INVESTIGATING THE PERFORMANCE OF EXPOSURE ASSESSMENT TECHNIQUES USED TO MONITOR AIR AND DERMAL EXPOSURES TO MONOMERIC AND POLYMERIC 1,6-HEXAMETHYLENE DIISOCYANATE

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ABSTRACT

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Investigating the Performance of Exposure Assessment Techniques Used to Monitor Air and Dermal Exposures to Monomeric and Polymeric 1,6-Hexamethylene Diisocyanate
(Under the direction of Leena A. Nylander-French)

Monomeric and polymeric 1,6-hexamethylene diisocyanate (HDI) is widely used in clearcoat products used in the automotive repair industry. Inhalation exposure has been considered the primary exposure route and the primary cause of isocyanate-induced sensitization in automotive refinishing industry workers. Although many studies have been performed to investigate inhalation exposure to HDI, the literature is conflicting as to what type of air sampling device most reliably measures exposure levels. More recently, concerns about the role of dermal exposure in isocyanate induced sensitization and asthma have been raised. Dermal exposure has been documented among these workers, yet methods to measure skin exposure are not validated or standardized, and the penetration patterns and absorption rates of monomeric and polymeric HDI are not known. The objective of this study was to evaluate inhalation and dermal sampling methods for monomeric and polymeric HDI. We conducted a study comparing 13 different air samplers, which are commonly used in research studies as well as by practicing industrial hygienists for regulatory purposes, for their ability to monitor air exposures to HDI. We also developed and evaluated a patch sampler to measure dermal exposures to HDI and compared it with the tape-strip method. Our results indicate that methods commonly used to measure air and dermal exposure to HDI likely underestimate exposure. We also investigated the time-dependent

penetration patterns of HDI in human skin. We observed that these compounds were readily absorbed and penetrated into the skin and that the composition of the clearcoat mixture may affect the penetration rate of the individual isocyanate compounds (both monomeric and polymeric). Our results indicate that the dose received through dermal exposure to HDI-containing clearcoats in the occupational setting has a significant potential to exceed the absorbed dose received at the equivalent air concentration corresponding to the established regulatory limits for inhalation exposure. A critical need exists to monitor dermal exposure quantitatively in exposed worker populations and to re-evaluate regulatory exposure limits for isocyanate exposures.

To my Grandma Martha

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LIST OF ABBREVIATIONS

1-2PP	1-(2-pyridyl) piperazine
ACGIH	American Conference of Governmental Industrial Hygienists
ACN	Acetonitrile
DMF	*N,N'*-dimethylformamide
FL	Fluorescence
GFF	Glass-fiber filter
GM	Geometric mean
GSD	Geometric standard deviation
HDI	1,6-Hexamethylene diisocyanate
HPLC	High performance liquid chromatography
HVLP	High-volume low-pressure
IOM	Institute of Medicine
IPDI	Isophorone diisocyanate
LC-MS	Liquid chromatography – mass spectrometry
L&I	Labor and Industries
LOD	Limit of detection
LOQ	Limit of quantitation
MAMA	9-(N-methylaminomethyl)anthracene
MDI	diphenylmethane diisocyanate
MMAD	Mass median aerodynamic diameter
MPP	1-(2-methoxyphenyl)piperazine
NCO	Isocyanate function group

ND	non-detectable
NIOSH	National Institute for Occupational Safety and Health
ODIU	Urea derivative of 1,8-octamethylene diisocyanate
OEL	Occupational exposure limit
OSHA	Occupational Safety and Health Administration
PP	Polypropylene
PPE	Personal protective equipment
PS	Polystyrene
PTFE	Polytetrafluoroethylene
RSE	Relative Standard Error
RSD	Relative Standard Deviation
TDI	Toluene diisocyanate
TLV	Threshold limit value
TOL	Toluene
TRIG	Total reactive isocyanate groups
TWA	Time weighted average
UV	Ultraviolet
WA	Washington State
WA-DOSH	Washington State Division of Safety and Health

CHAPTER 1

BACKGROUND AND SIGNIFICANCE

1.1 Isocyanates

Diisocyanates are a group of highly reactive, low-molecular-weight aromatic and aliphatic compounds, characterized by containing two isocyanate functional groups (N=C=O). The most common diisocyanates, 1,6-hexamethylene diisocyanate (HDI), toluene diisocyanate (TDI), and diphenylmethane diisocyanate (MDI) [1], are widely used in the production of polyurethane materials and paints [2]. Approximately 280,000 workers are either occupationally or potentially exposed to diisocyanates in various industries [3]. Occupational personal exposures to isocyanates in the United States is estimated to be 100,000 workers each year [4]. A large number of these workers are employed in the automotive refinishing industry where polyurethane-based paints and coatings containing monomeric and polymeric HDI are used. The most widely used isocyanates include HDI monomer as well as HDI polyisocyanates uretdione, biuret, and isocyanurate [3]. We will refer to both diisocyanates and polyisocyanates as isocyanates from this point forward.

1.2 Health Effects

Exposure to isocyanates may cause adverse health effects to the skin, mucous membranes, eyes, and respiratory tract [5-7]. The most common adverse health outcome is asthma due to

sensitization with less prevalent outcomes of contact dermatitis and hypersensitivity pneumonitis [5-10]. An exposed worker may be sensitized after a single acute exposure, but usually sensitization takes a few months to several years of exposure [5, 11-13]. Once a worker is sensitized, a subsequent exposure even to a low concentration can induce a severe asthmatic response or even death due to the aggravated acquired immune response [5, 14]. An asthmatic reaction due to sensitization may occur immediately (i.e., minutes following exposure) or several hours after exposure [11, 12]. Occupational or environmental asthma may account for as much as one-third of the more than 10 million adult asthma cases, with isocyanate-induced asthma accounting for between 5 to 30% of the occupational asthma cases [15-20].

Polyisocyanates, mainly of HDI and MDI with considerably lower vapor pressures than monomeric forms, are increasingly used to reduce inhalation exposures [21, 22]. Isocyanate asthma occurs in workers exposed to polyisocyanates [23-27] and specific inhalation challenge testing of individual patients has confirmed that polyisocyanates can cause asthmatic reactions [27-29]. However despite the extensive use of polyisocyanates, exposure-response associations have not been thoroughly investigated [21]. Automotive spray painters exposed to HDI polyisocyanate mixtures are among the workers with the highest incidence of occupational asthma in industrialized countries [21, 30-32].

1.3 Automotive Refinishing Industry

1.3.1 Isocyanate Sources and Uses

Polyurethane-based paints are frequently used in the automotive industry because of their outstanding technical features such as durability, color stability and resistance to abrasion,

chemical, and weather extremes. Coatings based on aliphatic isocyanates are more light-stable, durable, and tend to retain their gloss longer than coatings based on aromatic isocyanates [33]. Most automotive paints consist of isocyanates based on HDI, which contain trace amounts of HDI monomer (usually <0.5%) and much higher amounts of HDI polyisocyanates (2.5-20%) depending on the formulation [34]. HDI polyisocyanates commonly used in automotive paint include the dimer, uretdione, and the trimers, biuret and isocyanurate. Isophorone diisocyanate (IPDI)-based polyisocyanates may also be used in automotive coatings but are typically present at lower levels than HDI-based polyisocyanates.

Automotive paints are typically applied using a two-stage system, where the first stage is the base coat and the second stage is the clearcoat. Isocyanates may be present in the primer applied between the metal and the paint as well as in the clearcoat applied onto the paint [35]. Generally, hardener containing monomeric and polymeric HDI is added to the clearcoat. The isocyanates in the hardener react with polyols in the clearcoat solution to form polyurethane [36].

1.3.2 Application

Automotive painting is usually performed using compressed-air spray guns inside ventilated booths. The conventional spray booth is a heavy gauge, sheet metal enclosure with one open face. These may be crossdraft, downdraft, or semi-downdraft booths. The majority of the paint droplets produced by the spray gun land on the surface of the automobile to form a polyurethane coating. However, some of the droplets are captured by the airflow around the surface and become airborne, forming a paint mist or overspray that is likely to contain

unreacted isocyanates [37]. The overspray may be transported into the worker's personal space and result in dermal and inhalation exposure to isocyanates. To a large degree, the hazard in a given spray-paint operation depends on the transfer efficiency of the paint application method. Transfer efficiency represents the percentage of the paint that is deposited on the work piece with the remainder being lost to overspray and rebound [38].

High-volume low-pressure (HVLP) spray guns have largely replaced conventional spray guns in spray-paint applications due to their high transfer efficiencies (65-75%) [38]. Conventional spray guns use nozzle pressures greater than HVLP guns. The conventional system requires high airflow, generates fine particles, and has low transfer efficiency in the range of 25-30% and, therefore, the resulting overspray and rebound can result in dermal and inhalation exposures [38]. Since the air pressure for HVLP gun is lower than conventional air atomization, there is a reduction in overspray and rebound with resulting transfer efficiencies in excess of 65% [38].

1.3.3 Inhalation Exposures

Isocyanates volatilize at room temperature, thus the most common route of exposure for workers is considered to be inhalation of vapors. Respiratory exposures have been the primary route of exposure, and research, regulation, and prevention have focused almost exclusively on airborne exposures [39]. Airborne isocyanate exposures have been reduced through improved controls and use of less-volatile isocyanates (i.e., polyisocyanates). However, isocyanate asthma continues to occur, and is often observed in work settings where measured isocyanate respiratory exposures are very low and/or below the levels detectable by

commonly used methodologies [40]. This has prompted a focus on skin as a route of exposure [39, 40].

1.3.4 Dermal Exposures

In spray-painting operations, monomeric and polymeric HDI can be present in both aerosol and vapor forms. Most exposure assessments have only focused on the characterization of airborne exposures [41-46], however, aerosol and/or vapor deposition on the skin and skin contact with contaminated surfaces and liquid product also constitute an important route for exposure [40]. Isocyanates are commonly mixed with various solvents, polyols, and other substances, such as catalysts and blowing agents, which may affect isocyanate reactivity, skin absorption, and health effects [40]. The higher the volatility of the isocyanate, the shorter its residence time is on the skin. Therefore, the less-volatile polymeric isocyanates (HDI biuret and isocyanurate) potentially may have longer residence time on the skin and, thus, may have skin and systemic effects different from that of the monomer. Fent et al. postulated that differences between dermal exposures for polymeric HDI are likely due to different rates of skin absorption or chemical reactivity [47]. Exposure of the skin to isocyanates could contribute to a significant part of the total body burden. For example, Bello et al. estimated that 1% skin absorption of a small MDI droplet (10 mg) would result in a dose approximately 4.5-fold (450%) higher than a 15 min inhalation exposure to a concentration at the United Kingdom Health and Safety Executive short-term exposure limit (70 µg NCO/m^3), assuming 100% lung retention and a ventilation rate of 7 l/min [40].

Animal studies have linked respiratory sensitization due to dermal exposure to isocyanates [48, 49]. Respiratory sensitization was induced after epicutaneous exposure to HDI in mice

[50] and to TDI in guinea pigs [51] as well as after intradermal and topical exposure to MDI in guinea pigs [49]. Only one study investigating dermal penetration of diisocyantes (octyl isocyanate, MDI, polymeric HDI, and polymeric IPDI) has been conducted using guinea pig skin [52]. However, guinea pig skin, like mouse or rat skin is structurally and functionally very different from human skin. Therefore, the use of experimental animals for dermal penetration studies has only a limited value in human exposure and risk assessment.

1.4 Air Sampling Devices

Most exposure assessments have focused only on the characterization of airborne exposures [41-46]. A variety of air sampling devices and analytical methods were used in these studies, therefore, making it challenging to compare results. The measurement of airborne isocyanate-containing compounds continues to be a challenge in the industrial hygiene field. Streicher et al. [53] states that selecting the most appropriate sampling and analytical methods for isocyanates in a specific workplace environment is difficult for the following reasons: (1) isocyanates may be in the form of vapors or aerosols of various particle sizes; (2) the species of interest are reactive and unstable; (3) commercially available pure analytical standards exist only for monomeric diisocyanates; and (4) low limits of detection are needed. Selecting an inappropriate method may result in either over or underestimation of exposure or a failure to detect airborne isocyanates. The ability to select the best methodology (i.e., sampling and analytical) is critical for accurate assessment of the worker's exposure.

A further complication is that most exposure standards address only a few isocyanate monomer species, even though many isocyanate formulations commonly used in today's industry have been reformulated so that the monomers are only a small fraction (frequently

less than 1%) of the isocyanate species present. In the spray-painting environment, monomeric and polymeric HDI are present in both aerosol and vapor phases [54]. Isocyanates are very reactive and, therefore, unstable after collection and cannot simply be collected on a filter [55]. The isocyanates can be lost through reaction with other compounds on the aerosol particle or simultaneously collected on the filter. Therefore, it is necessary to derivatize the isocyanate species rapidly upon collection. Filters and sorbents impregnated with derivatizing reagent as well as an impinger or a bubbler containing solution of derivatizing reagent have been used for the collection of isocyanate aerosols. However, neither filters nor impingers appear to adequately sample for the entire range of isocyanate aerosols likely to be encountered in the workplace [55]. Particles smaller than 2 μm in diameter are not efficiently collected by an impinger and isocyanate species present in large particles are not efficiently derivatized when collected on reagent-coated filters [55-57].

Both single-stage and dual-stage samplers have been used to sample isocyanates in the occupational setting [58]. The dual-stage sampler typically contain a first stage that is loaded with an untreated polytetrafluoroethylene (PTFE) pre-filter (designed to collect isocyanate aerosols) and the second stage is loaded with a glass-fiber filter (GFF) impregnated with a derivatizing agent (designed to collect and derivatize isocyanate vapors). After sampling, the PTFE filter is placed into derivatizing solution. The dual-stage sampling system is designed primarily for short term monitoring (i.e., <30 min) because the isocyanates collected on the PTFE filter can polymerize over time. With single-stage samplers, a PTFE filter is not used. As a result, the impregnated GFF collects and derivatizes all phases of isocyanates. A commonly used and commercially available single-stage sampler is the OSHA42 sampler [59], which utilizes a GFF impregnated with 1-(2-pyridyl) piperazine (1-2PP) to sample

isocyanate monomer. A commonly used dual-stage sampler is IsoChek® (Omega Specialty Instrument Co., Houston, TX) that employs 9-(N-methylaminomethyl)anthracene (MAMA) for derivatization [42]. The analytical method provided by the commercially available IsoChek® reports diisocyanate monomer as well as all polyisocyanates expressed as total reactive isocyanates (TRIG). TRIG is defined as the sum of free NCO groups found in all isocyanate species of a sample [60]. In addition, there are several impinger methods (e.g., NIOSH 5521, NIOSH 5522, proposed NIOSH 5525), which have been modified for single-stage filter sampling of isocyanates [61].

A limitation of the OSHA42 is that it only identifies and quantitates the isocyanate monomer [62]. Studies have also shown that the OSHA42 may underestimate isocyanate in aerosol form when sampling for extended periods [63] and it has also been suggested that additional 1-2PP be added to the filter to prevent this [64]. In a comparison study of isocyanate sampling methods for monomeric and polymeric HDI in spray-painting environments, OSHA42 appeared to have the greatest variability when compared with NIOSH 5521, NIOSH 5522, Total Aerosol Mass Method (TAMM), the proposed NIOSH 5525, and the IsoChek® sampler [54].

There have been several criticisms of the IsoChek® sampler in the literature [56, 62]. In a controlled laboratory study, the IsoChek® sampler significantly underestimated TDI and MDI monomer concentrations and inaccurately apportioned them into vapor and aerosol phases [62]. The two-stage filter sampling system may produce biased results due to evaporation of aerosol off the PTFE filter and adsorption of vapor onto the PTFE filter [56]. However, in a field study performed by England et al., the IsoChek® sampler collected HDI monomer concentrations that did not differ significantly from four other commonly used sampling

methods (NIOSH 5521, NIOSH 5522, proposed NIOSH 5525, and OSHA42) [54]. However, for HDI-based polyisocyanates, England et al. observed that NIOSH 5522, NIOSH 5521, IsoChek®, and the TAMM were significantly different from one another [54]. They observed that TAMM collected the most followed by IsoChek® then NIOSH 5521, and finally NIOSH 5522 [54].

