



Development of an Interception Glove Sampler for Skin Exposures to Aromatic Isocyanates

Homero Harari^{1,2*}, Dhimiter Bello¹,
Susan Woskie¹ and Carrie Redlich²

1.Work Environment Department, University of Massachusetts Lowell, One University Avenue, Lowell, MA 01854, USA;

2.Yale Occupational and Environmental Medicine Program, Yale School of Medicine, 135 College Street, New Haven, CT 06510, USA;

*Author to whom correspondence should be addressed. Tel: +1-203-500-0630; e-mail: homero.harari@mssm.edu

Submitted 28 January 2015; revised 24 June 2016; revised version accepted 6 July 2016.

ABSTRACT

Objectives: Skin is an important exposure route for isocyanate chemicals and contributes to systemic sensitization. Methods for assessing skin exposure are currently limited and generally rely upon removal (e.g. tape-strip) techniques prone to underestimation. The aim of this study is to (i) develop and field test an interception-based hand exposure sampler to monitor potential skin exposure to isocyanates in the workplace, (ii) to develop an analytical method based on ultra-high-performance liquid chromatography–UV absorbance–tandem mass spectrometry (UHPLC–UV–MS/MS) for analyzing glove samples; and (iii) compare it with tape-stripping skin sampling method.

Methods: Laboratory investigations assessed different glove materials/fabrics, methods for impregnating with 1-(9-anthracenylmethyl)piperazine (MAP) derivatizing agent, methylene diphenyl diisocyanate (MDI) uptake and recovery, and durability. Following use, gloves were dissected into sections corresponding to different spatial regions (finger, palm) and analyzed using a newly developed UHPLC–UV–MS/MS method capable of differentiating and quantitating different MDI isomers with high sensitivity. Performance of the glove sampler was further assessed in a pilot field study using six workers.

Results: A MAP-impregnated thin cotton glove sampler and UHPLC–UV–MS/MS analytical method for detecting MDI were successfully developed in laboratory studies. In subsequent field studies, a total of 384 samples from 14 glove pairs identified full-shift exposures ranged from 0.01 to 306 µg of 4,4'-MDI/worker for each hand. Surface area adjusted MDI values measured with the glove sampler (0.13–572 ng MDI cm⁻²) were considerably higher (~400-fold) than values obtained with tape stripping.

Conclusion: A glove sampler and a novel UHPLC–UV–MS/MS analytical method were developed to quantitatively measure MDI skin exposure. The novel interception technique overcomes inherent limitations of removal techniques for measuring isocyanate skin exposure and may be useful in exposure surveillance and future research on isocyanate's health risks.

KEYWORDS: dermal sensitization; diisocyanate; polyurethane; occupational asthma; skin exposure assessment

INTRODUCTION

Isocyanates are reactive chemicals containing the functional group ($-N=C=O$) and can be classified according to the number of NCO groups contained in the molecule (Bello *et al.*, 2004; Creely *et al.*, 2006). Di- and poly-isocyanates are commercially used as essential cross-linking agents for generating polyurethane (PU). Methylene diphenyl diisocyanate (MDI) and polymeric MDI (PMDI) are the most abundantly produced diisocyanates (Ulrich, 1997; Allport *et al.*, 2003) and are commonly used in the production of rigid and flexible PU foams, as well as coatings and elastomers. Monomeric MDI (also called 'pure MDI') consists mainly of the monomer of 4,4'-MDI (~98%) and a small percentage of isomers (2,2'-MDI and 2,4'-MDI), while PMDI is a mixture of monomeric MDI and higher-molecular-weight 'oligomeric' species.

MDI, PMDI, and other di-/poly-isocyanates are potent sensitizers and among the most commonly reported causes of occupational lung disease (Chan-Yeung and Malo, 1995; Torén and Blanc, 2009; Vandenplas, 2011). With asthma as the primary adverse health effect, inhalational exposures have been the primary focus of research and regulatory efforts to date. However, a growing body of animal and workplace observational studies suggest that skin exposure to isocyanates elicits immune sensitization and exacerbates the subsequent development of isocyanate asthma (Bello *et al.*, 2007; Redlich and Herrick, 2008; Wisniewski *et al.*, 2011).

Despite evidence that skin exposure can contribute to isocyanate asthma pathogenesis, methods to sample and quantitate isocyanate skin exposure are limited (Liljelind *et al.*, 2010; ISO, 2011). Two studies (Thomassen *et al.*, 2011; Blake *et al.*, 2012) evaluated derivatizing reagent-impregnated felt patches or cotton sleeves and demonstrated potential utility for detecting isocyanate skin exposures but have not been further developed. Prior studies have relied almost exclusively on 'removal' techniques, primarily tape stripping (Fent *et al.*, 2009) and wiping of exposed skin (Bello *et al.*, 2008) to measure isocyanate skin exposure. 'Removal' techniques measure the (residual) unreacted isocyanate available at the time of sampling. Since isocyanates can react with proteins and water/sweat present in the skin, removal techniques are highly depending upon the timing of sample collection and are especially prone to underestimation.

In this study, we present a new approach to skin sampling, which—similar to current air monitoring approaches—is based on an 'interception' method, e.g. chemical exposure to the target tissue (skin) is intercepted by glove impregnated with a derivatizing agent that reacts with, captures, and stabilizes the agent of interest (isocyanate).

Analytical methods are equally important when deciding an optimal skin sampling method. Increased resolution and sensitivity are needed to be able to identify and quantitate isocyanates, especially with skin samples that contain more complex mixtures than airborne sampling. Practical experience with existing analytical methods based on high-pressure liquid chromatography (HPLC) with ultraviolet (UV)/fluorescence in our and other laboratories have shown that such methods lack the necessary sensitivity and specificity needed to handle the complex matrix composition and interferences introduced by the other chemicals (e.g. amines, oils, dyes, etc.) typically used in combination with isocyanates. As a result, hyphenated mass spectrometry techniques are a necessity.

The aim of this study is to (i) develop and field test an interception-based hand exposure sampler to monitor potential skin exposure to isocyanates in the workplace, (ii) to develop an analytical method based on LC-MS/MS for analyzing glove samples, and (iii) compare it with tape-stripping skin sampling method.

