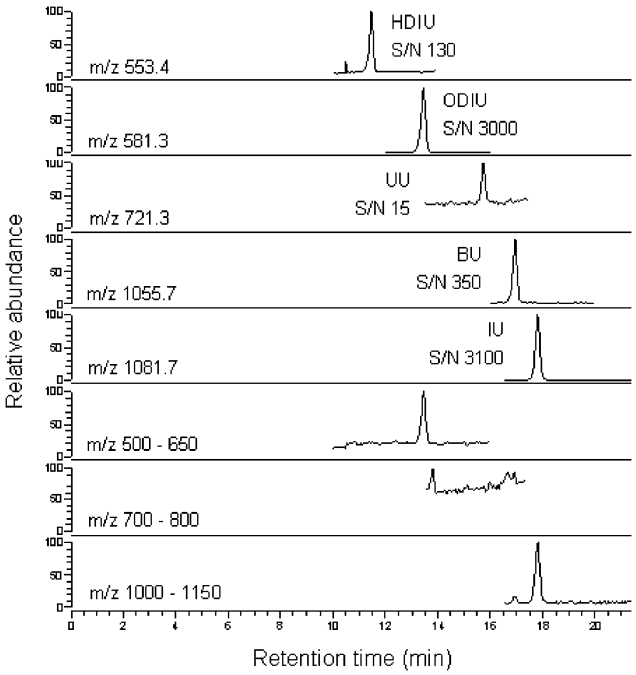

Fig. 3 Chromatograms from LC-MS analysis of a tape sample collected from the arm of an automobile painter who did not wear protective clothing during paint application. Four different isocyanates were collected from the skin and quantitated as the urea derivatives of hexamethylene diisocyanate (HDIU), uretidone (UU), biuret (BU), and isocyanurate (IU). The urea derivative of octamethylene diisocyanate (ODIU) was added for the internal standard.

Table 1 Intra- and inter-assay accuracy and precision [relative standard deviation (RSD)] for quality control standards of HDI, biuret, and isocyanurate

		Intra-assay		Inter-assay	
Analyte and standard curve for quantitation	Nominal concentration/pmol μl^{-1}	Accuracy (% of nominal)	RSD (%)	Accuracy (% of nominal)	RSD (%)
HDI monomer					
Linear	0.005	98	6.8	97	6.2
	0.1	111	1.2	110	3.8
	1	87	0.7	88	4.7
Biuret					
Linear	0.02	100	2.4	105	5.5
	0.5	105	4.1	99	0.8
	5	99	2.7	95	1.9
Isocyanurate					
Linear	0.02	100	4.7	106	13
	0.5	107	6.1	101	1.9
	5	93	1.8	89	2
Polynomial	0.02	100	4.4	106	13
	0.5	106	6.2	100	1.9
	5	99	1.9	94	2.2
	100	99	1.3	93	1.7

Recovery of polyisocyanates from tape samples

The average recovery of HDI monomer, uretidone, biuret, and isocyanurate from tape spiked with clearcoat was 106 ± 12, 116 ± 8, 110 ± 13, and 106 ± 12%, respectively. The sample mean for the tape ($N = 6$) did not differ significantly at a 0.05 level from the sample mean for the references ($N = 6$) for the measured polyisocyanates, except for uretidone.

Stability of derivatized polyisocyanates in tape samples

The tape samples used for the storage stability analysis did not contain uretidone, but did contain the other polyisocyanates of interest. Degradation was linear for all the urea derivatives of polyisocyanates tested. All three urea derivatives degraded at 6–7% per week at 4 °C. Degradation was less at lower temperature, to 2% per week for HDIU and IU, whereas BU losses were minimal at −40 °C (Table 2) over a 2 month period.

Exposure monitoring of automotive spray painters

Exposure assessments were carried out on workers performing 35 different paint tasks. Table 3 presents a summary of the exposure assessment results. Distributions of the exposure data are positively skewed. Thus, median values are the best measure of central tendency. Detectable levels of HDI and isocyanurate were found on the skin for 71 and 100% of the tasks, respectively. The other polyisocyanates were detected on skin for less than 25% of the tasks. Therefore, statistical analysis was confined to the HDI and isocyanurate exposure data exclusively.