Several studies have compared filter samplers with impinger samplers [54, 65-67]. Ekman et al. investigated the performance of filter and impinger samplers that used the same derivatizing agent [1-(2-methoxyphenyl)piperazine, MPP] to quantify total isocyanates under a simulated spray-painting environment and observed no significant difference ($\alpha = 0.05$) between single-stage filter and impinger sampling [66]. Bello et al. compared stainless steel Institute of Medicine (IOM) samplers loaded with 25-mm quartz fiber filters impregnated with 500 μg of 1-(9-anthracenylmethyl)piperazine (MAP) with MAP impinger samples [67]. They observed that impingers and treated filter IOM samplers performed equally well with respect to collection efficiency for the monomer and total polymeric HDI.

1.5 Dermal Sampling Techniques

Methods for monitoring dermal exposures are less advanced than those of air sampling techniques. However, several groups have measured isocyanate skin exposures using SWYPE™ colormetric indicators (CLI, Des Plains, IL) [1, 68], wipes [69], and tape-stripping [47, 70, 71]. These methods may underestimate exposures due to losses from absorption, chemical reactions, or poor removal efficiency [34, 40]. Dermal exposure has been documented among automotive spray painters [68, 69, 71, 72], yet methods to measure skin exposure are not validated or standardized.

1.6 Study Objectives

Inhalation exposure has been considered the primary route of contact and the primary cause of isocyanate-induced sensitization in exposed workers. However, more recently, concerns about the role of dermal exposure in isocyanate induced sensitization and asthma have been raised [39, 40]. Although many studies have been performed to investigate inhalation exposure to isocyanates, the literature is conflicting as to what type of air sampling device is most reliable at predicting exposure levels. In the automotive refinishing industry, polyurethane paints used typically contain monomeric and polymeric HDI; however, polymeric HDI compose the majority of isocyanates in automotive paints. Rates of absorption into tissue and toxicity may vary between monomeric and polymeric isocyanates. Dermal exposure to monomeric and polymeric HDI in the automobile 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. Although dermal sampling methods have been developed, the penetration patterns and absorption rates are not well understood for monomeric and polymeric HDI.

The primary goal of this research project was to investigate the performance of air and dermal exposure assessment techniques used to monitor exposures to monomeric and polymeric HDI. The study objectives were as follows:

Specific Aim 1: Determine the variability and errors associated with air sampling devices used to measure monomeric and polymeric (biuret and isocyanurate) HDI during the application of a slow- and fast-drying clearcoat (a mixture of monomeric and polymeric HDI) in the occupational field setting.

Specific Aim 2: Develop a sampling patch to quantify exposure to monomeric and polymeric HDI deposited on the skin in the spray-painting environment and to compare the method with the dermal tape-strip method as described by Fent et al [47, 70].

Specific Aim 3: Using excised full-thickness human skin, (1) demonstrate that monomeric and polymeric HDI penetrate into and beyond the stratum corneum, (2) determine the difference in penetration patterns with a fast- and slow-drying clearcoat, and (3) evaluate the efficiency of the tape-strip method to measure dermal exposures to monomeric and polymeric HDI.

CHAPTER 2

FIELD COMPARISON OF AIR SAMPLING METHODS FOR MONOMERIC AND POLYMERIC 1,6-HEXAMETHYLENE DIISOCYANATE

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2.1 Abstract

The purpose of this study was to critically compare 13 different air samplers for their ability to monitor air exposures to monomeric and polymeric 1,6-hexamethylene diisocyanate (HDI) in the automotive refinishing industry. Using both fast- and slow-drying clearcoat, we tested the following types of samplers: single- and dual-stage 37-mm polypropylene (PP) and polystyrene (PS) samplers (open- and closed-face), IOM (with plastic and stainless steel inserts), OSHA42, IsoChek®, and WA-DOSH samplers. Midget impingers with frit were used as reference samplers. We observed the PP, PS, and IOM samplers to measure greater levels of HDI monomer and biuret when a fast-drying clearcoat was applied compared to a slow-drying clearcoat. When a slow-drying clearcoat was applied, the open-face PP and PS samplers measured significantly more monomeric and polymeric HDI (2-fold; $p < 0.003$)

than the closed-face PP and PS samplers. We determined that significantly more monomeric and polymeric HDI were measured by impingers (1.3 – 1.9-fold) compared with single-stage PP/PS (N = 59), dual-stage PP/PS (N = 59), or IOM (N = 24) samplers. However, when stratified by cassette characteristics, the open-face single-stage PP and PS samplers performed equally to the impingers for HDI monomer when a fast-drying clearcoat was applied and for all analytes when a slow-drying clearcoat was applied. Significantly higher HDI monomer concentrations (1.2 – 3.1-fold; $p = 0.001$) were measured with OSHA42 compared to the impinger. The IsoChek® did not detect HDI monomer and of the three samplers analyzed by laboratories other than UNC (i.e., OSHA42, IsoChek®, and WA-DOSH), the WA-DOSH was in the best agreement with the impingers. The influence of clearcoat drying-time on the sampler's ability to measure monomeric and polymeric HDI emphasizes the importance of the speciation of diisocyanates in chemical analysis and the careful consideration for the selection of the air sampler to be used when measuring exposures during automotive spray-painting.

2.2 Introduction

Diisocyanates are a group of highly reactive, low-molecular-weight aromatic and aliphatic compounds, characterized by containing two isocyanate functional groups (N=C=O). Exposure to isocyanates may cause adverse effects in respiratory tract, skin, and eyes [7, 73]. Occupational or environmental asthma may account for as much as one-third of the more than 10 million adult asthma cases [74], with isocyanate-induced asthma accounting for between 5 to 30% of the occupational asthma cases [15-20]. A large number of these workers are employed in the automotive refinishing industry where polyurethane-based

paints and coatings containing monomeric and polymeric 1,6-hexamethylene diisocyanate (HDI) are used. The most widely used isocyanates include HDI monomer as well as the HDI polyisocyanates uretdione, biuret, and isocyanurate [3]. Throughout the paper we will refer to both diisocyanates and polyisocyanates as isocyanates.

The measurement of airborne isocyanate-containing compounds continues to be a challenge in the industrial hygiene field. Selection of the most appropriate sampling and analytical method for quantitative monitoring of isocyanate exposure in a specific workplace environment is difficult for the following reasons: (1) isocyanates may be in the form of vapors or aerosols of various particle sizes; (2) the species of interest are reactive and unstable; (3) commercially available pure analytical standards exist only for monomeric diisocyanates; and (4) low limits of detection are needed [53, 55]. Use of an appropriate sampling and/or analytical method is critical for accurate assessment of worker's exposure to isocyanates. Most of the exposure assessment studies evaluating airborne isocyanate exposure in the automotive refinishing industry have used a variety of air sampling devices and analytical methods, therefore making it challenging to compare the results [41-46].

In the spray-painting environment, monomeric and polymeric HDI are present in both aerosol and vapor phases [54]. Several sampling devices and analytical chemistry methods have been used for the measurement and analysis of monomeric and polymeric HDI present in both the aerosol and vapor phase. Some common sampling devices include a variety of cassettes with treated filters, such as Institute of Medicine (IOM) sampler, Occupational Safety and Health Administration Method 42 (OSHA42) sampler, and IsoChek® sampler, as well as impingers or bubblers filled with absorbing solution.

Both single-stage and dual-stage filter cassettes have been used to sample isocyanates in the occupational setting [71]. The dual-stage samplers typically contain a first stage that is loaded with an untreated polytetrafluoroethylene (PTFE) pre-filter (designed to collect isocyanate aerosols) and the second stage is loaded with a glass-fiber filter (GFF) impregnated with a derivatizing agent (designed to collect and derivatize isocyanate vapors). With single-stage sampling, a PTFE filter is not used and, as a result, the impregnated GFF collects and derivatizes isocyanates in both aerosol and vapor phase.

A commonly used single-stage sampling method is OSHA42 [75], which utilizes a GFF impregnated with 1-(2-pyridyl) piperazine (1-2PP) to sample diisocyanate monomer. A commonly used dual-stage sampler is IsoChek® (Omega Specialty Instrument Co., Houston, TX) that employs 9-(N-methylaminomethyl)anthracene (MAMA) for derivatization. The analytical method provided by the commercially available IsoChek® reports diisocyanate monomer as well as all polyisocyanates expressed as total reactive isocyanates (TRIG). TRIG is defined as the sum of free NCO groups (i.e., comprised of all isocyanate species) present in a sample [60]. However, this is a subjective definition that is dependent on the analytical scheme used to define total NCO. In addition, there are several impinger methods (e.g., NIOSH 5521, NIOSH 5522, proposed NIOSH 5525), which have been modified for single-stage filter sampling of isocyanates.

Several studies have been performed to evaluate and compare some of these sampler types [46, 53-55, 60, 63, 65-67, 76, 77]. However, the evidence is inconclusive regarding the suitability and accuracy of these methods for monitoring isocyanate exposure in different settings. Therefore, the objective of this study was to compare sampling devices commonly used to quantify exposure to monomeric and polymeric HDI in the spray-painting

environment. Our ability to measure isocyanate exposure properly is critical for exposure and risk assessment in order to predict systemic exposure, to develop sensitive and predictive models through multiple exposure routes, and ultimately to protect the health of workers.

2.3 Methods

2.3.1 Air Samplers

A total of 13 air samplers were compared for measuring monomeric and polymeric HDI levels. Along with glass midget impingers with frit (reference sampler), we evaluated the performance of single- and dual-stage 37-mm polypropylene (PP) and polystyrene (PS) samplers either open- or closed-face, IOM samplers with plastic and stainless steel inserts, as well as OSHA42, IsoChek®, and Washington State Division of Safety and Health (WA-DOSH) samplers. These samplers were selected because they have previously been used to monitor monomeric and polymeric HDI exposures in research studies as well as by practicing industrial hygienists for regulatory purposes. We decided to include both PP and PS materials because we found lower recoveries of HDI for PS as compared with PP in laboratory experiments (results not published), which supported Huynh et al. [78] findings that showed higher recovery from PP cassettes. The different samplers are described below and further summarized in **Table 2.1**. All chemicals used in this study were obtained from Sigma Aldrich (St. Louis, MO, USA) unless otherwise specified. All samplers analyzed at UNC laboratory were prepared no more than two weeks prior to sampling.

2.3.1.1 Single and dual-stage PP and PS samplers. The single-stage PP and PS samplers contained a 37-mm GFF (Type AE, SKC, Eighty Four, PA) impregnated with MPP solution

[1-(2-methoxyphenyl)-piperazine (MPP), 192.3 g/mol; 1.09 g/ml in toluene] designed to collect and derivatize isocyanate vapors. The dual-stage PP and PS samplers contained an untreated polytetrafluoroethylene (PTFE) pre-filter, designed to collect isocyanate aerosols, and an impregnated GFF. Before GFFs were placed into a single- or dual-cassette housing, they were impregnated with 400 µl of 12 g/l MPP in toluene, which corresponds to 4.8 mg of MPP, and allowed to dry for 15 min.

2.3.1.2 IOM Samplers. IOM samplers with either a stainless steel or plastic insert were prepared by impregnating a 25-mm GFF (SKC) with 400 µl MPP solution, which corresponds to 4.8 mg of MPP (consistent with PP/PS samplers). Before placing the GFFs into a sampler, 200 µl of 12 g/l MPP in toluene was applied to a GFF and allowed to dry for 15 min after which an additional 200 µl of 12 g/l MPP in toluene, was applied and allowed to dry for 15 min.

2.3.1.3 OSHA42. A 37-mm GFF was coated with 0.1 mg of 1-(2-pyridyl)piperazine (1-2PP) and housed in a PS cassette. These samplers (Galson Laboratories, Syracuse, NY) were used to monitor concentrations of HDI monomer.

2.3.1.4 IsoChek®. This sampler was a dual-stage 37-mm PS cassette obtained from Galson Laboratories. The first stage included a 5-µm PTFE filter designed to capture aerosols while the second stage included a 37-mm GFF impregnated with 9-(*N*-methylaminomethyl)anthracene (MAMA) designed to capture and derivatize isocyanate vapors.

Table 2.1. Summary of the samplers, experimental conditions, and analytical techniques

	Impinger	Single-Stage	Dual-Stage	IOM	Single-Stage OSHA42	Dual-Stage IsoChek®	Dual-Stage WA-DOSH
Housing	25-ml midget impinger with frit	37-mm PP or PS	37-mm PP or PS	25-mm SS or plastic	37-mm PS	37-mm PS	37-mm PS
Housing Mode	NA	Open- or closed-face	Open- or closed-face	Open-face	Open-face	Closed-face	Closed-face
Sample Medium	15 ml of 2 g/l MPP in 30% DMF/ACN (30 mg)	GFF with MPP (4.8 mg)	5 µm PTFE GFF with MPP (4.8 mg)	GFF with MPP (4.8 mg)	GFF with 1-2PP (0.1 mg)	5 µm PTFE GFF with MAMA (0.1 mg)	5 µm PTFE GFF with MAMA (0.1 mg)
Flow Rate	1 l/min	1 l/min	1 l/min	2 l/min	1 l/min	1 l/min	1 l/min
Analyte	HDI monomer HDI polyisocyanates (biuret and isocyanurate)	HDI monomer HDI polyisocyanates (biuret and isocyanurate)	HDI monomer HDI polyisocyanates (biuret and isocyanurate)	HDI monomer HDI polyisocyanates (biuret and isocyanurate)	HDI monomer	HDI monomer HDI polyisocyanate (total NCO)	HDI monomer HDI polyisocyanates (biuret and isocyanurate)
Analytical Technique	LC-MS	LC-MS	LC-MS	LC-MS	HPLC-UV or FL	HPLC-PDA UV	HPLC-DAD and FLD
LOD[A] (µg)	monomer 0.002 biuret 0.02 isocyanurate 0.02	monomer 0.002 biuret 0.02 isocyanurate 0.02	monomer 0.002 biuret 0.02 isocyanurate 0.02	monomer 0.002 biuret 0.02 isocyanurate 0.02	0.1[B]	monomer and polyisocyanate 0.03[B]	monomer 0.005 biuret 1.7 isocyanurate 1.2

Notes: PP = polypropylene; PS = polystyrene; SS = stainless steel; NA = not applicable; MPP = 1-(2-methoxyphenyl)-piperazine; DMF = *N*,*N*-dimethylformamide; ACN = acetonitrile; GFF = glass fiber filter; PTFE = polytetrafluoroethylene; 1-2PP = 1-(2-pyridyl) piperazine; MAMA = 9-(*N*-methylaminomethyl)anthracene; LC-MS = liquid chromatography/mass spectrometry; HPLC = high-performance liquid chromatography; UV = ultraviolet detector; FL = fluorescence detector; PDA = photodiode array detector; DAD = diode array detector.

A. Instrumental limit of detection: µg per filter.
B. Based on limit of quantitation (reporting limit) provided by accreditated laboratory.

2.3.1.5 WA-DOSH. The WA-DOSH sampler uses the same dual-stage cassette, filters and derivatizing agents as the commercially available IsoChek® sampler described above. A different analytical technique is utilized for the analysis of the WA-DOSH sampler by the Washington State DOSH laboratory, an AIHA accredited laboratory. The WA-DOSH Labor and Industry (L&I) method directly measures the mass of individual diisocyanate polymers and expresses each diisocyanate in units of $\mu g/m^3$.

2.3.1.6 Impinger. Glass midget impingers with frit (SKC 225-36-2) were filled with 15 ml of derivatizing solution, which was made by dissolving 2 g of MPP in 1 l of 30% v/v solution of *N*,*N*-dimethylformamide (DMF, 73.09 g/mol) in acetonitrile (ACN, 41.05 g/mol). The theoretical amount of derivatizing agent (MPP) in each impinger was 30 mg.