MATERIALS AND METHODS

Chemicals and supplies

1-(9-Anthracenylmethyl)piperazine (MAP) was obtained from National Institute for Occupational Safety and Health (NIOSH; Cincinnati, OH, USA) and CSL Lab (Miami, FL, USA), 99+% purified via sublimation. Internal standards (4,4'-MDI-d8-MAP) and MDI-MAP standards were supplied by NIOSH (NIOSH, Cincinnati, OH, USA). Mondur MR, a well-characterized commercial aromatic diisocyanate, with a total NCO content of 31.5% was used. Acetonitrile, methanol, and ethyl acetate HPLC grade were purchased from VWR (NJ, USA); trifluoroacetic acid and formic acid HPLC grade were purchased from Fisher Scientific (Waltham, MA, USA). Filters, 0.45 and 0.25 μ m, were purchased from VWR (NJ, USA). Cotton gloves were purchased from several vendors (VWR, NJ, USA; Darling Fire & Safety, Cincinnati, OH, USA).

Design of the MAP reagent-impregnated glove

Glove material selection

A field visit was conducted to a PU fabric coating plant in order to identify possible scenarios of skin exposure during coating operations. Based on field observations, skin exposures normally occurred on the unprotected hands of workers. Thus, the development of the sampler focused primarily on the assessment of skin exposure using an interception technique. A total of five types of cotton fabric gloves were selected for further evaluation based on ease of use, ability to impregnate with reagent, and cost. Several additional factors were also considered, including glove thickness and possible issues with hand dexterity, usability during work, cotton fabric resistance to the stresses of different tasks, variability in cotton density (g cm^{-2}), and commercial availability. Based on these criteria and feedback from workers at the plant, two types of cotton fabric (0.05 and 0.02 g cm^{-2} fabric density) were selected for further investigation of the possible reactions between the cotton fabric and the isocyanates used in the plant, as well as isocyanate recovery from the fabric.

Investigation of the reaction of PMDI with the sampler material

Attenuated total reflectance-Fourier transform infrared monitoring

Experiments were conducted on a Tensor 27 FTIR Spectrometer (Bruker, Billerica, MA, USA), equipped with an attenuated total reflectance (ATR) accessory with a ZnSe crystal ($\sim 3\text{-mm}$ diameter). One scan (10-s long) was collected every minute over a 10- to 15-min period. Resolution was set at 4 cm^{-1} , apodization to 'Happ-Genzel', phase correction to 'Mertz', and zero filling to 'none'. A background spectrum was collected for subtraction at the beginning of each run. OPUS Software was used for data acquisition and processing.

Fabric density (g cm^{-2}) was measured for the two types of cotton fabric tested. Prior to testing, the gloves were rinsed with deionized water and allowed to air dry. Cotton pieces ($5 \times 2\text{ cm}$) were cut in order to be tested. A dosing solution was prepared with Mondur MR (a known MDI polymeric blend) dissolved in ethyl acetate. A total of $0.6\text{-}\mu\text{g}$ NCO in $100\text{ }\mu\text{l}$ were spiked on the attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy

crystal ($\sim 3\text{ cm}^2$) and immediately a $5 \times 2\text{ cm}$ piece of cotton glove fabric was placed on top of the solution over the previously spiked crystal. Measurements were collected after the first spike; subsequently, a measurement was collected every minute during the following 15 min. A control, established by spiking the ATR-FTIR crystal without placing the fabric on top, was used for comparison purposes. All experiments were performed in triplicate.

Impregnation of the cotton fabric material with derivatizing reagent

MAP was chosen as the derivatizing agent for impregnation onto the cotton glove fabric because MAP offers higher sensitivity and greater uniformity in the UV response across different isocyanate species (Streicher *et al.*, 1996). This derivatizing agent is commercially available and is used in the NIOSH air sampling method 5525 (Streicher *et al.*, 1996; Bello *et al.*, 2002). Determination of optimal reagent concentration for impregnation was calculated based on concentrations used on glass fiber filters (GFF) for air monitoring according to NIOSH Method 5525. A total of 5.5 mg of MAP dissolved in acetonitrile (22 ml for spray impregnation) was applied to the glove, resulting in a MAP surface density of 37.2 nmol cm^{-2} based on a glove area of 535 cm^2 (hand surface area of 446 cm^2 according to Lee *et al.*, 2007) plus 20% increase used as a safety factor due to hand expansion and use of nitrile gloves underneath glove sampler (Lee *et al.*, 2007).

Two methods were considered for impregnation of the MAP derivatizing agent onto the cotton fabric: direct immersion and spraying of solution with an art brush. For each impregnation method, the volume of solvent required and drying times were evaluated and recorded. The air brush spray method was selected due to the reduced use of solvent (22 versus 45 ml), shorter drying times (40 versus 150 min), and the ease of application. The application method was optimized to ensure homogeneous coverage by spraying with a substitute solution of a commercial fabric dye dissolved in acetonitrile and also by using of a fluorescent tracer reagent (Glo Germ, GloGerm Co, Moab, UT, USA) under UV light.

To determine the availability of the MAP derivatizing agent on the fabric, 10 cm^2 of freshly impregnated fabric was processed as a sample and the MAP

peak was monitored to confirm its presence and availability. Chemical analysis was conducted with ultra-high-performance liquid chromatography–UV absorbance–tandem mass spectrometry (UHPLC–UV–MS/MS) according to the method described in a later section.

Sample extraction and recovery of MDI species

Manual mixing and sonication were evaluated as the two practical extraction methods of MAP derivatives from the cotton fabric. Recovery of MDI species was tested by spiking a freshly impregnated piece of fabric (5×2 cm) with a 100- μ l solution of Mondur MR dissolved in ethyl acetate, delivering two total concentrations of 168 and 1512 ng NCO cm^{-2} . These concentrations were chosen based on a nominal and a maximal isocyanate surface density from our previous field measurements. Chemical analysis was conducted with an UHPLC–UV–MS/MS according to the method described in a later section.

The recovery of the MAP-impregnated fabric was evaluated in triplicate at two storage conditions to determine best storage times. The two conditions for storage of the freshly impregnated fabric wrapped in aluminum foil were in a freezer (-4°C) and at ambient conditions (placed on a bench in the laboratory exposed to ambient light and temperature $\sim 25^{\circ}\text{C}$). Each condition was evaluated at three time points: 1 day, 1 week, and 2 weeks. After experimental conditions were met, 100 μ l of a solution containing 1.6 ng μl^{-1}

of Mondur MR was spiked directly onto the fabric over 1 cm^2 , and samples were processed the same day.