Table 2 Degradation rates of urea derivatives of polyisocyanates collected from painter skin, stored at different temperatures over a two month period

		Change in concentration (% week^{-1} ± 95% confidence interval)[b]		
Urea derivatives[a]	N	−40 °C	−20 °C	4 °C
HDI	4	−2.16 ± 1.32	−1.85 ± 1.46	−5.91 ± 0.98
Biuret	4	−0.01 ± 1.75	+0.56 ± 2.30	−6.60 ± 1.30
Isocyanurate	4	−1.72 ± 1.14	−1.24 ± 1.40	−6.83 ± 0.90

[a] Polyisocyanates were collected from painter's skin using tape-strips. Four tape-strips were derivatized, split into 3 storage groups, and then analyzed with LC-MS on a bimonthly basis. [b] Linear regression was used to estimate the percent change in concentration over time.

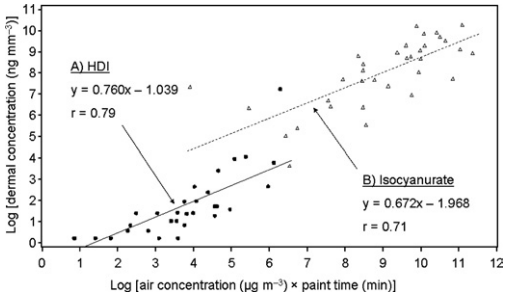

Fig. 4 Regression of log-transformed dermal concentration of (A) HDI and (B) isocyanurate on the log-transformed product of the respective air concentration and paint time for workers not wearing protective clothing or gloves.

A relationship was expected between dermal concentration and the product of breathing-zone concentration (intensity of overspray surrounding the painter) and paint time (duration of time in which overspray can deposit on the skin). Log-transformed dermal concentration correlated with the log-transformed product of breathing-zone concentration and paint time for HDI ($r = 0.79$, SE = 0.94, $p < 0.0001$, Fig. 4A) and isocyanurate ($r = 0.71$, SE = 1.14, $p < 0.0001$, Fig. 4B), respectively. A test for coincident lines ($\alpha = 0.05$) revealed that the two lines in Fig. 4 do not have significantly different slopes ($p = 0.580$), but do have significantly different intercepts ($p < 0.0001$).

The two-stage sampler allowed us to estimate the aerosol–vapor partitioning of HDI. While HDI oligomers exist primarily as aerosol in overspray, HDI monomers exist partially as vapor due to its high vapor pressure (0.05 mm Hg at 25 °C). Based on our measurements, the fraction of HDI aerosol in overspray averaged 57 ± 9.4% (95% confidence interval).

Discussion

The analytical method described in this study is specific (employing SIM to identify individual HDI-based polyisocyanate species) and sensitive (capable of detecting trace amounts of isocyanates in different media). By synthesizing and purifying urea derivatives of biuret and isocyanurate for use as standards,

Table 3 Assessments of exposure to HDI and related oligomers conducted on automotive spray-painters[a]

	Tape-strip sampling ($N = 35$)				Air sampling ($N = 34$)				Paint sampling ($N = 34$)			
	Dermal concentration/ng mm^{-3}				Breathing zone concentration/µg m^{-3}				Paint concentration/mg l^{-1}			
Analyte	Mean[b]	Median[b]	Range[c]	Non-detects (%)	Mean[b]	Median[b]	Range[c]	Non-detects (%)	Mean[b]	Median[b]	Range[c]	Non-detects (%)
HDI	48.5	3.92	nd–1400	29	20.2	7.24	nd–179	21	202	137	nd–530	3
Uretidone	35.9	9.51	nd–292	86	17.2	5.06	nd–124	61	2150	185	nd–17 000	32
Biuret	1320	13.5	nd–30 300	80	609	4.58	nd–7730	77	1760	8.12	nd–23 800	68
Isocyanurate	6950	4590	38.3–29 300	0	3540	2370	7.06–17 800	0	52 800	44 300	3980–154 000	0