2.3.2 Spray-painting Procedure and Sample Collection

As shown in **Figure 2.1**, six samplers (A – F) of the same type and three impingers (I1 – I3) were attached to a cardboard backing. The samplers were attached to a high-flow pump (SKC) operated at 1 or 2 l/min. The pumps were calibrated before and after sampling using a DryCal® primary flow meter (BIOS Corp., Butler, NJ). Clearcoat was sprayed directly above the samplers with a high-volume, low-pressure (HVLP) spray gun, producing an overspray that was deposited over the samplers. The same painter was used throughout the study and was instructed to stand approximately 1 m from the samplers. The painter sprayed the clearcoat over the top the samplers across the cardboard backing 4 times during each spray period. Spraying was conducted a total of three times throughout the 15 min sample period (once every 5 min) using a multi-use clear with either a HDI containing fast or slow

activator-reducer (3:1 clear to activator by volume). For quality control, blank sample controls were obtained by opening and closing prepared cassettes in the field setting. In addition, bulk samples (10 µl) of the clearcoat being sprayed were collected each time a new batch of clearcoat was mixed (N = 12).

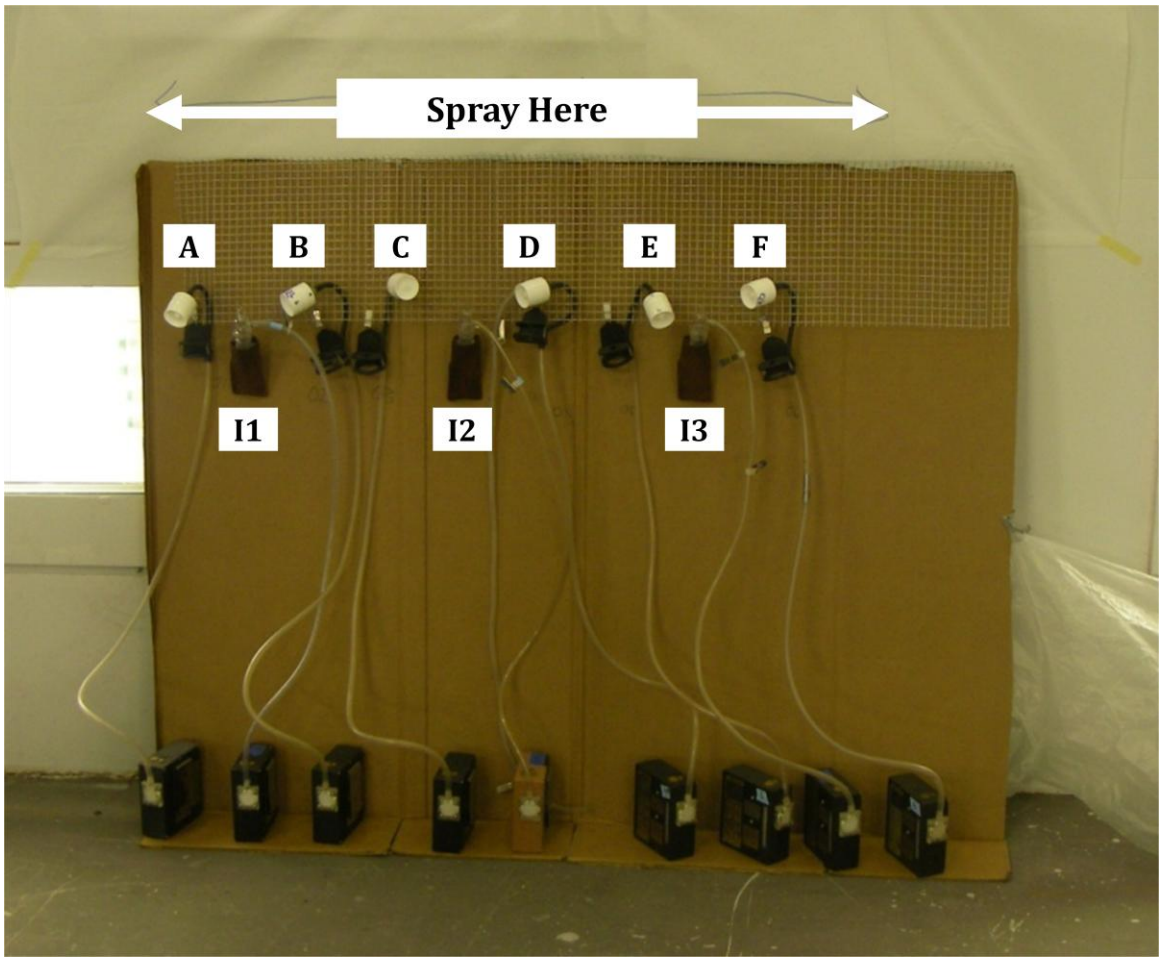

Figure 2.1. Sampler set-up during spray-painting. (I1 = reference impinger 1 compared with samplers A and B; I2 = reference impinger 2 compared with samplers C and D; I3 = reference impinger 3 compared with samplers E and F.

2.3.3 Sample Processing and Analysis

2.3.3.1 PP, PS, and IOM samplers. Immediately after sampling, both the PTFE and GFF from single- and dual-stage PP and PS as well as from IOM cassettes were placed into 20-ml

glass vials (I-Chem, Thermo Fisher Scientific, Rockwood, TN) containing 5 ml of derivatizing solution (2 g/l MPP in 30% DMF-ACN solution) to minimize the time for any competing reactions, such as isocyanate polymerization. Samples were shaken thoroughly and then stored in a cooler (~4 °C) until returned to UNC laboratory and stored at –40 °C until analyzed. For analysis, samples were returned to room temperature and acetic anhydride was added (100 µl) to acetylate residual MPP. After 15 min, internal standard (52 pmol/µl urea derivative of 1,8-octamethylene diisocyanate; ODIU) was added (100 µl) to give an internal standard concentration of 1 pmol/µl. Samples were analyzed for HDI monomer, biuret, and isocyanurate using liquid chromatography-mass spectrometry (LC-MS) as described elsewhere [47].

2.3.3.2. OSHA42. Samples were handled according to manufacturer's specifications. After sampling, the cassettes with GFFs in place were sealed and stored at –40 °C until shipment in a cooler (~4 °C) to Galson Laboratories (an AIHA-accredited laboratory) for analysis. Samples were processed and analyzed for HDI monomer by OSHA42 method, which utilizes solvent desorption and high-performance liquid chromatography (HPLC) analysis using ultraviolet (UV) or fluorescence (FL) detector [75].

2.3.3.3 IsoChek®. Samples were handled according to the manufacturer's specifications. After sampling, the PTFE filter was immediately placed into 5 ml of methoxy-2-phenyl-1-piperazine reagent (MOPIP) in toluene (0.1 mg/ml) to derivatize aerosols. The vials containing PTFE filters and the cassettes with GFFs in place were sealed and stored at –40 °C until shipment in a cooler (~4 °C) to Galson Laboratories for analysis. Both the PTFE

filters and GFFs were analyzed using HPLC with photodiode array detector (PDA) UV for HDI monomer and polyisocyanates. The IsoChek® method does not identify the types of polyisocyanates present based on specific polyisocyanate standards. Instead, polyisocyanates of HDI are identified by comparing a diode array scan of the associated monomer standard to a diode array scan of the samples in order to identify the presence of polyisocyanate peaks. Once identified, the areas of these peaks are summed and quantified using the response factor of the monomer peak and concentration calculated using the molecular weight of an NCO equivalent (42 g/mol).

2.3.3.4 WA-DOSH. Samples were handled according to the WA-DOSH L&I method specifications (L&I 0050 and L&I 0067). After sampling was completed, the PTFE filter was immediately removed from the cassette and placed in a jar with 4 ml of MOPIP in toluene solution (1 mg/ml) and the cassettes with GFFs in place were sealed. The samples were stored at –40 °C until analysis by WA-DOSH state laboratory using HPLC with diode array detector (DAD) and FL UV as described in WA-DOSH L&I methods 0050 and 0067 [79, 80]. The WA-DOSH L&I method directly measures the mass of individual diisocyanate polymers and expresses each diisocyanate in units of $\mu g/m^3$.

2.3.3.5 Impingers. After sample collection, the solution evaporated from the impinger during sampling was replaced with derivatizing solution (2 g/l MPP in 30% DMF/ACN) to obtain a total volume of 15 ml. The sampling solution was then transferred into a 20-ml glass vial (I-Chem) and placed in a cooler (~4 °C) and returned to UNC laboratory for storage at –40 °C until analyzed. For analysis, samples were returned to room temperature and acetic

anhydride was added (200 µl) to acetylate residual MPP. After 15 min, internal standard (77 pmol/µl ODIU) was added (200 µl) to given an internal standard concentration of 1 pmol/µl. Samples were analyzed using LC-MS as described elsewhere [47].

2.3.3.6 Bulk samples. Bulk sample of a mixed clearcoat (10 µl) was drawn into a 20-µl pipette and dispensed into a glass vial (I-Chem) filled with 15 ml of derivatizing solution (2 g/l MPP in 30% DMF/ACN). The pipette tip was also ejected into the solution to eliminate side-wall losses due to the viscosity of the clearcoat. The samples were then placed into a cooler (~4 °C) and returned to UNC laboratory for storage at –40 °C until analyzed. For analysis, samples were returned to room temperature and acetic anhydride was added (200 µl) to acetylate residual MPP. After 15 min, internal standard (2 pmol/µl ODIU) was combined (1:1 v/v ratio) with aliquots of each paint sample to give an internal standard concentration of 1 pmol/µl. Samples were analyzed using LC-MS as described elsewhere [47].

2.3.4 Data Analysis

The relative standard error (RSE) and relative standard deviation (RSD) for the three impinger samples and six cassette samples collected during each sampling run were calculated. We further calculated an overall mean RSE and RSD among the sampling runs for both fast- and slow-drying clearcoats. Paired *t*-test (α-level of 0.05) was used to determine if the variability among impingers and cassette samplers was significantly different.

2.3.4.1 PP, PS, and IOM samplers. For dual-stage samplers, the sum of the mass on the filters (PTFE and GFF) was calculated. Levels below the limit of detection (LOD) or quantitation (LOQ) were assigned values by dividing the respective limits by $\sqrt{2}$ [81]. The ratio of the HDI concentration in the filter(s) to the concentration measured with the impinger was determined and the ratios were natural log-transformed to satisfy the normality assumption. Shapiro-Wilks tests for normality indicated that the ratios were approximately log-normally distributed for HDI monomer ($W = 0.99$), biuret ($W = 0.95$), and isocyanurate ($W = 0.96$).

The data were analyzed using SAS 9.1 statistical software (Cary, NC) at α-level of 0.05. A paired *t*-test was used to determine if samplers were significantly different from the adjacent impinger samples. General linear modeling (SAS PROC GLM) with the least squares approach was used to investigate the differences in the measured amounts of HDI monomer, biuret, and isocyanurate between the samplers. GLM analysis of covariance (ANCOVA) type III sum of squares, with adjustment for reference impinger, was utilized to determine if the variables (1) clearcoat (fast- or slow-drying), (2) sampling style (open- or closed-face), (3) cassette stage (single- or dual-stage), or (4) cassette type (PP or PS; IOM plastic or IOM steel) were influencing the observed cassette to impinger ratios for HDI monomer, biuret, and isocyanurate. Differences in the least square means of the sampler to impinger ratios for HDI monomer, biuret, and isocyanurate were investigated for six sampler combinations: (1) one-stage open-face, (2) one-stage closed-face, (3) dual-stage open-face, (4) dual-stage closed-face, (5) IOM plastic, and (6) IOM steel. Tukey-Kramer adjustment for multiple comparisons was utilized and the data were stratified by clearcoat drying time (fast or slow).

2.3.4.2 OSHA42, IsoChek®, and WA-DOSH samplers. The results of the OSHA42 and IsoChek® (Galson Laboratories) and WA-DOSH (WA State DOSH Laboratory) samples were compared with the impinger samples analyzed at UNC. For the impinger samples corresponding with IsoChek® samples, the results for HDI polyisocyanate are expressed in mg/m^3 NCO in air calculated as the concentration (C) of the compound divided by its molecular weight (MW) and multiplied by the number (N) of NCO groups and the MW of NCO (42 g/mol):

$$(C_{compound}/MW_{compound}) \times N_{NCO} \times MW_{NCO}\ [82].$$

The results for HDI biuret and isocyanurate were converted to mg/m^3 NCO in air and summed for the impinger samples corresponding with IsoChek® samples only. Levels below the LOD or LOQ were assigned values by dividing the respective limits by $\sqrt{2}$ [81]. Paired *t*-test (α-level of 0.05) was used to determine if these samplers measured significantly different amounts of monomeric and polymeric HDI than the adjacent impinger samples. Student's *t*-test (α-level of 0.05) was used to determine if these samplers measured significantly different amounts of monomeric and polymeric HDI when using a fast- or slow-drying clearcoat.

2.3.4.3 Bulk sample. The bulk paint samples were analyzed at UNC. A Student's *t*-test (α-level of 0.05) was used to determine if significant differences existed between concentrations of HDI monomer, biuret, and isocyanurate between a fast- and slow-drying clearcoat.

2.4 Results

All samplers measured levels of monomeric and polymeric HDI that were within ranges reported in the occupational field settings [41, 58]. HDI monomer, biuret, and isocyanurate

concentration ratios (i.e., the sampler concentration divided by the impinger concentration) by clearcoat type for samplers are summarized in the box and whisker plots in **Figure 2.2, 2.3,** and **2.4,** respectively. It should be noted that OSHA42 and IsoChek® samplers are not plotted for HDI biuret and isocyanurate as these analytes were not measured by these samplers. It should also be noted that the HDI monomer levels measured by IsoChek® (N = 12) and the HDI biuret levels measured by WA-DOSH (fast-drying clearcoat only, N = 6) were below respective LOD or LOQ and were therefore calculated using respective LOD or LOQ divided by the √2 [81].