To evaluate whether environmental conditions (such as temperature and humidity) and photo-oxidation impact the recovery of MAP derivatives captured on the glove cotton fabric, 100 μ l of a solution containing 1.6 ng μl^{-1} of Mondur MR in acetonitrile was spiked onto the cotton fabric (5×2 cm) freshly sprayed with MAP and allowed to be kept at lab room temperature at the following target times: 1, 2, 4, and 8 h. Immediately after the target times were reached, the samples were placed into a 20-ml scintillation vial containing 10 ml of 5×10^{-4} M MAP solution in acetonitrile and processed for analysis (sample preparation is described in a later section). The target times were selected to simulate sections of an 8-h work shift. For comparison purposes, the same experiment was conducted on GFF impregnated with the same concentration of MAP as in the NIOSH Method 5525. All experiments were conducted in triplicate.

Sampled glove preparation for analysis

After field collection, gloves were placed into a 120-ml glass jar, capped with a polytetrafluoroethylene lid, stored in a cooler before transport to the laboratory for storage in a freezer at -4°C until analysis. Once in the laboratory, gloves were cut into 9–18 pieces with clean scissors representing specific sections of the hand (Fig. 1). Samples were prepared following an adapted version of NIOSH Method 5525. Each glove

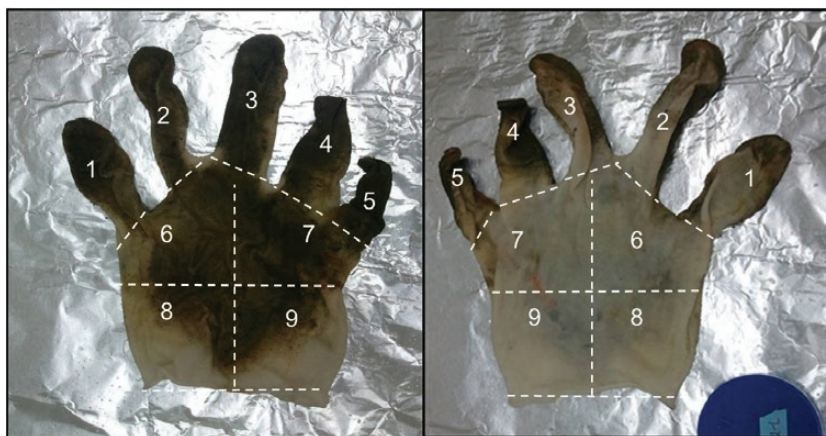


Figure 1 Example of glove sampler collected during field testing showing heavier contamination on the palmar versus dorsum. Dashed line indicates criteria for analysis of glove sampler collected in the field. Each region (1–9) represents an individual sample analyzed separately.

piece was placed into a 20-ml scintillation vial containing 10 ml of 5×10^{-4} M MAP in acetonitrile. Vials were shaken for 5 min at moderate speed to homogenize the sample and then placed in a sonication bath during 30 min to extract MAP derivatives from the fabric. After sonication, 10 ml of the MAP containing solution was extracted from the cotton fabric and filtered through a 0.45- μ m filter. Depending on the conditions of the cotton fabric, samples were filtered twice, once through a 0.45- μ m filter and later through a 0.25- μ m filter. Solutions were concentrated in a Visiprep (Sigma-Aldrich, USA) under vacuum with a stream of N_2 (Airgas, Billerica, MA, USA) to a final volume of 1 ml. The remaining 1 ml was placed in a 2-ml LC vial and 10 μ l of acetic anhydride was added to each sample and allowed to react overnight to consume the excess MAP. Glove cotton fabric samples used for the MAP stability and MAP recovery experiments were prepared similarly.

Chemical analysis with UHPLC–UV–MS/MS

Initially, the impregnated gloves were analyzed with NIOSH Method 5525. However, it was difficult to identify and accurately quantify the MDI species given other interfering substances collected by the glove. Due to these challenges, and the low sensitivity and specificity observed during HPLC analysis, a new UHPLC–UV–MS/MS method was developed. The Prominence UPLC (Shimadzu, Japan) consisted of a solvent delivery unit (LC-20AB), degasser (DGU-20A3), along with an autosampler (SIL-20AC) and column oven (CTO-20AC). Chromatographic separation was carried out on a Kinetex C18

column 100 $\times$ 4.6 mm I.D., 2.6- μ m particle size column (Phenomenex, CA, USA) preceded by a the matching phase guard column (Phenomenex, CA, USA). Gradient elution was performed using mobile phase A: 100% water; 0.1% ammonium acetate; and mobile phase B: 100% acetonitrile; 0.1% formic acid; 0.01% trifluoroacetic acid. Column oven temperature was set to 40°C and injection volume of 10 μ l. The compounds were eluted with a flow rate of 0.6 ml min⁻¹ using a gradient from 60 to 90% B from 0.01 to 15 min, from 90 to 95% B from 15.01 to 25 min followed by 3-min post-analysis re-equilibration at 65% B. For quality controls purposes double injection was performed in >400 samples yielding a coefficient of variation <0.5%. Mondur MR, a model isocyanate mixture containing the MDI monomers (4,4'-, 2,4'-, and 2,2'-MDI), trimer, and other oligomers, was used as a reference material. The UHPLC system was attached to an Applied Biosystems–MDS SCIEX API 3200 triple quadrupole equipped with a Turbo V IonSpray source. Chromatographic separation, optimized multiple reaction monitoring (MRM) transitions for each analyte, and the internal standard are presented in Table 1 and Fig. 2.

The optimized MS source parameters were as follows: curtain gas (CUR) 30, collision gas (CAD) 5, ion spray voltage 5500, source temperature 600°C, ion source gas 1 and 2 (GS1, GS2) 60 and 50, respectively, and interface heater was ON.

Quantitation of MDI and trimer species was based on their respective pure standards calibrations (4,4'-MDI-MAP and MDI Trimer-MAP), using MDI-d-MAP as the internal standard, spiked at 100 ng ml⁻¹.