[a] Samples were obtained from 13 workers performing 35 separate paint tasks. One air sample was excluded due to pump malfunction and one paint sample was lost in transport. [b] Levels below the limits of detection and quantitation were assigned values by dividing the respective limits by $\sqrt{2}$. [c] Levels below the limit of detection (non-detects) are represented by the symbol "nd".

we were able to confidently quantify the mass of individual polyisocyanates. The response ratios were linear over 2.3 orders of magnitude for each isocyanate, while a third-order polynomial equation was able to explain the response ratio for isocyanurate over 3.7 orders of magnitude. These dynamic ranges cover 100% of the levels in the occupational samples collected in this study. The linear and polynomial calibration curves were used to quantify levels of each isocyanate in quality control standards, demonstrating high precision and accuracy and consistency over a one-week time period.

The two-stage air sampling method used in this study is similar in design to the commercially available ISO-CHEK® method. The ISO-CHEK® method has been shown to perform similarly to other commonly used air sampling methods (*i.e.*, NIOSH 5521) during automotive spray-painting operations.[27] One advantage of a two-stage sampler is that it attempts to separate the vapor and aerosol portion of monomeric isocyanates (*i.e.*, HDI). The fraction of HDI aerosol measured with the two-stage sampler averaged 57 ± 9.4%. In contrast, Rando *et al.*[28] used denuder sampling in conjunction with impaction/filter sampling to estimate the aerosol fraction of HDI monomer in automotive paint overspray at 20%. These conflicting observations may reflect actual differences in sampled conditions or could be due to measurement bias.

Because of the uncertainty associated with measuring aerosol–vapor partitioning, the HDI air concentrations were reported as total HDI. The median breathing-zone concentrations of HDI, uretidone, biuret, and isocyanurate were 7.24, 5.06, 4.58, and 2370 μg m^{-3}, respectively. Because the breathing-zone concentrations represent task-based (20 min or less) time-weighted averages (TWAs), short term exposure limits (STELs) are appropriate for comparison. The sensitivity of the analytical method allows detection of HDI and its oligomers in air (sampling at 1 l min^{-1} for 15 min) at concentrations that are over 700 times lower than the NIOSH ceiling limit for HDI (140 μg m^{-3}) or the Oregon STEL for biuret and isocyanurate (1 mg m^{-3}). Oregon is the only government entity in the United States to promulgate an STEL for HDI-based polyisocyanates. The Oregon STEL was exceeded in 65% of the samples, with the highest isocyanurate air concentration (17 800 μg m^{-3}) being over 15 times greater than the recommended limit.

The workers in this study were protected by half-face respirators equipped with organic vapor cartridges. A recent study found that the average workplace protection factor for such respirators was 388 for HDI-based polyisocyanates.[29] Such protection would reduce the inhaled portion of the highest isocyanurate concentration to approximately 45 μg m^{-3}. This level of protection, however, can be achieved only when the respirator is worn and maintained properly, which is not always the case.

Likewise, personal protective clothing and gloves may be used to protect worker skin from exposure. Workers in this study, however, did not wear protective clothing or gloves. As a result, median dermal concentration levels of HDI, uretidone, biuret, and isocyanurate were 3.92, 9.51, 13.5, and 4590 ng mm^{-3}, respectively. Log-transformed dermal concentration was correlated with the log-transformed product of breathing-zone concentration and paint time for workers exposed to both isocyanurate and HDI. We did not find a significant correlation between dermal concentration and the product of paint concentration and paint time for HDI ($p = 0.0917$) or isocyanurate ($p = 0.308$). This underscores the important role played by factors other than the concentration in the paint, such as airflow in the booth and painter positioning, in determining both breathing-zone concentration and dermal concentration.