The overall mean RSE and RSD among the sampling runs for both a fast- and slow-drying clearcoat are summarized in **Table 2.2**. Paired *t*-test revealed that the impinger samplers were significantly less variable than cassette samplers for HDI biuret when a fast-drying clearcoat was applied ($p = 0.04$). Paired *t*-test also showed that the cassette samplers were significantly less variable than the impinger samplers for HDI monomer when a slow-drying clearcoat was applied ($p = 0.01$). For all other instances, the variability among impingers and cassette samplers was not significantly different**.**

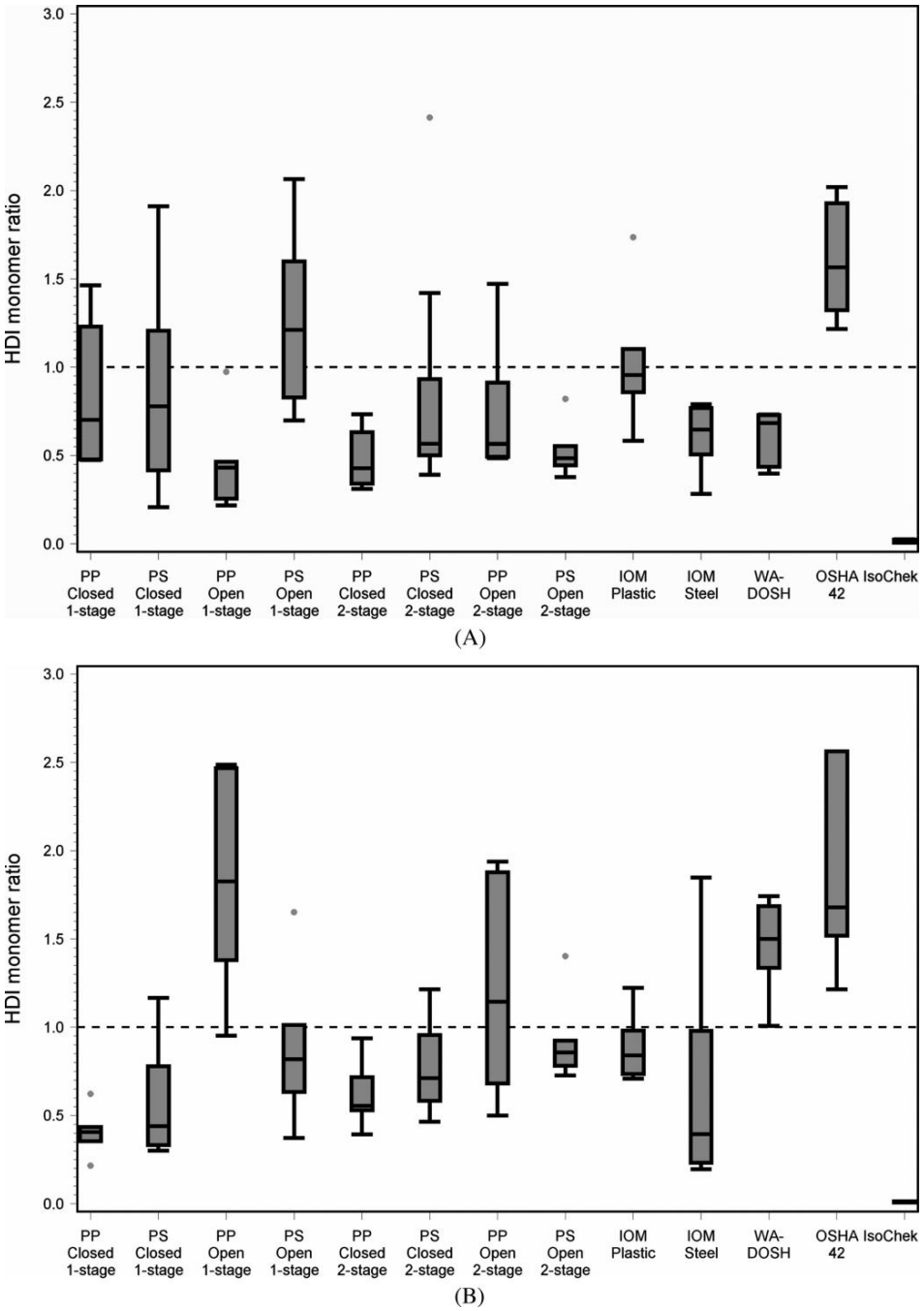

Figure 2.2. Box and whisker plots for HDI monomer for fast-drying (A) and slow-drying (B) clearcoat by sampler type. The top error bar represents the maximum observation below the upper fence (1.5 times interquartile range) and the bottom error bar the minimum observation. The top of the box is the 75th percentile while the bottom is the 25th percentile. The line in the box is the median and the dot is the maximum observation.

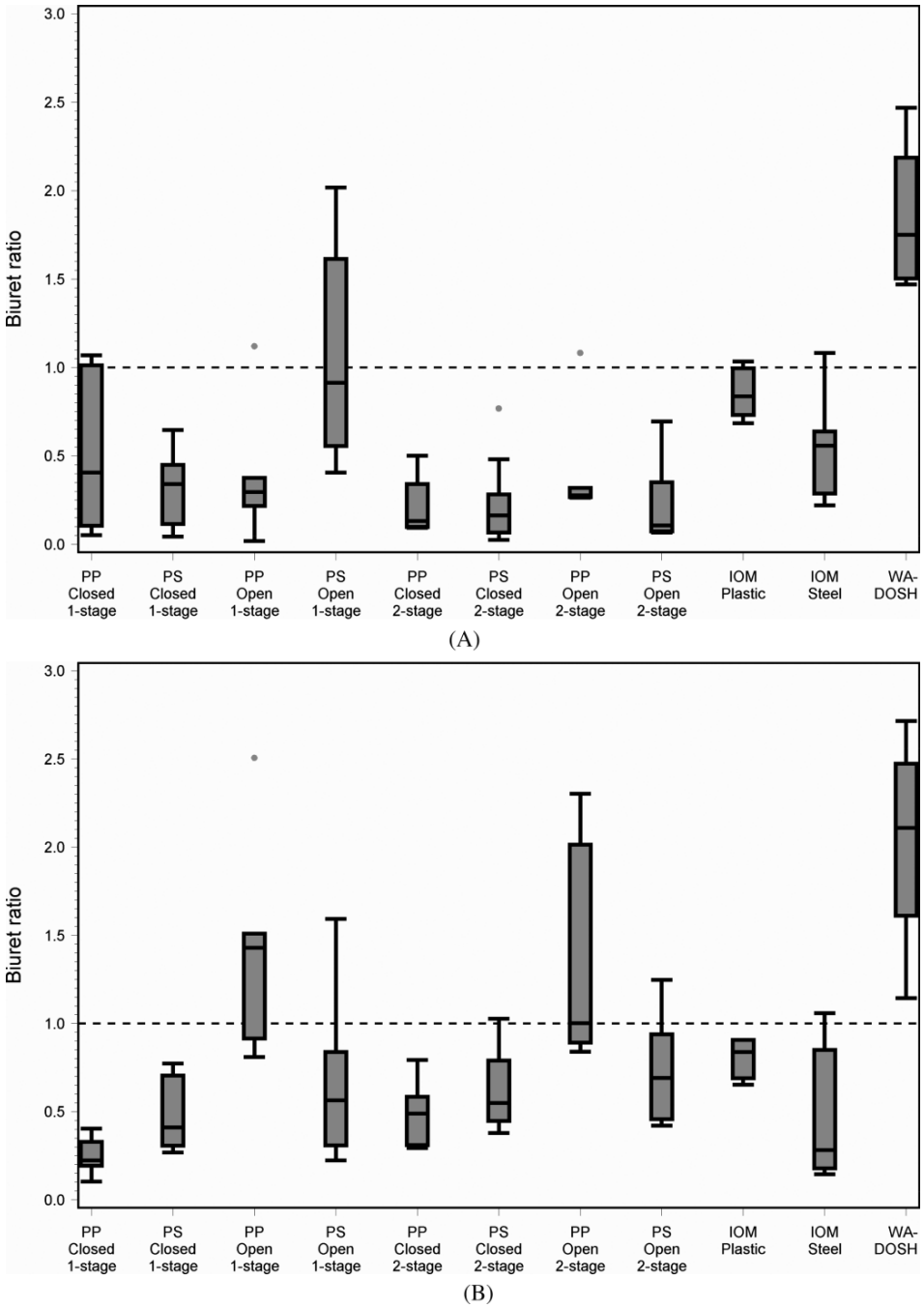

Figure 2.3. Box and whisker plots for biuret for fast-drying (A) and slow-drying (B) clearcoat by sampler type. The top error bar represents the maximum observation below the upper fence (1.5 times interquartile range) and the bottom error bar the minimum observation. The top of the box is the 75th percentile while the bottom is the 25th percentile. The line in the box is the median and the dot is the maximum observation.

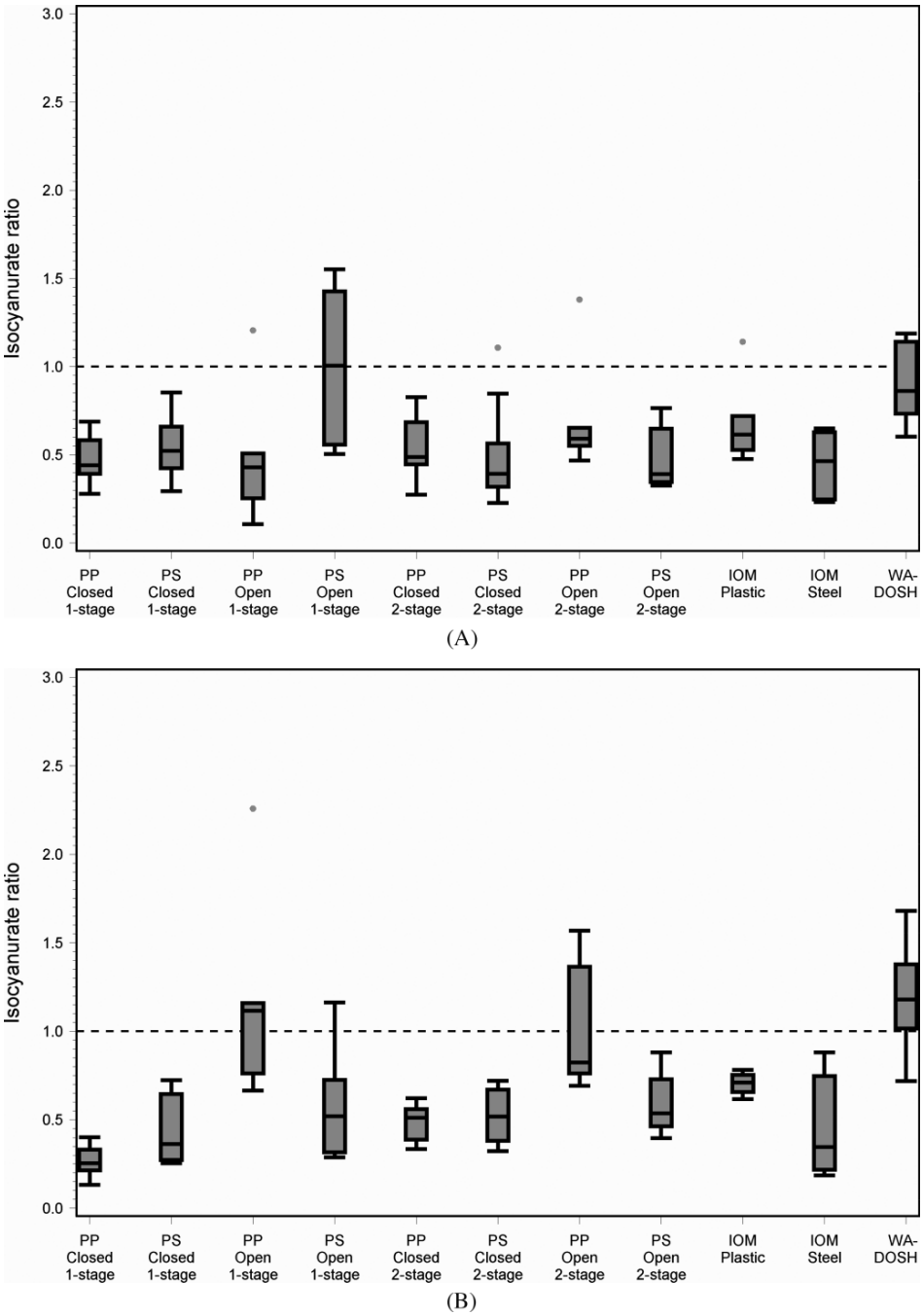

Figure 2.4. Box and whisker plots for biuret for fast-drying (A) and slow-drying (B) clearcoat by sampler type. The top error bar represents the maximum observation below the upper fence (1.5 times interquartile range) and the bottom error bar the minimum observation. The top of the box is the 75th percentile while the bottom is the 25th percentile. The line in the box is the median and the dot is the maximum observation.

Table 2.2. Overall relative standard error (RSE) of impingers and cassettes across sampling runs.

	Overall RSE ± RSD (%)		
	HDI	**Biuret**	**Isocyanurate**
Fast-Drying Clearcoat			
Impinger (N = 15)	19.1 ± 8.2	15.3 ± 9	11.8 ± 4.9
Cassette (N = 15, 13, 14)[A]	12.9 ± 7.6	25.5 ± 13.3	14.2 ± 10.1
Slow-Drying Clearcoat			
Impinger (N = 15)	17.6 ± 6.8	15.4 ± 8.5	12.6 ± 7.7
Cassette (N = 15, 13, 14)[A]	11.7 ± 6.9	15.6 ± 7.0	14.0 ± 10.8

Notes: RSE = relative standard error; RSD = relative standard deviation.
A. For HDI, biuret and isocyanurate, respectively.

2.4.1 PP, PS, and IOM Samplers

The geometric mean (GM) air levels (μg/m^3) of monomeric and polymeric HDI measured by impingers as well as PP, PS, and IOM samplers stratified by clearcoat drying-time are summarized in **Table 2.3**. As depicted in **Figure 2.2-2.4**, when a fast-drying clearcoat was applied, the single-stage open-face PS sampler was in best agreement with impinger samplers for HDI monomer, biuret, and isocyanurate [GM (95% Confidence Interval; 95%CI): 1.19 (0.78 – 1.8), 0.92 (0.48 – 1.8), 0.92 (0.56 – 1.5), respectively]. For slow-drying clearcoat, the dual-stage open-face PP sampler was in best agreement with impinger samplers for HDI monomer, biuret, and isocyanurate [GM (95%CI): 1.07 (0.60 – 1.9), 1.54 (0.80 – 3.0), 1.30 (0.59 – 2.9), respectively]. For biuret, GM ratios for all samplers were about 2-fold higher when a slow-drying clearcoat was sprayed compared with fast-drying clearcoat (Student's *t*-test, $p < 0.0001$); this significant trend was not observed for HDI monomer and isocyanurate.

Table 2.3. Geometric mean (GM) air levels (µg/m^3) of monomeric and polymeric HDI measured with PP, PS, and IOM samplers and impingers stratified by clearcoat.

Cassette Type	Sample Size (N)	HDI (µg/m^3)			Biuret (µg/m^3)			Isocyanurate (µg/m^3)		
		GM	Range	No. <LOD	GM	Range	No. <LOD	GM	Range	No. <LOD
Fast-Drying Clearcoat										
Impinger	36	8.58	3.55 – 22.8	0	28.4	14.15 – 69.6	0	3,425	1,986 – 6,708	0
IOM Plastic	6	5.86	3.46 – 7.82	0	24.5	14.7 – 37.0	0	2,207	1,434 – 4,348	0
IOM Steel	6	5.19	3.46 – 9.93	0	17.8	9.00 – 33.6	0	1,715	994 – 3,110	0
1-Stage Closed-Face PP	6	3.72	2.38 – 6.99	0	5.81	0.92 – 18.9	1	1,283	907 – 1,979	0
1-Stage Open-Face PP	6	5.64	2.38 – 16.3	0	9.96	0.75 – 52.8	1	2,149	560 – 8,081	0
2-Stage Closed-Face PP	6	2.87	2.37 – 3.72	0	2.70	1.33 – 7.98	0 (6)[A]	1,181	722 – 2,029	0 (1)[A]
2-Stage Open-Face PP	5	8.31	6.07 – 9.80	0 (1)[B]	13.8	7.92 – 32.0	0 (5)[A]	2,861	2,286 – 4,828	0
1-Stage Closed-Face PS	12	5.02	2.25 – 9.40	0	6.32	0.84 – 15.9	1	1,594	982 – 2,408	0
1-Stage Open-Face PS	6	10.9	6.81 – 14.0	0	21.8	14.3 – 40.1	0	2,913	2,178 – 4,423	0
2-Stage Closed-Face PS	12	7.18	3.59 – 13.6	0	5.55	1.74 – 19.0	0 (5)[A]	1,493	712 – 2,492	0
2-Stage Open-Face PS	6	5.78	3.79 – 7.15	0	3.76	1.77 – 19.2	2 (3)[A]	1,632	1,111 – 3,199	0
Slow-Drying Clearcoat										
Impinger	36	14.6	2.84 – 64.8	0	207	56.7 – 926.4	0	3,032	1,041 – 12,090	0
IOM Plastic	6	10.5	8.19 – 12.6	0	165	143 – 193	0	1,901	1,552 – 2,394	0
IOM Steel	6	8.29	4.75 – 19.5	0	104	65.8 – 182	0	1,369	864 – 2,100	0
1-Stage Closed-Face PP	6	4.80	3.23 – 6.24	0	50.6	26.3 – 92.3	0	810	508 – 1,259	0
1-Stage Open-Face PP	6	26.9	15.8 – 40.1	0	301	177 – 551	0	3,260	1813 – 8,177	0
2-Stage Closed-Face PP	6	8.40	5.82 – 11.3	0	71.0	41.6 – 116	0 (6)[A]	1,142	734 – 1,491	0
2-Stage Open-Face PP	6	12.9	9.08 – 37.6	0	208	128 – 674	0 (5)[A]	2,714	1,650 – 12,686	0
1-Stage Closed-Face PS	11	13.8	4.68 – 40.6	0	168	47.1 – 324	0	2,233	739 – 4,558	0
1-Stage Open-Face PS	6	3.79	2.87 – 5.10	0	62.4	39.3 – 101	0	934	639 – 1,325	0
2-Stage Closed-Face PS	12	17.6	10.8 – 24.8	0	159	93.5 – 247	0 (11)[A]	2,154	1498 – 2,970	0
2-Stage Open-Face PS	6	6.43	5.00 – 11.6	0	74.4	39.7 – 139	0 (3)[A]	1,077	739 – 1,642	0

[A] Dual-stage filters results are the sum of both filters, however bottom filter (GFF) <LOD but top filter (PTFE) >LOQ. Numbers in parenthesis represent the number of GFFs <LOD.