Table 1. Isocyanate species parameters for MDI-MAP multiple reaction monitoring transitions using UHPLC–UV–MS/MS

Isocyanate species	Primary ion Q1 (amu) (<i>m/z</i>)	Product ion Q3 (amu)	DT (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
4,4'-MDI-MAP	803.3	191.2	250	70	5	60	5
MDI Trimer	1210.8	191.2	250	90	10	100	5
MDI-d8-MAP, internal standard	819.4	191.2	250	70	5	60	5

CE, collision energy; CEP, cell exit potential; CXP, collision cell exit potential; DP, declustering potential; EP, entrance potential.

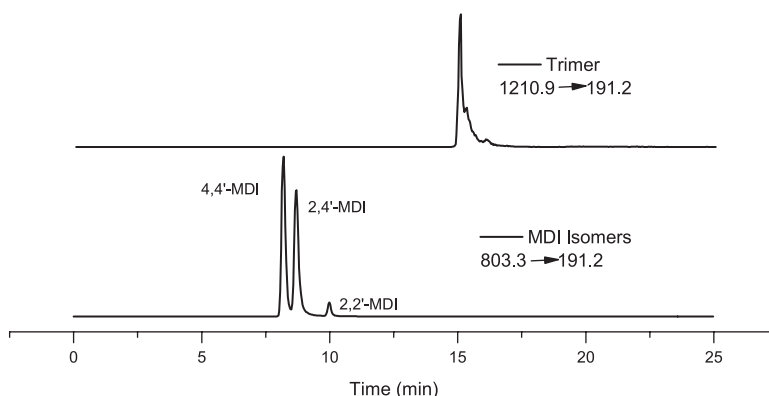


Figure 2 UHPLC–MS/MS chromatograms and MRM transitions of MDI isomers and trimer of an interception glove sample.

Twelve standards in the range of 250 pg ml^{-1} to $1 \text{ } \mu\text{g ml}^{-1}$ MDI were routinely used for the calibration curve. The calibration curve for 4,4'-MDI-MAP was linear up to $\sim 1 \text{ } \mu\text{g ml}^{-1}$ ($R = 0.9995$). The 2,4'- and 2,2'-MDI isomers were quantitated in MS based on the calibration curve of 4,4'-MDI and a correction factor in their MS responses derived from the online UV254 detector using a series of sequential dilutions of Mondur MR. The MS correction factors were 8.70 ($\text{SD } \pm 0.8$) and 9.6 ($\text{SD } \pm 1.0$) for 2,4'- and 2,2'-MDI, respectively, and were used to increase the slope coefficient of the 4,4'-MDI-MAP MRM calibration curve. This implies that the MS/MS response factor for 2,4'- and 2,2'-MDI were close to an order of magnitude higher than for 4,4'-MDI. The trimer calibration curve was linear to $>2 \text{ } \mu\text{g ml}^{-1}$ ($R = 0.997$), but its sensitivity in MS/MS was much lower than that of the monomer, a finding also reported by other laboratories using 2-PP reagent (White, 2006). The limit of detection of MDI isomers (as three times the S/N ratio) were as follows: 25 pg ml^{-1} for 4,4'-MDI, 5 pg ml^{-1} for 2,4'-MDI, and $\sim 1 \text{ ng ml}^{-1}$ for MDI-trimer. Recovery of MDI species with this method, over the calibration range, was determined from independent spikes of Mondur MR and subsequent treatment as regular samples (triplicate experiments).

Field testing of the MAP reagent-impregnated glove

The MAP reagent-impregnated glove was field tested in workers with exposure to MDI in a PU fabric coating plant. Recruitment and consent of subjects followed procedures approved by the Institutional Review Board of Yale University

and University of Massachusetts Lowell. Freshly impregnated glove samplers were prepared in the laboratory and transported in a cooler to the coating plant for field testing. A total of six workers were recruited and consented to participate in the study. Pairs of freshly prepared gloves were tested on two workers in a mixing area for MDI coating solutions and on four MDI fabric coating machine operators. Workers wore nitrile gloves underneath the MAP reagent-impregnated cloth glove to avoid contact between the MAP reagent and the skin of the hand. Sampling was conducted during a single 8-h shift for all the workers. At least two pairs of gloves (one pair every 4 h) were worn by each worker (additional pairs were used if gloves became soiled or torn). Machine operators mixed 50–100 ml of a PMDI solution [sometimes dissolved in methyl ethyl ketone (MEK)] into the basic PU formulation (polyol and other additives) in order to create the coating mixture (4 l). The mixture was then poured into the dispenser that coats the fabric running through the coating machine. Workers were in charge of preparing the mix (between 15 and 25 times per hour, depending on the thickness of the coating and the speed of the rolling fabric) and ensuring the dispenser is providing an even coating onto the fabric. Mixing area workers, on the other hand, dissolved solid flakes of MDI in a reactor and prepared different polyol formulations that also contained solvents such as MEK, toluene, and tetrahydrofuran. Mixing area workers performed the task throughout the 8 h of their work shift.

Comparison of MAP-impregnated glove with the tape-stripping method

The performance of the MAP reagent-impregnated glove was compared with the tape-stripping dermal exposure assessment technique in three workers exposed to MDI. The PU film production carried out by the worker consisted of mixing ~4 l of a previously made PU base formulation (polyol and additives) with 50–100 ml of pure PMDI. Mixing was carried out with a manual mixer equipped with flexible mixing blades. After mixing, the formulation was poured into molds that later were placed into an oven for curing. The cycle was repeated throughout the work shift. Production during the two periods during the shift was similar. Sampling was performed during an entire 8-h shift. The first 4 h we used one pair of MAP-impregnated glove samplers and the second 4 h we used the tape strips.

In the fabric coating plant previously described, two coating machine operators were sampled. Sampling was performed during 2 days with the same two machine operators using the same PU (MDI based) formulation on the same coating machines: one day (first 4 h of the day) for tape strips and the next day (first 4 h of the day) with one pair of MAP-impregnated glove samplers. Work was similar during the 4-h mixing period each day (50- to 100-ml PMDI repeatedly mixed with ~4 l PU polyol formulation, then poured into coating machine dispenser).