The regression models (Fig. 4) demonstrate the potential for using the product of breathing-zone concentration and paint time as a predictor for dermal concentration in unprotected workers. The similar slopes of the regression lines suggest that the effect of air concentration and paint time on dermal concentration is the same for HDI and isocyanurate. However, because the regression lines have significantly different intercepts ($p < 0.0001$), one would expect lower dermal concentration levels for HDI than for isocyanurate (on the order of about 92%) at the same level of predictor. Assuming the regression lines describe actual differences in the exposure pathways, there are several possible explanations for these differences. Firstly, because HDI exists partially as vapor in overspray, HDI may supply less exposure to the skin than isocyanurate, which exists solely as aerosol in overspray. Secondly, once on the skin, body temperature and air currents may cause HDI, with its high vapor pressure (0.005 mm Hg at 25 °C), to evaporate off the skin. Lastly, HDI may absorb into the skin and/or react with macromolecules in the skin or with alcohols in the paint more rapidly than isocyanurate.

Determining the cause of the differences between predicted dermal concentrations of HDI and isocyanurate is complicated by the high vapor pressure of HDI. Oligomers of HDI, on the other hand, have relatively low vapor pressures. Thus, any differences between predicted dermal exposure levels among HDI oligomers are likely due to different rates of skin absorption or chemical reactivity. According to Marzulli *et al.*,[30] compounds less than 400 amu are more likely than larger molecules to penetrate the skin. Thus, we would expect uretidone to penetrate the skin more rapidly than the other oligomers we measured. However, neither uretidone nor biuret was quantified in enough tape samples to perform regression modeling in this study.

In addition to investigating the differences in dermal concentration levels among the HDI-based polyisocyanates, future studies are warranted to explore the effectiveness of various types of protective clothing and gloves. The regression models we have developed for predicting dermal exposure to HDI and isocyanurate may serve as the basis for more complex models that consider the role of protective clothing and gloves, as well as other workplace factors. Such models may help to identify the main determinants of dermal exposure and the most effective controls to reduce those exposures.

Conclusion

The LC-MS method described is capable of measuring individual HDI monomer and oligomers in a variety of samples (*i.e.*, air, tape-strip, and paint samples). Because the same analytical method was used to analyze all the samples, we are confident in the comparability between measurements from different routes of exposure. As a result, we were able to characterize the exposure profiles in automotive spray painters, investigate the relationships among different markers for specific isocyanates, and identify important differences in predicted dermal concentration levels through regression modeling. The

regression models we developed may serve as the basis for more complex statistical models, including predictive models and models that consider the protective effects of clothing and gloves. This method allows us to achieve a more complete understanding of the complexity of dermal and inhalation exposure to HDI and its oligomers.