[B] Dual-stage filter results are the sum of both filters, however top filter (PTFE) <LOD but bottom filter (GFF) >LOQ. Numbers in parenthesis represent the number of PTFEs <LOD.

Significantly more HDI monomer, biuret, and isocyanurate were measured by impingers (1.3 – 1.9 fold) compared with the PP/PS single-stage (N = 59), PP/PS dual-stage (N = 59), and IOM (N = 24) samplers (p-values <0.005; data not shown). However, significant differences were not always observed between impingers when the samplers were stratified by different characteristics. For example, when fast-drying clearcoat was applied, the open-face single-stage samplers (N = 12) did not differ from the impingers for HDI monomer (p = 0.11). When slow-drying clearcoat was applied, the open-face single- (N = 12) and dual-stage (N = 12) samplers did not differ from the impingers for HDI monomer (p = 0.38 and p = 0.90, respectively), biuret (p = 0.73 and p = 0.90, respectively), and isocyanurate (p = 0.42 and p = 0.47, respectively). Regardless of the clearcoat formulation, the IOM plastic samplers (N = 12) did not differ from the impingers for HDI monomer (p = 0.35).

The influence of clearcoat type, sampling style, cassette stage, and cassette type on the observed cassette to impinger ratios for HDI monomer, biuret, and isocyanurate are presented in Table IV. For PP and PS samplers, the type of clearcoat was a significant variable influencing HDI monomer (p = 0.0064) and biuret (p < 0.0001) ratios. The ratios when the slow-drying clearcoat was applied were 1.1- and 2.5-fold higher than the fast-drying clearcoat for HDI monomer and biuret, respectively. Significant differences were observed between sampling styles (open- or closed-face) for all analytes (p-values <0.01) but not between cassette stages (single- or dual-stage) or cassette types (PP or PS) (p-values >0.22). For all analytes, the open-face samplers had ratios 1.4 – 1.9-fold higher compared with those of the closed-face samplers. However, when stratified by clearcoat (data not shown), significant differences between open- and closed-face samplers (Student's *t*-test, p < 0.05)

were only observed for all analytes when a slow-drying clearcoat was applied and for HDI biuret when a fast-drying clearcoat was applied (open-face samplers 2-fold higher).

Although we did not observe significant difference between the cassette stages (single- or dual-stage), we stratified the data by cassette stage because the sampler systems are different in construction (data not shown). We observed significantly greater ratios (1.6-fold) for all analytes when using a closed-face dual-stage PP/PS sampler (N = 18) compared with a closed-face single-stage PP/PS sampler (N = 17) only when slow-drying clearcoat was sprayed (Student's *t*-test, p-values <0.02). In a previous study [58], single-stage PS samplers were observed to measure significantly more HDI monomer and isocyanurate than dual-stage PS samplers. Thus, we stratified the data to compare closed-face single- and dual-stage PS samplers (data not shown). Only when slow-drying clearcoat was applied did we observe significantly greater HDI monomer ratios with the dual stage [N = 12, GM (95%CI) = 0.74 (0.61 – 0.90)] compared with the single-stage [N = 11, GM (95%CI) = 0.49 (0.36 – 0.68)] PS samplers (Student's *t*-test, p = 0.04).

For IOM samplers, clearcoat drying time significantly affected HDI monomer (p = 0.036) and biuret (p = 0.001) concentrations (**Table 2.4**). When a fast-drying clearcoat was sprayed, the ratios for IOM samplers were 1.3 and 1.4-fold higher for HDI monomer and biuret, respectively, than when a slow-drying clearcoat was applied. Significant differences were observed between the IOM plastic and IOM steel samplers for biuret (p = 0.015) and isocyanurate (p = 0.050) (**Table 2.4**). For all analytes, the ratios for IOM plastic samplers were about 2.0-fold higher than the IOM steel samplers.

Table 2.4. The effect of clearcoat type, sampling style, cassette stage, and cassette type on the sampler-impinger ratios for HDI monomer, biuret, and isocyanurate.

Variable	Analyte		
	HDI (p-value)	**Biuret (p-value)**	**Isocyanurate (p-value)**
PP and PS Samplers			
Clearcoat (fast- or slow-drying)	**0.0064**	**<0.0001**	0.7377
Sampling style (open- or closed-face)	**0.0078**	**0.0011**	**0.0004**
Cassette stage (single- or dual-stage)	0.6746	0.2217	0.7757
Cassette type (PP or PS)	0.2229	0.9578	0.9053
IOM Samplers			
Clearcoat (fast- or slow-drying)	**0.0359**	**0.0011**	0.3264
Cassette type (plastic or steel)	0.2696	**0.0154**	**0.0504**

Note: **Bold =** significant at α-level of 0.05

We did not observe significant difference in monomeric and polymeric HDI ratios between the material types of the samplers (PP or PS); thus, we combined these samplers into one group to assess the differences in the least square means of the sampler to impinger ratios for HDI monomer (**Table 2.5**), biuret (**Table 2.6**), and isocyanurate (**Table 2.7**) with the six samplers analyzed at UNC (PP, PS, and IOM). Some notable differences are that for slow-drying clearcoat, the single-stage and dual-stage open-face samplers measured more HDI monomer, biuret, and isocyanurate than the single-stage closed-face samplers. For fast-drying clearcoat, the single-stage open-face samplers measured more HDI biuret and isocyanurate than the dual-stage closed-face samplers.

Table 2.5. Comparison of the least square means of the sampler-impinger ratios for HDI monomer (p-values).

	Single-stage closed-face	Single-stage open-face	Dual-stage closed-face	Dual-stage open-face	IOM plastic	IOM steel
Fast-drying clearcoat						
Single-stage closed-face	1.0000					
Single-stage open-face	0.5103					
Dual-stage closed-face	0.9999	0.5554				
Dual-stage open-face	0.9292	0.9771	0.9589			
IOM plastic	0.8154	0.9999	0.8949	0.9993		
IOM steel	1.0000	0.7401	1.0000	0.9734	0.9242	1.0000
Slow-drying clearcoat						
Single-stage closed-face	1.0000					
Single-stage open-face	**(0.0007)**					
Dual-stage closed-face	0.1159	0.1918				
Dual-stage open-face	**(0.0085)**	0.9550	0.6417			
IOM plastic	0.1042	0.8718	0.9533	0.9981		
IOM steel	0.9999	0.0168	0.5870	0.0901	0.3339	1.0000

Note: **Bold =** significant at α-level of 0.05. Values in parentheses indicate higher ratios for the sampler on the left side of the table.

Table 2.6. Comparison of the least square means of the sampler-impinger ratios for HDI biuret (p-values).

	Single-stage closed-face	Single-stage open-face	Dual-stage closed-face	Dual-stage open-face	IOM plastic	IOM steel
Fast-drying clearcoat						
Single-stage closed-face	1.0000					
Single-stage open-face	0.0623					
Dual-stage closed-face	0.9401	**0.0032**				
Dual-stage open-face	0.9516	0.4160	0.5063			
IOM plastic	0.0799	0.9998	**(0.0171)**	0.5177		
IOM steel	0.2819	0.9999	0.0615	0.7839	0.9975	1.0000
Slow-drying clearcoat						
Single-stage closed-face	1.0000					
Single-stage open-face	**(0.0015)**					
Dual-stage closed-face	0.2571	0.1581				
Dual-stage open-face	**(0.0003)**	0.9980	0.0540			
IOM plastic	**(0.0444)**	0.9912	0.6673	0.9334		
IOM steel	1.0000	**0.0163**	0.6340	0.0054	0.1385	1.0000

Note: **Bold** = significant at α-level of 0.05. Values in parentheses indicate higher ratios for the sampler on the left side of the table.

Table 2.7. Comparison of the least square means of the sampler-impinger ratios for HDI isocyanurate (p-values).

	Single-stage closed-face	Single-stage open-face	Dual-stage closed-face	Dual-stage open-face	IOM plastic	IOM steel
Fast-drying clearcoat						
Single-stage closed-face	1.0000					
Single-stage open-face	0.1090					
Dual-stage closed-face	0.9924	0.2223				
Dual-stage open-face	0.3092	0.9974	0.5270			
IOM plastic	0.8435	0.9340	0.9757	0.9925		
IOM steel	1.000	0.2703	0.9965	0.5023	0.9162	1.0000
Slow-drying clearcoat						
Single-stage closed-face	1.0000					
Single-stage open-face	**(0.0009)**					
Dual-stage closed-face	0.4027	0.0682				
Dual-stage open-face	**(0.0005)**	1.0000	**(0.0479)**			
IOM plastic	0.0544	0.9672	0.5969	0.9508		
IOM steel	0.9983	0.0321	0.9324	**0.0243**	0.2970	1.0000

Note: **Bold =** significant at α-level of 0.05. Values in parentheses indicate higher ratios for the sampler on the left side of the table.

2.4.2 OSHA42, IsoChek®, and WA-DOSH Samplers

The mean air levels ($\mu g/m^3$) of monomeric and polymeric HDI measured by OSHA42, IsoChek® and WA-DOSH samplers and corresponding impingers are summarized in **Table 2.8**. The results of paired *t*-tests between samplers and impingers are also summarized in **Table 2.8**. The HDI monomer concentrations measured with the OSHA42 samplers and analyzed by Galson Laboratories were significantly higher (1.2 – 3.1-fold; $p = 0.001$) than the corresponding impinger samples, which were analyzed at UNC.

Table 2.8. Mean air levels of monomeric and polymeric HDI (µg/m^3) measured with OSHA42, IsoChek®, WA-DOSH samplers with sampler-impinger paired *t*-test results.

Sampler	Clearcoat	HDI Monomer (µg/m^3)				HDI Biuret (µg/m^3)				HDI Isocyanurate (µg/m^3)				HDI Polyisocyanates (µg/m^3 NCO)			
		Sampler[A]		Impinger[B]		Sampler[A]		Impinger[B]		Sampler[A]		Impinger[B]		Sampler[A]		Impinger[B,C]	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
OSHA42	***Fast-drying***	14	9 – 20	7	6 – 8	NA	NA	18	15 – 23	NA	NA	3,071	2,642 – 3,493	NA	NA	NA	NA
	Slow-drying	15	12 – 17	8	7 – 10	NA	NA	124	95 – 160	NA	NA	1,993	1,652 – 2,382	NA	NA	NA	NA
					p = 0.001				ND				ND				ND
IsoChek®	***Fast-drying***	<LOQ	<LOQ	9	4 – 12	NA	NA	23	17 – 35	NA	NA	3,461	2,755 – 4,697	1,030	770 – 1,300	870	700 – 1,200
	Slow-drying	<LOQ	<LOQ	10	7 – 12	NA	NA	120	115 – 130	NA	NA	2,042	1,974 – 2,140	750	560 – 910	540	520 – 570
					ND[D]				ND				ND				**p = 0.03**
WA-DOSH	***Fast-drying***	9	6 – 12	15	11 – 18	<LOD	<LOD	42	30 – 52	4,727	3,551 – 5,622	5,446	4,100 – 6,352	NA	NA	NA	NA
	Slow-drying	17	12 – 24	12	9 – 17	277	212 – 515	140	98 – 189	2,667	2,079 – 4,869	2,258	1,691 – 2,896	NA	NA	NA	NA
					p = 0.78				**p = 0.007**[E]				p = 0.66				ND

Notes: **Bold p-value =** significant at α-level of 0.05. Abbreviations: LOQ = limit of quantitation; LOD = limit of detection; NA = not applicable; ND = not determined

[A] Sample size (N) = 6.

[B] Impingers were analyzed using a different analytical method [47]; N = 3

[C] Impingers converted to NCO using equation 1.

[D] p-value not calculated because IsoChek® data <LOQ.

[E] p-value calculated using LOD/√2.

For HDI monomer, all samples collected with IsoChek® and analyzed by Galson Laboratories were below the LOQ (0.03 µg). IsoChek® samplers measured significantly more HDI polyisocyanates (i.e., total NCO concentration) than did the impingers ($p = 0.03$). Although not significant, the IsoChek® measured more HDI polyisocyanates when a slow-drying clearcoat was applied (Student's *t*-test, $p = 0.44$; data not shown).

Significant difference was observed between WA-DOSH samplers, analyzed by WA DOSH laboratory, and the impingers, analyzed by UNC, for biuret ($p = 0.007$) but not for HDI monomer ($p = 0.78$) and isocyanurate ($p = 0.66$). Further, significant difference between the fast- and slow-drying clearcoat was observed for HDI monomer ($p < 0.001$) and biuret ($p = 0.032$) but not for isocyanurate ($p = 0.12$) (data not shown). All of the WA-DOSH samples ($N = 6$) analyzed for biuret when a fast-drying clearcoat was applied were below the LOD (1.7 µg). The ratios for WA-DOSH samplers were greater when a slow-drying clearcoat was applied compared to a fast-drying clearcoat for HDI monomer (2.4-fold) followed by biuret (1.5-fold).

2.4.3 Bulk Samples

Six samples of both fast- and slow-drying clearcoat were collected during this study. The results of the bulk paint sample analyses are presented in **Table 2.9**. The concentration (mg/l) of HDI monomer and biuret in the slow-drying clearcoat was significantly greater than that of the fast-drying clearcoat (Student's *t*-test, $p = 0.016$ and $p = 0.001$, respectively). No significant difference in the concentration of isocyanurate was observed between the fast- and slow-drying clearcoat (Student's *t*-test, $p = 0.349$).

Table 2.9. Summary of the bulk-paint sample analyses.

	Mean ± STDV	95% CI	p-value
HDI (mg/l)			
Fast-drying (N = 6)	200 ± 20	170 – 220	**0.016**
Slow-drying (N = 6)	430 ± 160	260 – 590	
Biuret (mg/l)			
Fast-drying (N = 6)	1,020 ± 150	860 – 1,190	**0.001**
Slow-drying (N = 6)	7,470 ± 2,350	5,000 – 9,940	
Isocyanurate (mg/l)			
Fast-drying (N = 6)	134,090 ± 8,980	124,700 – 143,510	0.349
Slow-drying (N = 6)	125,400 ± 19,710	104,700 – 146,100	

Note: **Bold =** significant at α-level of 0.05.

2.5 Discussion

In this study, we evaluated 13 different air samplers (i.e., midget impinger with frit, single- and dual-stage PP and PS samplers in both open- and closed-face mode, IOM samplers with plastic and stainless steel insert, OSHA42, IsoChek®, and WA-DOSH samplers) to quantitatively measure exposure to monomeric and polymeric HDI in the spray-painting environment. In order to account for variability between each sampling run and variability of the paint spray across the samplers, we used midget impinger with frit as a reference sampler and standardized the cassette sampler results by dividing the measured concentration by the concentration measured with the adjacent impinger.

The impingers measured significantly more HDI monomer, biuret, and isocyanurate than the cassette samplers analyzed at UNC. This finding likely reflects the belief that impingers collect isocyanate species more efficiently than filter cassettes because the sample is drawn into a liquid solution of derivatizing agent, thereby reducing the potential for polymerization [55]. Further, aerosols dissolve in the impinger solution, facilitating the reaction between

isocyanates and derivatizing agent. Neither filters nor impingers adequately sample for the entire range of isocyanate species likely to be encountered in the workplace [55]. Particles smaller than 2 μm and greater than 20 μm in diameter are not efficiently collected by an impinger and isocyanate species present in large particles are not efficiently derivatized when collected on reagent-coated filters [55-57, 77].