Tape strip collection

For each worker, six areas in the hand were tape stripped twice in the following way: each finger (locations 1–5 from Fig. 1) and the palm of the hand (locations 6–7 and 8–9 from Fig. 1). Sample collection is described elsewhere (Fent *et al.*, 2006; Liljelind *et al.*, 2010). After collection, tape strips were placed in 20-ml scintillation vials containing 15 ml of 5×10^{-4} MAP solution in acetonitrile and shipped to the laboratory in a cooler. The sample preparation is described in the Sampled glove preparation for analysis section, except no sonication was used, and chemical analysis was conducted with UHPLC–UV–MS/MS as described for the gloves.

Statistical analysis

Data were analyzed using traditional descriptive statistics. For 4,4'-MDI, the levels in samples were 100%

above the limit of detection (25 pg ml^{-1}) listed in the section on Chemical analysis with UHPLC–UV–MS/MS. Prior to analysis, the data were log-transformed, which resulted in a more normal distribution. Paired *t*-tests were used to determine differences between dominant and non-dominant hand and between tape strips and glove samplers. All statistical analyses were conducted using SAS 9.3 statistical software (Cary, NC, USA), and statistical significance was determined at a $P \leq 0.05$ level.

RESULTS

Reaction of PMDI with the glove material

Recovery of MDI from the two glove types (thin and thick), surface wipe, and tape strip is shown in [Supplementary Appendix Figure S1](#), available at *Annals of Occupational Hygiene* online. Thin and thick glove types had substantially higher recovery of MDI than the tape strip or surface wipe. The thin cotton gloves showed minimal loss, comparable to the reference MDI solution and expected NCO decay. Consequently, the thin cotton fabric (density = 0.02 g cm^{-2}) was chosen for impregnation with the derivatizing agent. An additional benefit of thin fabric is better dexterity, which was preferred by the workers.

Recovery of MDI species from the glove sampler

Thirty-minute sonication of freshly prepared MAP-impregnated gloves spiked with 168 and 1512 ng NCO cm^{-2} yielded mean recoveries $>92.8\%$ for all of the MDI isomers. Stability of the MAP-impregnated fabric was further compared at different storage times (1 week, 2 weeks) and at different temperatures (room temperature and freezer at -4°C). The results indicate that the unused glove sampler can be stored for at least a week in the freezer before use as recoveries remain $\geq 92.8\%$ for 4,4'-MDI spiked and analyzed after the storage period. This is comparable to the freshly prepared glove recoveries.

Recovery of MDI was examined at 1, 2, 4, and 8 h after MDI spiking of freshly prepared gloves and GFF used in the NIOSH 5525 method. MDI-MAP derivatives recovery declined to 80% after 1 h, 35% after 4 h, and 25% after 8 h. The results for 8 h after spiking were comparable to the spiking of the GFF used in NIOSH Method 5525. These data suggest that lower recovery could be a result of the spiking method, not the

derivatizing agent or underlying material resulting in insufficient derivatization.

Field testing of the glove sampler

After the design experiments were completed, the glove sampler was tested in the field by six workers. A total of 14 pairs of gloves were collected from the 6 workers. Upon collection and as part of the physical evaluation of the gloves, it was found that the majority of the gloves were wet. Each glove was cut

into an average of 14 pieces, 7 dorsum, and 7 palmar samples (382 total samples), which were processed to determine load on different anatomical regions of the hand (Fig. 1). Results from field testing are summarized in Table 2 and show the spatial distribution of MDI levels for each hand in the different anatomical regions of the hand. The palm and thumb region of the hand showed the highest MDI exposures accounting for at least 50% of skin exposure on each hand. Those regions showed consistently high levels across all

Table 2. Field study results describing spatial distribution of hand exposure to 4,4'-MDI (μg) among workers in a polyurethane fabric coating operation

	Hand location						Total
	Thumb (μg)	Index (μg)	Middle (μg)	Ring (μg)	Little (μg)	Palm (μg)	
Estimated surface area (cm^2)	76	76	84	76	55	168	535
Machine operator A ($n = 3$)							
Right hand	43.3	33.5	28.3	27.7	29.4	134.4	296.5
Left hand ^a	43.3	34.0	29.9	30.8	25.8	142.8	306.6
Machine operator B ($n = 1$)							
Right hand ^a	2.5	1.8	1.8	1.6	1.6	7.6	16.8
Left hand	2.2	0.9	1.3	1.1	1.1	4.9	11.5
Machine operator C ($n = 2$)							
Right hand ^a	0.01	0.01	0.01	0.01	0.01	5.1	5.1
Left hand	0.01	0.01	3.9	1.3	1.2	6.2	12.6
Machine operator D ($n = 2$)							
Right hand ^a	4.1	4.2	4.3	4.3	4.3	13.5	34.7
Left hand	2.2	1.3	1.7	2.3	2.3	6.8	16.6
Mixer 1 ($n = 3$)							
Right hand ^a	4.0	3.4	2.7	4.1	2.9	4.2	21.3
Left hand	0.9	0.7	3.4	2.4	0.6	1.1	9.0
Mixer 2 ($n = 3$)							
Right hand ^a	27.7	24.5	23.1	20.1	19.0	90.1	204.6
Left hand	22.6	14.5	13.8	12.9	14.2	59.2	137.2

n , number of hand exposure samplers collected over a full work shift used to explain daily exposure.

^aDominant hand.

workers for both job titles. Paired *t*-tests showed that the dominant hand had significantly higher amounts of 4,4'-MDI ($P \leq 0.05$).

The spatial distribution of 4,4'-MDI on the palmar and dorsum area from the gloves from a machine operator applying a single coating is shown in Fig. 3, expressed as total load and mass adjusted for size of the glove piece (Fig. 3a, b). The total palmar area load was approximately twice the amount of total 4,4'-MDI as the highest total load (~13 700 ng) on the dorsum side (~7600 ng). The thumb (2970 ng) and ring (2980 ng) finger presented the highest exposures among all the regions of the hands evaluated, representing ~43% of the hand exposure, although combined the thumb and ring finger cover ~28% of the surface area of the hand (152 cm²). When the exposure data were normalized to surface area (ng cm⁻²), the thumb, ring, and little finger showed the highest exposures.