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References

1 P. L. Piirila, H. Nordman, H. M. Keskinen, R. Luukkonen, S. P. Salo, T. O. Tuomi and M. Tuppurainen, *Am. J. Respir. Crit. Care Med.*, 2000, **162**, 516.
2 L. Belin, U. Hjortsberg and U. Wass, *Scand. J. Work, Environ. Health*, 1981, **7**, 310.
3 J. L. Malo, G. Ouimet, A. Cartier, D. Levitz and C. R. Zeiss, *J. Allergy Clin. Immunol.*, 1983, **72**, 413.
4 S. M. Wilkinson, P. H. Cartwright, J. Armitage and J. S. English, *Contact Dermatitis*, 1991, **25**, 94.
5 C. J. Morgan and A. E. Haworth, *Contact Dermatitis*, 2003, **48**, 224.
6 D. Bello, C. A. Herrick, T. J. Smith, S. R. Woskie, R. P. Streicher, M. R. Cullen, Y. Liu and C. A. Redlich, *Environ. Health Perspect.*, 2007, **115**, 229.
7 A. Pronk, F. Yu, J. Vlaanderen, E. Tielemans, L. Preller, I. Bobeldijk, J. A. Deddens, U. Latza, X. Baur and D. Heederik, *Occup. Environ. Med.*, 2006, **63**, 624.
8 M. R. Flynn, Y. Koto, K. Fent and L. A. Nylander-French, *J. Occup. Environ. Hyg.*, 2006, **3**, 475.
9 K. W. Fent, K. Jayaraj, A. Gold, L. M. Ball and L. A. Nylander-French, *Scand. J. Work Environ. Health*, 2006, **32**, 225.
10 Y. Liu, D. Bello, J. A. Sparer, M. H. Stowe, R. J. Gore, S. R. Woskie, M. R. Cullen and C. A. Redlich, *Ann. Occup. Hyg.*, 2007, **51**, 429.
11 M. Janko, K. McCarthy, M. Fajer and J. van Raalte, *Am. Ind. Hyg. Assoc. J.*, 1992, **53**, 331.
12 J. Lesage, N. Goyer, F. Desjardins, J. Y. Vincent and G. Perrault, *Am. Ind. Hyg. Assoc. J.*, 1992, **53**, 146.
13 W. E. Rudzinski, B. Dahlquist, S. A. Svejda, A. Richardson and T. Thomas, *Am. Ind. Hyg. Assoc. J.*, 1995, **56**, 284.
14 J. Sparer, M. H. Stowe, D. Bello, Y. Liu, R. J. Gore, F. Youngs, M. R. Cullen, C. A. Redlich and S. R. Woskie, *J. Occup. Environ. Hyg.*, 2004, **1**, 570.
15 A. Pronk, E. Tielemans, G. Skarping, I. Bobeldijk, V.A.N.H. J, D Heederik and L. Preller, *Ann. Occup. Hyg.*, 2006, **50**, 1.
16 D. Karlsson, M. Spanne, M. Dalene and G. Skarping, *Analyst*, 1998, **123**, 117.
17 A. Marand, D. Karlsson, M. Dalene and G. Skarping, *J. Environ. Monit.*, 2005, **7**, 335.
18 D. A. Bagon, C. J. Warwick and R. H. Brown, *Am. Ind. Hyg. Assoc. J.*, 1984, **45**, 39.
19 D. Bello, R. P. Streicher and S. R. Woskie, *J. Environ. Monit.*, 2002, **4**, 351.
20 R. J. Rando, H. G. Poovey and R. A. Gibson, *J. Liq. Chromatogr.*, 1995, **18**, 2743.
21 D. Bello, S. R. Woskie, R. P. Streicher, Y. Liu, M. H. Stowe, E. A. Eisen, M. J. Ellenbecker, J. Sparer, F. Youngs, M. R. Cullen and C. A. Redlich, *Am. J. Ind. Med.*, 2004, **46**, 480.
22 NIOSH, Method 5521, isocyanates, monomeric, issue 2, in *NIOSH Manual of Analytical Methods*, ed. P. M. Eller and M. E. Cassinelli, NIOSH, Cincinnati, OH, USA, 4th edn, 1994.
23 D. A. Schwindt, K. P. Wilhelm and H. I. Maibach, *J. Invest. Dermatol.*, 1998, **111**, 385.
24 R. Marks, S. Nicholls and C. King, *Int. J. Cosmet. Sci.*, 1981, **3**, 251.
25 A. M. Almeida, M. M. Castel-Branco and A. C. Falcao, *J. Chromatogr., B: Biomed. Appl.*, 2002, **774**, 215.
26 C. A. Reilly, D. J. Crouc, G. S. Yost and A. A. Fatah, *J. Chromatogr., A*, 2001, **912**, 259.
27 E. England, R. Key-Schwartz, J. Lesage, G. Carlton, R. Streicher and R. Song, *Appl. Occup. Environ. Hyg.*, 2000, **15**, 472.
28 R. J. Rando and H. G. Poovey, *Am. Ind. Hyg. Assoc. J.*, 1999, **60**, 737.
29 Y. Liu, M. H. Stowe, D. Bello, S. R. Woskie, J. Sparer, R. Gore, F. Youngs, M. R. Cullen and C. A. Redlich, *J. Occup. Environ. Hyg.*, 2006, **3**, 234.
30 F. N. Marzulli, D. M. Anjo and H. I. Maibach, *Food Cosmet. Toxicol.*, 1981, **19**, 743.