Average mass mean aerodynamic diameters (MMAD) of over-spray paint mists have been measured in the breathing zone when HVLP spray guns were used. Carlton and Flynn [83] reported an average MMAD 18.9 μm, while Sabty-Dailey et al. [84] reported an average MMAD of 5.9 μm. Based on these studies, impingers should perform reasonably well for the particle sizes generated during automotive spray-painting.

Filter cassette samplers have been compared with impingers in several studies [54, 65-67]. Under a simulated spray-painting environment, Ekman et al. [66] observed no significant difference ($\alpha = 0.05$) in the performance of closed-face single-stage filter sampler and an impinger when the same derivatizing agent (MPP) was used to quantify HDI monomer. However, we observed a significant difference between the closed-face single-stage filter samplers and impingers containing the same derivatizing agent. The open-face single-stage sampler was in best agreement with the impinger. This sampler was not significantly different from impinger for all analytes when a slow-drying clearcoat was applied or for HDI monomer when a fast-drying clearcoat was applied. When open-face sampling is employed, there are concerns regarding the potential for aerosols depositing or impacting on the filters instead of being drawn into the filters. This potential was minimized in this study because the sampler cassettes were positioned to minimize deposition of the overspray.

The variability across the impingers and filter cassette samplers during each sampling run was similar. Although we did not collect replicate sampling runs for all of the sampler types, we collected replicate sampling runs for the closed-face single- and dual-stage PS samplers. As expected, the variability for impinger and cassette samplers was not significantly different for the replicate sampling runs. The main source of this variability is likely the result from potentially uneven spraying during sampling runs. Therefore, we used the impinger as a reference sampler across all spray applications and sampling runs.

As may be expected in field sampling conditions, the measurements made by each sampler type were not extremely precise (Figure 2.2-2.4). Because the same analytical method was used for single- and dual-stage PP and PS samplers, IOM samplers, and impingers, the respective sampling results are directly comparable and the analytical method is unlikely to be a source of any differences. The PP and PS sampler ratios for HDI monomer and biuret were significantly greater when a slow-drying clearcoat was applied, compared to a fast-drying clearcoat. Although we observed significantly less HDI monomer and biuret in the bulk paint samples for the fast-drying clearcoat, it is unlikely that the paint concentration is responsible for these differences because the results were standardized using reference impinger concentrations.

However with a fast-drying clearcoat, losses may have occurred because of rapid polymerization on the filters' surfaces. This rapid polymerization may also explain why we did not observe a significant difference between open- and closed-face samplers when a fast-drying clearcoat was applied. An open-face sampler distributes aerosols and vapors over a greater filter surface area, increasing the isocyanates contact with the derivatizing agent. This may explain why the open-face samplers measured significantly more monomeric and

polymeric HDI than the closed-face samplers when slow-drying clearcoat was applied. When fast-drying clearcoat was applied, this effect was likely diminished due to rapid polymerization, resulting in similar performance for open- and closed-face samplers.

Our previous investigations of breathing-zone concentrations of monomeric and polymeric HDI in North Carolina (NC) and Washington State (WA) revealed that closed-face single-stage PS samplers measured significantly higher levels of monomeric and polymeric HDI than closed-face dual-stage PS samplers [58]. Further investigation of this data (not published previously) suggested that there could be other causes for the observed sampling bias. We observed significant differences in variables associated with air concentrations between the single- and dual-stage sampling events for air samples collected in WA in downdraft booths. We observed significantly greater levels ($p < 0.05$) of all analytes in the paint, longer paint times, and greater intensity of exposure when single-stage samplers were used compared with dual-stage samplers. We also observed significantly greater booth flow rates ($p = 0.02$) with dual-stage samplers, which could also contribute to the observed sampling bias.

When we previously performed side-by-side sampling using single- and dual-stage PS samplers in NC, we observed that the single-stage samplers measured significantly higher levels of HDI monomer and isocyanurate [58]. Although this finding supports a true sampling bias, the results may have been influenced by worker practices as well as insufficient number and diversity of side-by-side samples. The positioning of the sampler on the worker, painter orientation, and geospatial distribution of paint overspray are possible factors associated with these differences. Factors associated with worker practices were eliminated in this current study because filter cassettes and impingers were not worn by

workers and we used reference impinger samples to account for the distribution and intensity of paint overspray.

Using a more controlled study design, we found significantly greater ratios for closed-face dual-stage (N = 12, GM = 0.74) compared with the single-stage (N = 11, GM = 0.49) PS samplers for HDI monomer when a slow-drying clearcoat was applied. In our previous study [58], we observed that in NC the closed-face dual-stage PS samplers measured higher HDI monomer concentrations than the closed-face single-stage PS samplers. Further investigation of the NC data set (not published previously) indicated no significant differences ($p > 0.05$) between single- and dual-stage sampling events in paint concentrations, paint times, intensity of exposure, or booth flow rates.

When we combined the PP and PS samplers from the current study, we observed significantly lower concentrations measured with closed-face single-stage samplers (N = 17) compared with closed-face dual-stage samplers (N = 18) when a slow-drying clearcoat was applied. Removing the aerosol with untreated PTFE filters may allow for greater contact of vapor with the derivatizing agent, thus allowing for more complete derivatization with the impregnated GFF. Breakthrough may be more likely with the single-stage samplers than with the dual-stage samplers. It is important to note that these filters were extracted immediately after sampling, and therefore, the potential for losses due polymerization was minimized. Nevertheless, we observed significant differences between impinger and dual-stage closed-face samplers (both PP and PS) for all analytes when a slow-drying clearcoat was applied. This indicates that closed-face dual-stage as well as the closed-face single-stage samplers do not perform as well as the impinger in collecting monomeric and polymeric HDI.

We observed significantly lower ratios of HDI monomer, biuret, and isocyanurate when an IOM sampler with a stainless steel insert was used, rather than a plastic insert. Bello et al. [67] compared IOM steel samplers loaded with 25-mm quartz fiber filters impregnated with 500 μg of 1-(9-anthracenylmethyl)piperazine (MAP) to impingers containing MAP. They observed that impingers and treated filter IOM steel samplers perform equally well with respect to collection efficiency for the monomer and total polymeric HDI.

However, in our study we observed significant differences between impingers and IOM steel samplers for all analytes. We also observed significant differences between impingers and IOM plastic samplers for biuret and isocyanurate, but not for HDI monomer. After sampling, Bello et al. [67] immediately transferred filters and inserts into a jar containing MAP derivatizing agent. In our study, we did not extract the inserts in derivatizing agent. Consequently, losses may have occurred from the isocyanate species sticking to the walls of the inserts. This could also account for the differences between the plastic and steel inserts. Plastic is more likely than steel to become negatively charged and polyurethane paint aerosols may also become negatively charged. Consequently, the improved collection efficiency of the plastic inserts may have reflected the generation of static electricity during sampling, which prevented paint aerosols from sticking to the surface. HDI monomer vapor would not necessarily be attached to paint aerosols and hence would not become negatively charged; this could further explain why the measurement of HDI monomer did not differ between IOM sampler insert types.

The OSHA42, IsoChek®, and WA-DOSH samplers were analyzed by different laboratories using different analytical methods and procedures, which most likely accounts for the observed variability. Consequently, we cannot compare these results directly to those

for PS, PP, and IOM samplers. Nonetheless, some important observations can be made. First, significantly higher HDI monomer concentrations (1.2 – 3.1-fold) were measured with OSHA42 compared to the impinger. A potential exists for overestimation of HDI monomer concentration with OSHA42 because this method utilizes a less specific/sensitive UV detection, compared with the MS detection used for the impinger samples. It is unlikely that the differences between OSHA42 and impinger are because of sampler design.

We did not observe significant differences between impingers and single-stage open-face samplers (same design as OSHA 42) that utilized the same derivatizing agent, therefore, the differences between the impingers used in this study and OSHA42 are likely due to differences in the analytical methods. Levine [76] stated that when using the UV detector, the baseline noise is high at the quoted method LOD. Another limitation of the OSHA42 method is that it only identifies and quantifies the isocyanate monomer [62]. However, many automotive coatings based on HDI polyisocyanates typically contain small amounts (<1%) of volatile monomers and larger amounts (>99%) of non-volatile polyisocyanates [67, 85, 86]. Because OSHA42 may also underestimate isocyanate in aerosol form when sampling for extended periods [63], it has been suggested that additional derivatizing agent (1-2PP) should be added to the filter [64].

However, underestimation in our study is unlikely because we sampled for only 15 minutes. In a comparison study of isocyanate sampling methods for monomeric and polymeric HDI in spray-painting environments, OSHA42 had the greatest variability when compared with NIOSH Method 5521, NIOSH Method 5522, Total Aerosol Mass Method (TAMM), the proposed NIOSH Method 5525, and IsoChek® [54]. We did not observe much variability in OSHA42 samplers, however, this may reflect the lack of sensitivity and

specificity of the analytical method and the fact that the measured levels were close to the detection limit.

Second, the results for all the IsoChek® samplers were below the LOQ for HDI monomer although the corresponding impinger samples measured levels above the LOQ for IsoChek®. This is concerning as adverse health effects can occur at very low exposures levels, and this information is critical when conducting exposure and risk assessment. Several concerns of the performance of the IsoChek® have been indicated in the literature [56, 62], and it has been stated that the dual-stage filter sampling system may produce biased results due to evaporation of aerosol from the PTFE filter and adsorption of vapor onto the PTFE filter [56].

In a controlled laboratory study, IsoChek® was observed to significantly underestimate TDI and MDI monomer concentrations and inaccurately apportioned them into vapor and aerosol phases [62]. However, in a field study performed by England et al. [54], the IsoChek® measured HDI monomer concentrations that did not differ significantly from four other commonly used sampling methods (NIOSH 5521, NIOSH 5522, proposed NIOSH method, and OSHA42). HDI monomer levels measured with the impinger were 3–10 times greater than the IsoChek® LOQ. It appears that IsoChek® greatly underestimates HDI monomer in the spray-painting environment. When using IsoChek®, losses may occur because GFF is not immediately extracted in the field, accounting for some of the differences in HDI monomer levels.

England et al. [54] also reported that IsoChek®, NIOSH 5522, NIOSH 5521, and the TAMM were significantly different from one another when sampling for HDI-based polyisocyanates. They concluded that TAMM measured the most HDI polyisocyanates

followed by IsoChek® then NIOSH 5521 and finally NIOSH 5522 [54]. We observed that the IsoChek® measured more HDI polyisocyanates than the respective impingers. This is likely due to the fact that the method used to analyze the impinger samples is specific for the HDI polyisocyanates biuret and isocyanurate, while the IsoChek® reports HDI polyisocyanates in the form of TRIG or the sum of free NCO groups found in all isocyanate species of a sample [60], which could include higher molecular weight polyisocyanates and prepolymers.

Third, of the three samplers that were analyzed with different methods by laboratories other than UNC (i.e., IsoChek®, OSHA42, and WA-DOSH), the WA-DOSH samplers were in the best agreement with impingers. The reason why the WA-DOSH samplers detected HDI monomer while the IsoChek® did not likely reflects the fact that the LOD for HDI monomer is 6 times lower in the WA-DOSH L&I method. Like the IsoChek®, the GFF in the WA-DOSH sampler was not immediately extracted in the field. Therefore, the observed difference in HDI monomer levels between IsoChek® and impinger is most likely due to the poorer sensitivity of the IsoChek® method. The WA-DOSH samplers underestimated HDI monomer concentrations when a fast-drying clearcoat was applied. However, this is not unexpected for reasons previously discussed such as species reactivity and losses as a result of polymerization on the surface of filters.

Although we observed slightly higher levels of biuret and isocyanurate as compared to the impinger when a slow-drying clearcoat was applied, this may reflect the lack of pure analytical standards to analyze the WA-DOSH samples. All of the WA-DOSH samplers analyzed for biuret when a fast-drying clearcoat was applied were below the LOD (1.7 µg),

which provides additional evidence of the high reactivity of biuret and respective sampling challenges.

The ability to measure isocyanate exposure accurately is critical for exposure and risk assessment in order to predict systemic exposure, to develop sensitive and predictive models through multiple exposure routes, and ultimately to protect the health of workers. In summary, we observed significant differences in sampler performance between fast- and slow-drying clearcoat. We also observed open-face sampling to be the most effective when sampling for monomeric and polymeric HDI. Like any study, this one has its limitations and additional studies should be performed where larger sample sizes are tested. We did not control for wind currents in this study or the amount of spray being applied. However, we attempted to control for these confounders by using impingers as reference samples. Future studies should aim to better control for these variables and employ additional impingers so that each sampler is only paired with one impinger.

2.6 Conclusions

Overall, the single-stage open-face sampler was in best agreement with the impinger for measuring monomeric and polymeric HDI during spray-painting in the automotive refinishing industry. Of all the three samplers analyzed by laboratories other than UNC (i.e., OSHA42, IsoChek®, and WA-DOSH), the WA-DOSH was in the best agreement with the impingers. When selecting a sampling device for monomeric and polymeric HDI in the automotive refinishing industry, one must take into consideration the product being sampled, specifically the clearcoat drying time. Caution should be used when interpreting filter cassette sampler results, especially when atmospheres containing fast-drying clearcoat

aerosols are sampled. When fast-drying clearcoat was applied, almost all samplers used in this study underestimated HDI polyisocyanate concentrations. Further investigation of the sampling techniques used to monitor isocyanates is warranted.

CHAPTER 3

DEVELOPMENT OF A SAMPLING PATCH TO MEASURE DERMAL EXPOSURES TO MONOMERIC AND POLYMERIC 1,6-HEXAMETHYLENE DIISOCYANATE: A PILOT STUDY

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3.1 Abstract

The purpose of this study was to develop and evaluate a patch sampler to monitor dermal exposures to monomeric and polymeric 1,6-hexamethylene diisocyanate (HDI) in the automotive refinishing industry. Different patch materials were used to construct the patches and patches impregnated with a derivatizing solution were compared to those that were not impregnated. We observed that impregnated felt patches measured significantly more HDI monomer ($p = 0.04$) than non-impregnated patches in a controlled experiment. Both impregnated and non-impregnated patches were compared to the tape-strip method by monitoring three spray painters' dermal exposure to monomeric and polymeric HDI. Isocyanurate was the predominant species measured by all three sampler types with detectable levels in >86% of samples. Overall, tape-strips of exposed skin measured lower levels of monomeric and polymeric HDI than impregnated patch samplers at the same sampling site on the skin. Unlike tape-strips, impregnated patches are not as prone to

evaporative or reactive losses, or losses due to rapid penetration into the skin. Further investigations are warranted to evaluate these and other methods to measure dermal exposure to workers under occupational conditions in order to better understand the relationship between dermal exposure and internal dose.

3.2 Introduction

Respiratory exposure to diisocyanates has long been considered the primary route of exposure, and, thus, research, regulation, and prevention have focused almost exclusively on airborne isocyanate exposures [39]. Airborne isocyanate exposures have been reduced through improved controls and use of less-volatile isocyanates, however, isocyanate asthma continues to be prevalent in workplaces where measured isocyanate inhalation exposures are very low or non-detectable but where there is a clear opportunity for dermal exposure [40]. Animal studies have linked respiratory sensitization with prior dermal exposure to isocyanates [48, 49]. Respiratory sensitization was induced after epicutaneous exposure to 1,6-hexamethylene diisocyanate (HDI) in mice [50] and to toluene diisocyanate (TDI) in guinea pigs [51] and after intradermal and topical, but not inhalation, exposure to diphenylmethane diisocyanate (MDI) in guinea pigs [49].