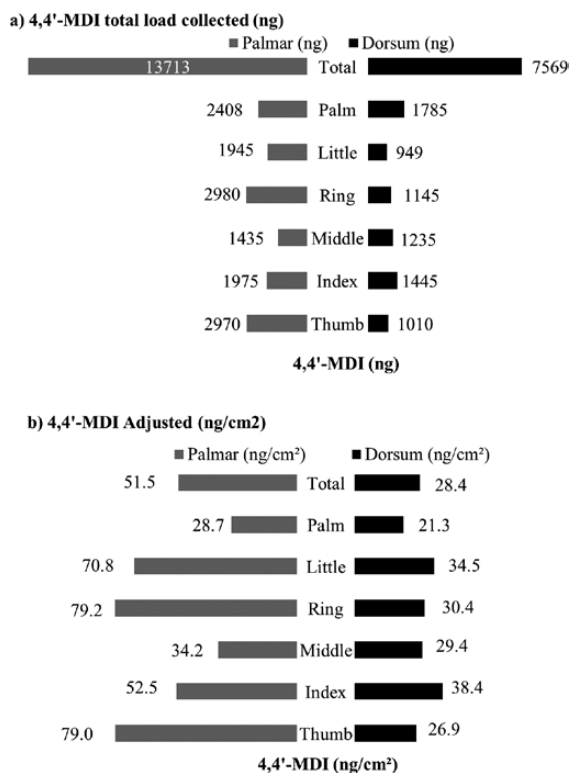


Figure 3 Skin exposure of MDI mixer in different areas of the hand. (a) 4,4'-MDI total load collected (ng); (b) 4,4'-MDI adjusted (ng cm⁻²).

Figure 4 shows the 4,4'-MDI levels found in one machine operator applying a single coating over a single work shift and several different tasks.

A total of three separate pairs of gloves were collected from the machine operator. During the first half of the work shift, from 8 am to 12 noon (240 min), the operator performed regular polyol-PMDI mixing and coating tasks and continued to perform these tasks after lunch time, from 1:30 pm to 2:25 pm (115 min). Over the last 30 min of the shift, when the machine operator cleaned his work area and coating machine with a rag wet with solvent, the 4,4'-MDI levels were considerably higher than levels collected for the most part of the shift. This last short-duration task produced >50% of the total exposure over the shift, demonstrating the variability of exposure over time.

Comparison of the MAP-impregnated glove with tape stripping

Results of the comparison between the glove sampler and tape-stripping methods are presented in Table 3. All samples for both exposure scenarios were above the detection limit for MDI. For all three workers sampled in this comparison study, the glove sampler measured >3 orders of magnitude higher 4,4'-MDI levels than the tape-stripping method. The mean ratio of the glove to tape-strip levels varied from 326 to 888, depending on the hand (right or left) and the location on the hand. Paired *t*-tests of MAP reagent-impregnated gloves and tape strips showed that the gloves consistently produced higher quantities of 4,4'-MDI across all anatomical regions ($P \leq 0.05$).

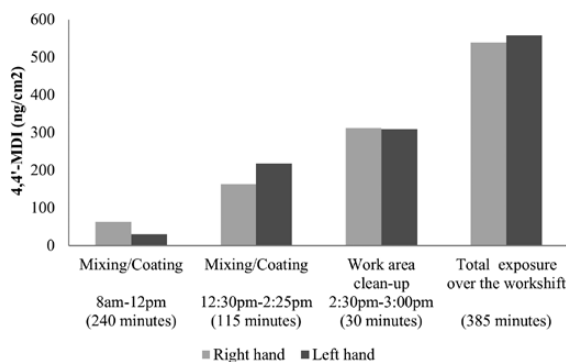


Figure 4 Temporal variations in skin exposure to 4,4'-MDI (ng cm⁻²) for a fabric coating machine operator over a single work shift.

Table 3. Performance comparison of interception glove sampler versus tape strip for collection of 4,4'-MDI for three coating machine operators

Hand/location		4,4'-MDI						Mean ratio glove/tape strip
		Worker number 1		Worker number 2		Worker number 3		
		Tape strip (ng cm ⁻²)	Glove (ng cm ⁻²)	Tape strip (ng cm ⁻²)	Glove (ng cm ⁻²)	Tape strip (ng cm ⁻²)	Glove (ng cm ⁻²)	
Left hand	Thumb	0.02	35.8	0.3	2.8	1.8	139.6	626
	Index finger	0.03	39.6	0.2	31.9	3.5	160.6	508
	Middle finger	0.02	32.4	0.5	9.9	1.8	125.6	570
	Ring finger	0.02	39.8	0.1	33	2.4	100.5	787
	Little finger	0.02	47.9	0.1	15.8	1	109.9	888
	Palm	0.01	17.4	10.7	15.4	1.6	189.5	620
	Total	0.12	213	11.9	108.8	12	825.9	618
Right hand	Thumb	0.03	33.5	18.9	63.6	3.8	95.5	382
	Index finger	0.02	27.9	4.9	21.4	3.6	92.3	475
	Middle finger	0.01	23.9	4.4	19.3	8.7	81.3	801
	Ring finger	0.01	26.7	0.3	9.8	1.3	88.3	924
	Little finger	0.02	38.4	1.4	1.3	14.3	123.4	643
	Palm	0.02	12.2	0.2	71.8	8	61.8	326
	Total	0.11	162.5	30.1	187.1	39.7	542.5	499

DISCUSSION

The aim of this study was to develop a novel hand skin dosimeter for isocyanates using an interception approach. The resulting prototype, a MAP (derivatizing reagent)-impregnated glove sampler, functions by reacting with NCO that lands on its surface, effectively capturing and stabilizing the diisocyanate chemical until analysis can be completed in the laboratory. The type of glove, reagent application method, and pre- and post-sampling storage were optimized and recoveries determined. The feasibility of using the gloves to measure MDI exposures in the field was evaluated among workers in a fabric coating factory. The final glove sampler prototype is a surrogate hand skin exposure sampling method that can quantitate different isomers of MDI (4,4'-MDI; 2,4'-MDI; 2,2'-MDI; and trimer).

Surrogate (interception-type) skin exposure methods based on cotton gloves are not unprecedented and have been used to measure other types of skin

exposures. For example, untreated gloves (without derivatizing agent) have been used to characterize exposure to pesticides, beryllium, drilling fluids, and crude oil (Fenske *et al.*, 1989, 1999; Day *et al.*, 2007; Galea *et al.*, 2014). However, the use of an interception glove sampler with a reactive chemical such as isocyanates has been limited. One study reported using rubber gloves to assess hexamethylene diisocyanate skin exposure in auto body shop workers, but noted problems with solvent compatibility and likelihood of underestimation due to isocyanates' inherent instability (Pronk *et al.*, 2006). The presently described reagent-impregnated glove expands upon these earlier studies and offers the advantage of monitoring exposure to the hand, perhaps the most likely location of occupational skin exposure. The analytical method described here is also significantly more specific and sensitive than previous HPLC or LC-MS methods for detecting derivatized isocyanate chemical.

The interception method of evaluating isocyanate skin exposure, as developed here, also offers substantial advantages over previously described methods that rely upon removal (e.g. tape stripping) of chemical. In our comparison between the MAP-treated cotton glove and tape stripping, we noted that tape-strip samples detected much lower levels of MDI, on average over 400-fold lower. Thus, prior estimates of workplace dermal exposures based on tape-stripping methods may substantially underestimate skin exposure.

Particular features of the present interception glove design and methodology are critical to their capacity to detect MDI. One important consideration is the material of the glove sampler, which affects the capture and collection efficiency. In our experimental evaluation, not all cotton fabrics were appropriate as a sampling material; some possessed greater inherent reactivity with isocyanate, leading to unextractable products and underestimation of exposure levels (see [Supplementary Figure S1](#), available at *Annals of Occupational Hygiene* online). Additionally, it was important to ensure an even distribution of the derivatizing agent on the material. The glove sampler described here had similar high recovery efficiency (92.8%) at both low and high spiking levels (168 and 1512 ng NCO cm⁻², respectively) and still contained an excess of MAP derivatizing agent, as long as concentrations did not exceed 1680 ng cm⁻². However, it is likely that there are still derivatization losses, and even with these recoveries, actual exposures may be underestimated.

Strengths and weaknesses of the present interception glove design and methodology are important with regard to interpreting the present data and future application of the approach toward exposure surveillance. Major strengths as mentioned above are the focus on hand skin, the non-invasiveness of the technique (versus tape stripping), and the sensitivity/specificity of the UHPLC–UV–LS–MS/MS method of analysis. The most important limitation of the glove is how long it remains reactive, as the derivatizing agent is sensitive to UV light. Based on the current findings, we recommend gloves should be used for no more than 4 h to ensure adequate capturing capacity. In scenarios where the derivatizing capacity of the glove is exceeded due to a single high-level exposure or repeated contact between the glove and MDI-contaminated surfaces, exposure might be underestimated. Solvents used along with MDI could also be a concern, as they could potentially remove MAP from the glove or damage the fabric. Although not a

problem in the present studies, glove (fabric) durability could interfere with tasks involving excessive and forceful material handling. To prevent the potential issues noted above, it is important to understand the tasks being performed and to have additional gloves that can be used when such concerns arise. Future studies in a larger number of workers should better define the limits of our newly developed skin exposure dosimeter under normal working conditions and its utility in other work settings/industries where isocyanates are used.

CONCLUSION

A MAP reagent-impregnated cotton glove has been designed as a sampler to measure MDI skin exposures. The combination of this sampler with a newly developed, highly sensitive, and specific UHPLC–UV–MS/MS analytical method enables quantitation of potential spatial variation and loading of MDI to the 25 pg ml⁻¹ level. In a limited field study, the glove sampler was ~400-fold more sensitive than tape stripping, currently the prevailing method for assessing isocyanate skin exposure in the field. The non-invasive glove sampler will facilitate the collection of detailed information on variation in exposures over time and between different tasks, enabling targeted preventive efforts. Thus, our novel, reagent-impregnated glove interception technique provides a highly sensitive and specific approach to skin exposure assessment and offers numerous advantages over current existing methods for assessing isocyanate skin exposure.

SUPPLEMENTARY DATA

Supplementary data can be found at <http://annhyg.oxfordjournals.org/>.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Robert Streicher (NIOSH) and Dr Adam Wisniewski (Yale Occupational and Environmental Medicine Program) for reviewing and providing valuable comments to this manuscript, Dr Aleksandr Stefaniak (NIOSH) for the support during the design stages of the study, and Dr Meredith Stowe (Yale Occupational and Environmental Medicine Program) for assistance throughout the study. We also thank the factory workers and management for their participation in and support of this study. The study was funded through NIOSH Grants R01 OH009375-01 and R01 OH009364-01. The authors have no potential competing interests to disclose.