In spray-painting operations, monomeric and polymeric HDI can be present in both aerosol and vapor forms. Most exposure assessments have only focused on the characterization of airborne exposures [41-46]. However, aerosol deposition on the skin and skin contact with contaminated surfaces and liquid product also constitute an important contact site for exposure. Isocyanates are commonly mixed with various solvents, polyols, and other substances, such as catalysts and blowing agents, which may affect isocyanate

reactivity, skin absorption, and health effects [40]. Urine and blood biomarkers of isocyanate exposure can potentially be used to assess internal dose, but not to distinguish whether exposure is due to the dermal or respiratory route [40]. The higher the volatility of the isocyanate, the shorter its residence time is on the skin. Therefore, the less-volatile polymeric isocyanates (HDI biuret and isocyanurate) may have potentially longer residence time on the skin and, thus, may have skin and systemic effects different from that of the monomer. Fent et al. postulated that differences between dermal exposures for polymeric HDI are likely due to different rates of skin absorption or chemical reactivity [47]. Exposure of the skin to isocyanates could contribute to a significant part of the total body burden. For example, Bello et al. estimated that 1% skin absorption of a small MDI droplet (10 mg) would result in a dose approximately 4.5-fold (450%) higher than a 15 min inhalation exposure to a concentration at the United Kingdom Health and Safety Executive short-term exposure limit (70 μg NCO/m^3), assuming 100% lung retention and a ventilation rate of 7 l/min [40].

Methods for monitoring dermal exposures are less advanced than those for air sampling techniques. However, several groups have measured exposure of the skin to isocyanates using qualitative SWYPE™ colorimetric indicators (CLI, Des Plains, IL) [1, 68], quantitative wipes [69], and quantitative tape strips [47, 70, 71]. These methods may underestimate exposures due to losses from absorption, chemical reactions, or poor removal efficiency [34, 40]. The objective of this study was to develop a sampling patch to quantify exposure to monomeric and polymeric HDI deposited on the skin in the spray-painting environment and to compare the method with the dermal tape-strip method as described by Fent et al [47, 70]. The tape-strip method has the ability to quantify monomeric and polymeric HDI that has

penetrated into the stratum corneum and that covers the skin's surface. Comparison of the tape-strip and the patch methods allowed us to investigate the potential limitations of these sampling techniques and to improve our understanding of the relationship between dermal exposure, penetration, and the contribution of this route of exposure to the total body burden. Our ability to measure dermal isocyanate exposure accurately is critical for exposure and risk assessment in order to predict systemic exposure, develop sensitive and predictive models through multiple exposure routes, and ultimately protect the health of workers.

3.3 Materials and Methods

3.3.1 Laboratory Studies

Three materials (polyester felt, wool, and 37-mm glass fiber filter) were tested to determine their suitability for use as a patch sampler. We evaluated reactivity of these materials with a derivatizing solution and for the recovery of HDI monomer.

3.3.1.1 Reactivity of materials with derivatizing solution. A piece of either felt or wool (5 cm^2) was placed in a 20 ml glass jar (I-Chem, New Castle, DE) with 5 ml of derivatizing solution, which was made by dissolving 2 g of 1-(2-methoxyphenyl)-piperazine (MPP; 192.3 g/mol) in 1:1 of 30% v/v solution of *N*,*N*-dimethylformamide (DMF; 73.09 g/mol) in acetonitrile (ACN; 41.05 g/mol). The materials were left at room temperature for 24 h. Materials were visually assessed periodically throughout the 24 h for signs of breakdown, discoloration, and disintegration.

3.3.1.2 Recovery of HDI monomer with felt, wool, and GFF. Pieces of wool (N = 6) and felt (N = 6) (2.5 cm × 4 cm) along with 37-mm glass fiber filters (GFF; Type AE, SKC, Eighty Four, PA) (N = 3) were placed separately into 20 ml glass jars (I-Chem, New Castle, DE). Each material type was spiked with 40 µl of a mixture of HDI in toluene [1,550 pmol/µl HDI in toluene (TOL; 92.14 g/mol)]. After spiking, the lids of the jar were affixed and samples held at room temperature for 15 min. Reference samples were prepared by spiking the same amount of HDI/TOL onto the glass in an empty glass jar. Following the 15 min period, 10 ml of derivatizing solution (2 g/l MPP in 30% DMF-ACN solution) was added to each glass jar. Acetic anhydride (200 µl) was added to acetylate residual MPP. After 15 min, the internal standard (2 pmol/µl urea derivative of 1,8-octamethylene diisocyanate; ODIU) was combined (1:1 v/v ratio) with aliquots of each sample to give an internal standard concentration of 1 pmol/µl. All samples were analyzed for HDI monomer using liquid chromatography-mass spectrometry (LC-MS) as described elsewhere [47]. Wool, felt, and GFF samples were compared to reference samples to determine the percent recovery from each material type.

3.3.1.3 Recovery of HDI monomer from impregnated felt. Pieces of felt (2.5 cm × 4 cm) were impregnated with a derivatizing solution (1.09 g/ml MPP in TOL) designed to collect and derivatize isocyanate vapors. The felt pieces were impregnated with 1,200 µl of 6 g/l MPP in TOL and allowed to dry for 20 min. Each impregnated felt was placed into a 20 ml glass jar and spiked with 40 µl of HDI/TOL (1,550 pmol/µl). After spiking, the lids of the jars were affixed and jars held at room temperature for 15 min. Reference samples were collected by spiking the same amount of HDI/TOL onto the glass in an empty glass jar.

Following the 15 min period, 10 ml of derivatizing solution (2 g/l MPP in 30% DMF-ACN solution) was added to each glass jar. Sample processing and analysis were performed as described previously. All samples were analyzed for HDI monomer using LC-MS as described elsewhere [47]. Felt patch samples were compared to reference samples to determine percent recovery.

3.3.2 Field Studies

3.3.2.1 Comparison of impregnated and non-impregnated patches

Clearcoat spiking**:** Felt patches (2.5 cm × 4 cm) were impregnated as described above. Both impregnated and non-impregnated felt patches were placed separately in 20 ml glass jars. A mixed clearcoat (15 µl; BASF®, Münster, Germany), used by an automotive spray painter, was spiked on each patch using a 20-µl pipette. After spiking, the open jars with samples were allowed to sit at room temperature for 15 min. Leaving the jars open mimicked more closely the conditions of field sampling and allowed us to investigate evaporation. The pipette tips were ejected into a separate glass vial containing 15 ml of derivatizing solution (2 g/l MPP in 30% DMF/ACN) to account for total mass. Following the 15 min period, glass jars were filled with 15 ml of derivatizing solution (2 g/l MPP in 30% DMF/ACN). A reference sample of a mixed clearcoat (15 µl) was drawn into a 20-µl pipette and dispensed into a glass vial (I-Chem) filled with 15 ml of derivatizing solution (2 g/l MPP in 30% DMF/ACN). The pipette tip was also ejected into the solution to eliminate side-wall losses due to the viscosity of the clearcoat. All samples were then placed into a cooler (~4 °C) and returned to UNC laboratory for storage at –40 °C until analyzed. Sample processing and analysis were performed as described previously. A total of 9 impregnated and 9 non-

impregnated felt patches and respective pipette tips were analyzed for HDI monomer, biuret, and isocyanurate using LC-MS as described elsewhere [47]. The percent recovery for each patch sample was calculated by adding the sum of mass measured for each patch sample and respective pipette tip. This mass was then compared to a specific reference sample to calculate the percent recovery.

Side-by-side spray-painting: Felt patches (2.5 cm × 4 cm) were impregnated with 2,000 µl of impregnating solution (6 g/l MPP in TOL) as described above. Both impregnated (N = 4) and non-impregnated felt patches (N = 4) were lined up alternating on a cardboard backing. A spray painter sprayed the patches with a Deltron® (Strongsville, OH) clearcoat mixture. After spraying, the patches were allowed to remain in the spray booth for 12 min, which is the approximate time it takes to apply one coat of clearcoat to an automobile. After this time, patches were placed into glass jars containing 10 ml of derivatizing solution (2 g/l MPP in 30% DMF/ACN) and capped. All samples were then placed into a cooler (~4 °C) and returned to UNC laboratory for storage at –40 °C until analyzed. Sample processing and analysis were performed as described previously. Samples were analyzed for HDI monomer, biuret, and isocyanurate using LC-MS as described elsewhere [47] and the results of the impregnated and non-impregnated felt patches compared.

3.3.2.2 Comparison of Patches and Tape-Strips during Application of Clearcoat. Patch sampling was performed on three workers during 11 spray-painting tasks in central North Carolina. These painters were enrolled in our exposure assessment study described elsewhere [58]. Patches (size 5.5 cm × 3.5 cm with the sample collection surface of 2.5 cm × 4 cm) were constructed with felt, aluminum foil, and Cover-Roll® adhesive tape (Beiersdorf

AG, Hamburg, Germany). The felt was impregnated with 2,000 µL of impregnating solution (6 g/l MPP in TOL) as described above. The impregnated felt was backed with two layers of aluminum foil and the foil was folded around the edges to prevent any potential run off of the gel-like MPP and contact with the skin. The adhesive tape was attached to the back of the aluminum foil for easy placement of the patch to the skin of the worker.

Sample collection**:** Both impregnated and non-impregnated patches were placed on the right and left volar forearm of workers during spray-painting during 11 spray-painting tasks (22 samples sets). As depicted in **Figure 3.1**, a space in between the patches was left for the collection of tape-strip samples after the paint task. The locations of the impregnated and non-impregnated patches on the worker's arm were randomized. Painters in this study did not wear coveralls (as part of their normal work practice) and hence their arms were exposed to spray paint mist. The painters in this study used BASF®, Dupont® Nason® (Wilmington, DE), and Deltron® products. After each paint task, patches were immediately placed in 10 ml of derivatizing solution (2 g/l MPP in 30% DMF/ACN). Additionally, five successive tape-strips (Cover-Roll®, 2.5 cm × 4 cm) were collected adjacent to each patch sampler site and each tape-strip placed in 5 ml of derivatizing solution (2 g/l MPP in 30% DMF/ACN) as described elsewhere [47]. Each tape-strip represents approximately one cell layer, thus we removed approximately 5 cell layers. Bulk sample of mixed basecoat or clearcoat (10 µl) was drawn into a 20-µl pipette and dispensed into a glass vial (I-Chem) filled with 15 ml of derivatizing solution (2 g/l MPP in 30% DMF/ACN) to confirm the presence of isocyanates. The pipette tip was also ejected into the solution to eliminate side-wall losses due to the viscosity of the product. All samples were then placed into a cooler (~4 °C) and returned to UNC laboratory for storage at –40 °C until analyzed.

Sample analysis: For analysis, samples were returned to room temperature and acetic anhydride was added (200 µl for patch and bulk; 100 µl for tape-strips) to acetylate residual MPP. After 15 min, internal standard (2 pmol/µl ODIU) was combined (1:1 v/v ratio) with aliquots of each bulk paint and patch sample to give an internal standard concentration of 1 pmol/µl. For tape-strips, after 15 min internal standard (52 pmol/µl ODIU) was added (100 µl) to give an internal standard concentration of 1 pmol/µl. Samples were analyzed for HDI monomer, biuret, and isocyanurate using LC-MS as described elsewhere [47]. Comparisons were made between impregnated patch, non-impregnated patch, and tape-strip results.

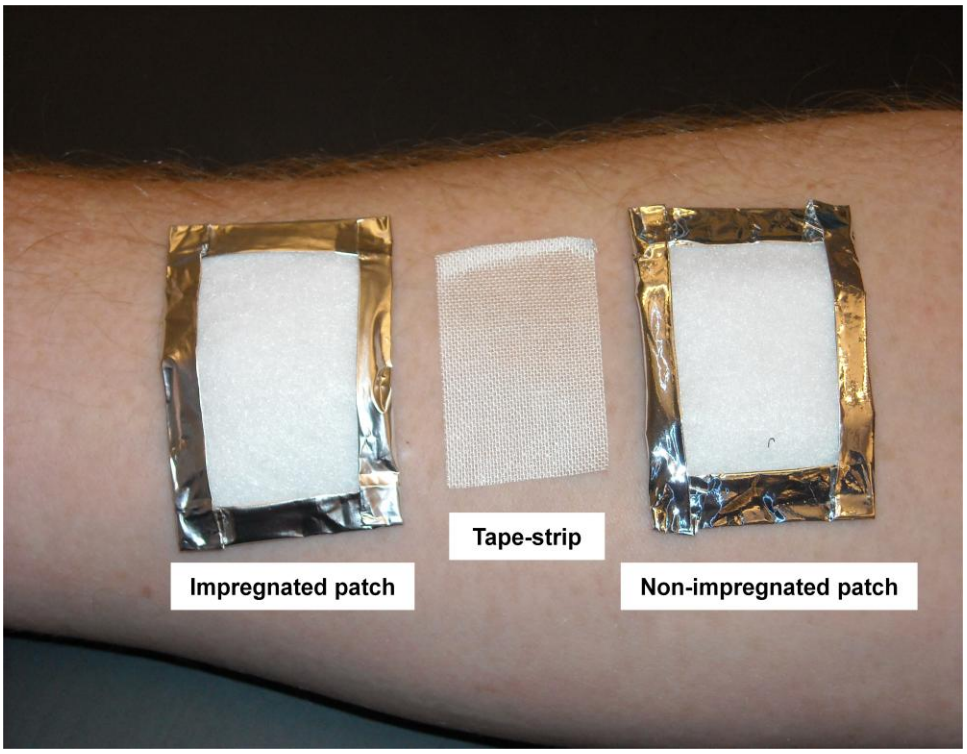

Figure 3.1. Right volar forearm of a worker depicting the location of the patches during spraying and the tape-strip after spraying. The exposed patch surfaces and the tape-strips were each 2.5 cm × 4 cm.

3.4 Results

3.4.1 Laboratory Studies

Based on visual inspection for reactivity of the three patch materials (polyester felt, wool, and GFF) with derivatizing solution, we did not observe the material types to be reactive with the derivatization solution. The average percent recovery (sample/reference × 100%) for each patch type compared to the reference samples for HDI monomer for all material types were >94%. The average percent recoveries ± standard deviations for the felt, wool, and GFF were 104 ± 5, 99 ± 4, and 94 ± 8 %, respectively. We observed that HDI did not react with this material type (recoveries ~100%), thus the recovery of HDI monomer with impregnated felt was determined. The average percent recovery and standard deviation for HDI monomer with the impregnated felt was 105 ± 9 %. The laboratory results provided evidence of sufficient recovery with the impregnated felt. Therefore, we proceeded to test the felt patch design in an occupational field setting.

3.4.2 Field Studies

3.4.2.1 Comparison of impregnated and non-impregnated felt patches. The average percent recovery and standard deviation for each analyte when either felt patch type was spiked with the clearcoat are presented in **Table 3.1**. Although not significant (two-sample means *t*-test, α-level of 0.05), the impregnated patches measured more monomeric and polymeric HDI compared to non-impregnated patches. **Table 3.2** provides the results from the side-by-side spray-painting experiment for each analyte reported as ratios of impregnated patches to non-impregnated patches. Paired *t*-test (α-level of 0.05) indicated that the impregnated patches measured significantly more HDI monomer ($p = 0.04$) than non-impregnated patches.

Table 3.1. Recovery of HDI monomer, biuret, and isocyanurate from impregnated and non-impregnated felt patches (N = 9) after application of 15 µl of clearcoat[A].

Analyte	Average Percent Recovery[B] ± Standard Deviation		
	Impregnated Patch	**Non-Impregnated Patch**	***P*-value**
HDI monomer	117 ± 12	108 ± 15	0.18
Biuret	90 ± 13	82 ± 16	0.26
Isocyanurate	92 ± 14	82 ± 17	0.67

[A] Following application of clearcoat, the patches were allowed to sit for 15 min at room temperature before adding derivatizing solution.
[B] Sample amount / reference amount × 100.

Although not significant, the impregnated patches generally measured more HDI biuret and isocyanurate than non-impregnated patches.

3.4.2.2 Comparison of felt patches and tape-strips during application of clearcoat. The results of the felt patch and tape-strip sampling performed on the three workers during 11 spray-painting tasks are presented in **Table 3.3**. The percent recovery of tape-strips compared to the patches was calculated for each of the 22 sample sets collected by summing the mass of analyte measured by the five consecutive tape-strips and comparing it to the masses measured in the patch samplers. The results of the 5 successive tape-strips show a decreasing trend in analyte mass, thus, indicating penetration into the stratum corneum (data not shown). Bulk paint sample analysis confirmed the presence of HDI (187 ± 172 mg/l), biuret (3,331 ± 7,274 mg/l), and isocyanurate (48,482 ± 45,250 mg/l) in all spray-painting tasks.