REFERENCES

- Allport DC, Gilbert DS, Outterside SM. (2003) *MDI and TDI: safety, health and the environment: a source book and practical guide*. West Sussex, UK: John Wiley & Sons Ltd.
- Bello D, Herrick CA, Smith TJ *et al.* (2007) Skin exposure to isocyanates: reasons for concern. *Environ Health Perspect*; 115: 328–35.
- Bello D, Redlich CA, Stowe MH *et al.* (2008) Skin exposure to aliphatic polyisocyanates in the auto body repair and refinishing industry: II. A quantitative assessment. *Ann Occup Hyg*; 52: 117–24.
- Bello D, Streicher RP, Woskie SR. (2002) Evaluation of the NIOSH draft method 5525 for determination of the total reactive isocyanate group (TRIG) for aliphatic isocyanates in autobody repair shops. National Institute for Occupational Safety and Health. *J Environ Monit*; 4: 351–60.
- Bello D, Woskie SR, Streicher RP *et al.* (2004) Polyisocyanates in occupational environments: a critical review of exposure limits and metrics. *Am J Ind Med*; 46: 480–91.
- Blake C, Johnson G, Abritis A *et al.* (2012) Isocyanate and VOC exposure analysis using Flexane®. *Regul Toxicol Pharmacol*; 63: 363–70.
- Chan-Yeung M, Malo J-L. (1995) Occupational asthma. *N Engl J Med*; 333: 107–12.
- Creely KS, Hughson GW, Cocker J *et al.* (2006) Assessing isocyanate exposures in polyurethane industry sectors using biological and air monitoring methods. *Ann Occup Hyg*; 50: 609–21.
- Day GA, Dufresne A, Stefaniak AB *et al.* (2007) Exposure pathway assessment at a copper–beryllium alloy facility. *Ann Occup Hyg*; 51: 67–80.
- Fenske RA, Birnbaum SG, Methner MM *et al.* (1989) Methods for assessing fieldworker hand exposure to pesticides during peach harvesting. *Bull Environ Contam Toxicol*; 43: 805–13.
- Fenske RA, Simcox NJ, Camp JE *et al.* (1999) Comparison of three methods for assessment of hand exposure to azinphos-methyl (Guthion) during apple thinning. *Appl Occup Environ Hyg*; 14: 618–23.
- Fent KW, Jayaraj K, Gold A *et al.* (2006) Tape-strip sampling for measuring dermal exposure to 1,6-hexamethylene diisocyanate. *Scand J Work Environ Health*; 32: 225–40.
- Fent KW, Trelles Gaines LG, Thomasen JM *et al.* (2009) Quantification and statistical modeling—part II: dermal concentrations of monomeric and polymeric 1,6-hexamethylene diisocyanate. *Ann Occup Hyg*; 53: 691–702.
- Galea KS, McGonagle C, Sleeuwenhoek A *et al.* (2014) Validation and comparison of two sampling methods to assess dermal exposure to drilling fluids and crude oil. *Ann Occup Hyg*; 58: 591–600.
- ISO. (2011) ISO/TR 14294:2011 workplace atmospheres—measurement of dermal exposure—principles and methods.
- Lee J-Y, Choi J-W, Kim H. (2007) Determination of hand surface area by sex and body shape using alginate. *J Physiol Anthropol*; 26: 475–83.
- Liljelind I, Norberg C, Egelrud L *et al.* (2010) Dermal and inhalation exposure to methylene bisphenyl isocyanate (MDI) in iron foundry workers. *Ann Occup Hyg*; 54: 31–40.
- Pronk A, Yu F, Vlaanderen J *et al.* (2006) Dermal, inhalation, and internal exposure to 1,6-HDI and its oligomers in car body repair shop workers and industrial spray painters. *Occup Environ Med*; 63: 624–31.
- Redlich CA, Herrick CA. (2008) Lung/skin connections in occupational lung disease. *Curr Opin Allergy Clin Immunol*; 8: 115–9.
- Streicher RP, Arnold JE, Ernst MK *et al.* (1996) Development of a novel derivatization reagent for the sampling and analysis of total isocyanate group in air and comparison of its performance with that of several established reagents. *Am Ind Hyg Assoc J*; 57: 905–13.
- Thomasen JM, Fent KW, Nylander-French LA. (2011) Development of a sampling patch to measure dermal exposures to monomeric and polymeric 1,6-hexamethylene diisocyanate: a pilot study. *J Occup Environ Hyg*; 8: 709–17.
- Torén K, Blanc PD. (2009) Asthma caused by occupational exposures is common—a systematic analysis of estimates of the population-attributable fraction. *BMC Pulm Med*; 9: 7.
- Ulrich H. (1997) *Chemistry and technology of isocyanates*. West Sussex, UK: John Wiley & Sons Ltd.
- Vandenplas O. (2011) Occupational asthma: etiologies and risk factors. *Allergy Asthma Immunol Res*; 3: 157–67.
- White J. (2006) MDHS 25 revisited; development of MDHS 25/3, the determination of organic isocyanates in air. *Ann Occup Hyg*; 50: 15–27.
- Wisniewski AV, Xu L, Robinson E *et al.* (2011) Immune sensitization to methylene diphenyl diisocyanate (MDI) resulting from skin exposure: albumin as a carrier protein connecting skin exposure to subsequent respiratory responses. *J Occup Med Toxicol*; 6: 6.

Development of an Interception Glove Sampler for Skin Exposures to Aromatic Isocyanates

Homero Harari^{1,2,*}, Dhimiter Bello^{1,3}, Susan Woskie¹, Carrie Redlich².

¹University of Massachusetts Lowell, Work Environment Department, Lowell, MA, USA;

²Yale School of Medicine, Yale Occupational and Environmental Medicine Program, New Haven, CT, USA;

³Harvard School of Public Health, Molecular and Integrated Physiology Sciences, Boston, MA, USA.

*Author to whom correspondence should be addressed. E-mail: homero.harari@mssm.edu

Annex 1 – Supplemental information

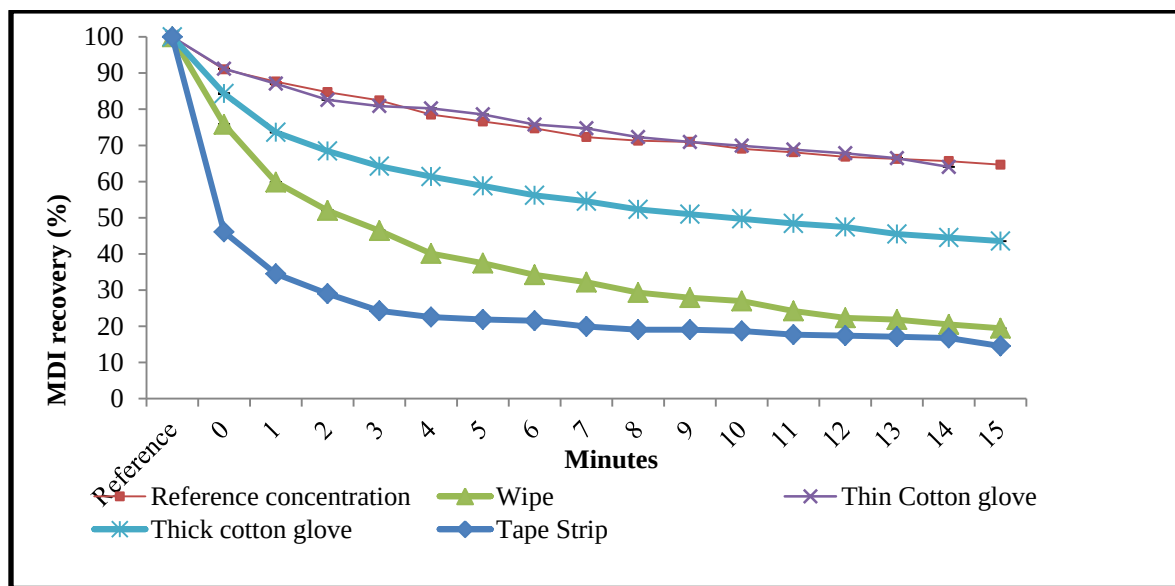


Figure S1. Recovery of MDI with different sampling material analyzed by FTIR-ATR