Table 3.2. Comparison of measured isocyanates from clearcoat sprayed onto impregnated and non-impregnated felt patches.

Analyte	Sample Number	Impregnated Patch (µg)	Non-Impregnated Patch (µg)	Ratio Impregnated/Non-Impregnated	*P*-value
HDI monomer	1	0.86	0.48	1.8	0.04
	2	0.58	0.41	1.4	
	3	1.35	0.65	2.1	
	4	1.55	0.78	2.0	
Biuret	1	2.23	2.19	1.0	0.23
	2	1.92	2.24	0.9	
	3	5.36	3.34	1.6	
	4	6.64	3.99	1.7	
Isocyanurate	1	239.65	221.69	1.1	0.29
	2	177.87	214.07	0.8	
	3	327.69	248.48	1.3	
	4	359.94	282.37	1.3	

Overall, the impregnated patches measured more monomeric and polymeric HDI than non-impregnated patches or the tape-strip samples. Impregnated patches detected HDI monomer in 63% of the samples while non-impregnated patches and tape-strips measured detectable levels of monomer in 9% and 36% of the samples, respectively. At most, the tape-strips recovered 35% of HDI monomer measured by impregnated patches. Biuret was detectable in 18% of impregnated and non-impregnated patch samples and 14% of tape-strip samples. The amount of biuret measured by the tape-strip samples varied from 11 to 60% of the amount measured by the impregnated patches.

Isocyanurate was the predominant species measured with all samplers. Tape-strip samples detected isocyanurate in 100% of the samples while the impregnated and non-impregnated

patches measured detectable levels of isocyanurate in 91% and 86% of the samples, respectively. Due to the much greater detection for isocyanurate than all other species, paired *t*-test (α-level of 0.05), in which samples below the limit of detection (LOD) or limit of quantitation (LOQ) were assigned values by dividing the respective limits by $\sqrt{2}$ [81], was performed. Values were natural log-transformed to satisfy the normality assumption. Shapiro-Wilks tests for normality indicated that isocyanurate levels measured with impregnated patches ($W = 0.93$), non-impregnated patches ($W = 0.93$), and tape-strips ($W = 0.81$) were approximately log-normally distributed. Geometric mean (GM) levels for each sampler type were calculated. Paired *t*-test indicated that significantly greater amounts of isocyanurate were measured using impregnated patches (GM = 1.4 µg; $p < 0.01$) and non-impregnated patches (GM = 1.0 µg; $p = 0.01$) than tape-strips (GM = 0.43 µg). However, for 2 tasks (worker 3, task 2 and 3), the tape-strips measured greater levels of isocyanurate than the impregnated patches. Although only borderline significant ($p = 0.07$), the impregnated patches collected more isocyanurate than non-impregnated patches.

Table 3.3. The summary of felt patch and tape-strip measurements obtained from adjacent sample areas from three workers during different spray-painting tasks.

Worker	Task	Arm Location	HDI (µg)			Biuret (µg)			Isocyanurate (µg)		
			Impregnated Patch[A]	Non-Impregnated Patch[A]	Tape[B]	Impregnated Patch[A]	Non-Impregnated Patch[A]	Tape[B]	Impregnated Patch[A]	Non-Impregnated Patch[A]	Tape[B]
1	1	Left	4.72 (35%)	1.75 (95%)	1.66	60.6 (34%)	26.95 (77%)	20.8	972 (34%)	369 (91%)	334.1
		Right	0.22	0.03	<LOQ	2.35 (11%)	0.25 (100%)	0.25	40.5 (6%)	9.7 (24%)	2.32
	2	Left	<LOQ	nd	nd	<LOQ	nd	nd	2.71 (39%)	2.32 (46%)	1.06
		Right	<LOQ	nd	nd	nd	<LOQ	nd	4.71 (17%)	3.65 (22%)	0.79
2	1	Left	nd	nd	nd	nd	nd	nd	2.73 (16%)	1.31 (34%)	0.44
		Right	nd	nd	<LOQ	nd	nd	nd	0.76 (54%)	0.89 (46%)	0.41
	2	Left	0.09	nd	<LOQ	nd	nd	nd	1.74 (27%)	2.32 (20%)	0.47
		Right	0.12 (8%)	nd	0.01	nd	nd	nd	4.71 (14%)	3.65 (18%)	0.65
	3	Left	0.03	nd	nd	nd	nd	nd	8.53 (12%)	7.37 (14%)	1.06
		Right	0.02	nd	nd	nd	nd	nd	8.81 (18%)	7.15 (23%)	1.62
3	1	Left	nd	nd	nd	nd	nd	nd	nd	nd	<LOQ
		Right	nd	nd	<LOQ	nd	nd	nd	nd	nd	0.06
	2	Left	nd	nd	nd	nd	nd	nd	0.62 (16%)	0.38 (26%)	0.1
		Right	nd	nd	nd	nd	nd	nd	0.30 (274%)	nd	0.82
	3	Left	nd	nd	nd	nd	nd	nd	0.74 (46%)	0.60 (56%)	0.34
		Right	nd	nd	nd	nd	nd	nd	0.44 (205%)	0.32 (286%)	0.9
	4	Left	0.32	nd	nd	nd	nd	nd	0.54	<LOQ	<LOQ
		Right	0.12	nd	<LOQ	nd	nd	nd	<LOQ	0.35	<LOQ
	5	Left	0.33	nd	<LOQ	nd	nd	nd	1.07 (27%)	0.7 (41%)	0.29
		Right	0.2	nd	nd	nd	nd	nd	1.23 (59%)	0.89 (82%)	0.73
	6	Left	<LOQ	nd	nd	nd	nd	nd	1.40	1.40	<LOQ
		Right	0.02	nd	nd	0.25 (60%)	0.19 (79%)	0.15	1.50 (11%)	1.80 (9%)	0.16
LOD			0.003	0.003	0.002	0.04	0.04	0.02	0.04	0.04	0.02
LOQ			0.008	0.008	0.004	0.1	0.1	0.05	0.1	0.1	0.05

Notes: Abbreviations: nd = below the limit of detection (LOD); LOQ = limit of quantitation

A. Percent measured by five consecutive tape-strips compared with the respective patch sampler provided in parentheses.

B. Summation of 5 consecutive tape-strips..

3.5 Discussion

In this study, we developed and evaluated a patch sampler to quantitatively measure dermal exposure to monomeric and polymeric HDI in the spray-painting environment. Impregnated felt patches collected more than non-impregnated felt patches and tape-strips for the majority of the analytes and experiments. When clearcoat was directly sprayed onto the samplers, impregnated felt patches collected significantly more HDI monomer than non-impregnated patches. The comparison of samplers worn by painters during spray-painting provided additional evidence that the impregnated patches may be more efficient at collecting isocyanates than the tape-strips or non-impregnated patches.

When worn by painters, impregnated patches detected more HDI monomer (63%) than the non-impregnated patches (9%) and tape-strips (36%). For HDI monomer, it is no surprise that the impregnated patch collected more than the non-impregnated patch and tape-strips because HDI monomer is more volatile than the polymeric forms and, thus, may evaporate more rapidly. The impregnated patches should quickly derivatize isocyanates into more stable molecules thereby minimizing evaporative or reactive losses. Tape-strip samples, on the other hand, can exhibit losses due to evaporation, polymerization, reactivity, and penetration into the skin (beyond the layers of the stratum corneum sampled). Because the felt used to construct the patches did not react with HDI monomer, the non-impregnated patches should only exhibit losses due to evaporation/polymerization and not reactivity. Therefore, the differences between the non-impregnated patch and the tape-strip are likely due to chemical reactions that are occurring on the skin's surface or penetration into the skin. Our results indicate uptake and penetration into the stratum corneum as we measured HDI in successive tape-strip samples as also indicated in our previous publication [70]. Bello et al.

observed that octyl isocyanate, used as a surrogate for HDI monomer, disappeared from the skin surface relatively fast (~70% of the applied dose in 10 min) [52]. Their data indicated that the losses due to evaporation were minimal, thus suggesting rapid penetration of this low molecular weight isocyanate.

When the patches were sprayed directly with clearcoat, we observed less than 2-fold difference between the impregnated and non-impregnated patches for the HDI polymers. Greater differences were observed in a few instances when the patches were worn by the painters. For worker 1 task 1, the impregnated patches measured 2.3 and 9.4 times more biuret and 2.6 and 4.2 times more isocyanurate than the non-impregnated patches. However, for the other workers and tasks, the levels of biuret and isocyanurate were similar for impregnated and non-impregnated patches. It is possible that the results observed for worker 1 may be due to unusual spatial variability in clearcoat overspray, accidental touching of the impregnated sampler with contaminated hands, or some other sampling error/malfunction.

Biuret was detected in 18% of the patch samples and 14% of the tape-strip samples. The impregnated-patch samplers measured 40 – 89% more biuret than the tape-strips. The tape-strips measured similar levels of biuret as the non-impregnated patch samplers (77 – 100%). In a previous study, we found evidence of high reactivity and sampling challenges for biuret [87]. Biuret is likely more difficult to measure than HDI monomer and isocyanurate. We measured biuret the least despite the confirmation of its presence in the bulk paint samples. Similarly, we previously measured detectable levels of biuret in 83% of the bulk paint samples but in only 9% of the tape-strips collected from the lower arms of painters who did not wear protective clothing in a larger study of automotive spray-painters [71].

A borderline significant difference ($p = 0.07$) was observed between the impregnated and non-impregnated patches and thus may suggest some polymerization. Evaporation is possible, but unlikely, given the low vapor pressure (5.3×10^{-9} mmHg) of isocyanurate. Compared to the patches, on average, the tape-strips recovered half the amount of isocyanurate ($p < 0.01$). These results indicate that isocyanurate is either reacting with the skin or rapidly penetrating into the deeper layers of the skin, thus leaving less of the compound for sampling with the tape-strips. Bello et al. investigated dermal penetration of polymeric HDI, polymeric isophorone diisocyanate (IPDI), and MDI and found that chemical reaction was minimal, thus, suggesting permeation into the deeper layers of the skin [52]. Our results confirm penetration into the stratum corneum as we measured isocyanurate in the successive tape-strip samples. Another possibility is that the tape-strip is not efficient at removing isocyanurate from the skin's surface. However, this seems unlikely because we have previously observed that the tape-strip technique removes >95% of a high molecular weight compound [88]. In addition, if the tape-strips had poor collection efficiency, we would expect to see similar results with biuret; yet, 77 – 100% of biuret measured with the non-impregnated patches was also measured with tape-strips.

We previously used tape-strip sampling to measure dermal exposure to HDI-based polyisocyanates in 47 automotive spray-painters performing a total of 296 paint tasks [71]. The results of tape-strip sampling performed on the lower arms of painters who did not wear protective clothing was directly comparable with the tape-strip sampling results presented here. The distribution of isocyanurate we collected with tape-strips (GM = 0.43 µg, GSD = 7.3, N = 22) was comparable to the distribution measured in our previous study [71] (GM = 1.5 µg, GSD = 8.1, N = 332). Although we cannot reliably calculate the distributions for

HDI monomer and biuret due to the large number of non-detects and a small sample size, our detection rates (36, 14, and 100% for HDI monomer, biuret, and isocyanurate, respectively) were similar to the respective detection rates (44, 9, 96%, respectively) in our previous study [71]. Hence, our measurements may be representative of the automotive refinishing industry.

Although the tape-strip is capable of measuring monomeric and polymeric HDI on the skin surface and that has penetrated into the skin, it appears that penetration into deeper layers of the skin may be occurring. Knowledge obtained on the penetration and absorption of monomeric and polymeric HDI into human skin is required to further our understanding on the effect of dermal exposure to internal dose received. Studies investigating urinary biomarkers of isocyanate exposure have provided indirect evidence of dermal uptake. The HDI hydrolysis product 1,6-hexamethylene diamine (HDA) has been measured in both blood plasma [89] and urine [90] of workers using respiratory protection and elevated levels of urinary biomarkers have been detected in workers where isocyanate inhalation exposures were very low or non-detectable [91, 92].

Until we can establish dermal uptake, penetration patterns, and the fate of isocyanates in the skin, it seems prudent to measure dermal exposures with both impregnated patches and tape-strips. The tape-strip samples seem to underestimate dermal exposure due to the rapid penetration while non-impregnated patches suffer from losses due to evaporation/polymerization. Our ability to measure dermal isocyanate exposure accurately is critical in understanding the contribution of dermal exposure to the internal dose received and, thus, to the potential related health effects.

We acknowledge that in this study we only measured exposure to three workers. Future studies involving a larger population are required to further evaluate these and other methods

(e.g., wipe sampling method) to measure dermal exposure as well as to assess dermal penetration of isocyanates. Additionally, modification of the patch sampler design is necessary. The medical tape attached to the aluminum foil backing of the patch did not provide a sufficient hold on the workers' arms and resulted in the movement or loss of patches. In future studies, we will refine the design so that we can use Velcro® and stick the patches to arm bands worn by the worker.

3.6 Conclusions

We developed and evaluated a dermal patch designed to measure monomeric and polymeric HDI during spray-painting in the automotive refinishing industry. Although this study is limited in size, we demonstrate the potential use for the dermal patch sampling in the occupational setting. Overall, we measured greater levels of monomeric and polymeric HDI with impregnated patches compared to tape-strips. Further investigation comparing tape-strips to patch sampling as well as studies designed to further our understanding of dermal penetration and absorption patterns of monomeric and polymeric HDI in human skin are warranted.

CHAPTER 4

PENETRATION PATTERNS OF MONOMERIC AND POLYMERIC 1,6-HEXAMETHYLENE DIISOCYANTE

Jennifer M. Thomasen and Leena A. Nylander-French

(Manuscript)

4.1 Abstract

We investigated penetration patterns of monomeric and polymeric 1,6-hexamethylene diisocyanate (HDI) 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 composition of the clearcoat mixture may affect the penetration rate of the individual isocyanate compounds. The short-term absorption rates (10 and 60 min) 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). Based on the short-term absorption rates for a slow-drying clearcoat after 10 min (1.33 μg/cm^{2}h) or 60 min (0.219 μg/cm^{2}h), we calculated that ~3 and 18 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 (<14 min) than that for fast-drying clearcoat (274 min). Isocyanurate had the shortest skin absorption times regardless of clearcoat formulation (6 – 47 sec). These results indicate that the dose received through dermal exposure to HDI-

containing clearcoats in the occupational setting 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 worker populations and to re-evaluate regulatory exposure limits for isocyanate exposures. Additionally, the use of proper dermal protective equipment to reduce dermal exposures is necessary when working with these compounds.

4.2 Introduction

In the automotive refinishing industry, polyurethane paints used typically contain monomeric (usually <0.5%) and polymeric (i.e., uretdione dimer and biuret and isocyanurate trimers; 2.5 – 20%) 1,6-hexamethylene diisocyanate (HDI) [34]. Isophorone diisocyanate (IPDI)-based polyisocyanates may also be used in automotive coatings but are typically present at lower levels than HDI-based polyisocyanates [93]. 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 [40]. This observation has prompted a concerted investigation of dermal exposures [39, 40]. Dermal exposure to monomeric and 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 [86]. 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 [47, 70, 71]. 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 [71]. 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 [47, 71].

Previously, we compared dermal tape-strips with impregnated dermal patch samplers [94]. 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 [94]. Bello et al. investigated the dermal penetration of isocyanates using cadaver guinea pig skin [52]. 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 demonstrate that monomeric and polymeric HDI penetrate into and beyond the stratum corneum, determine the difference in penetration patterns with a slow- and fast-drying clearcoat, and evaluate the efficiency of the tape-strip method to measure dermal exposure to monomeric and polymeric HDI.

4.3 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 x 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 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 **Figure 4.1**.

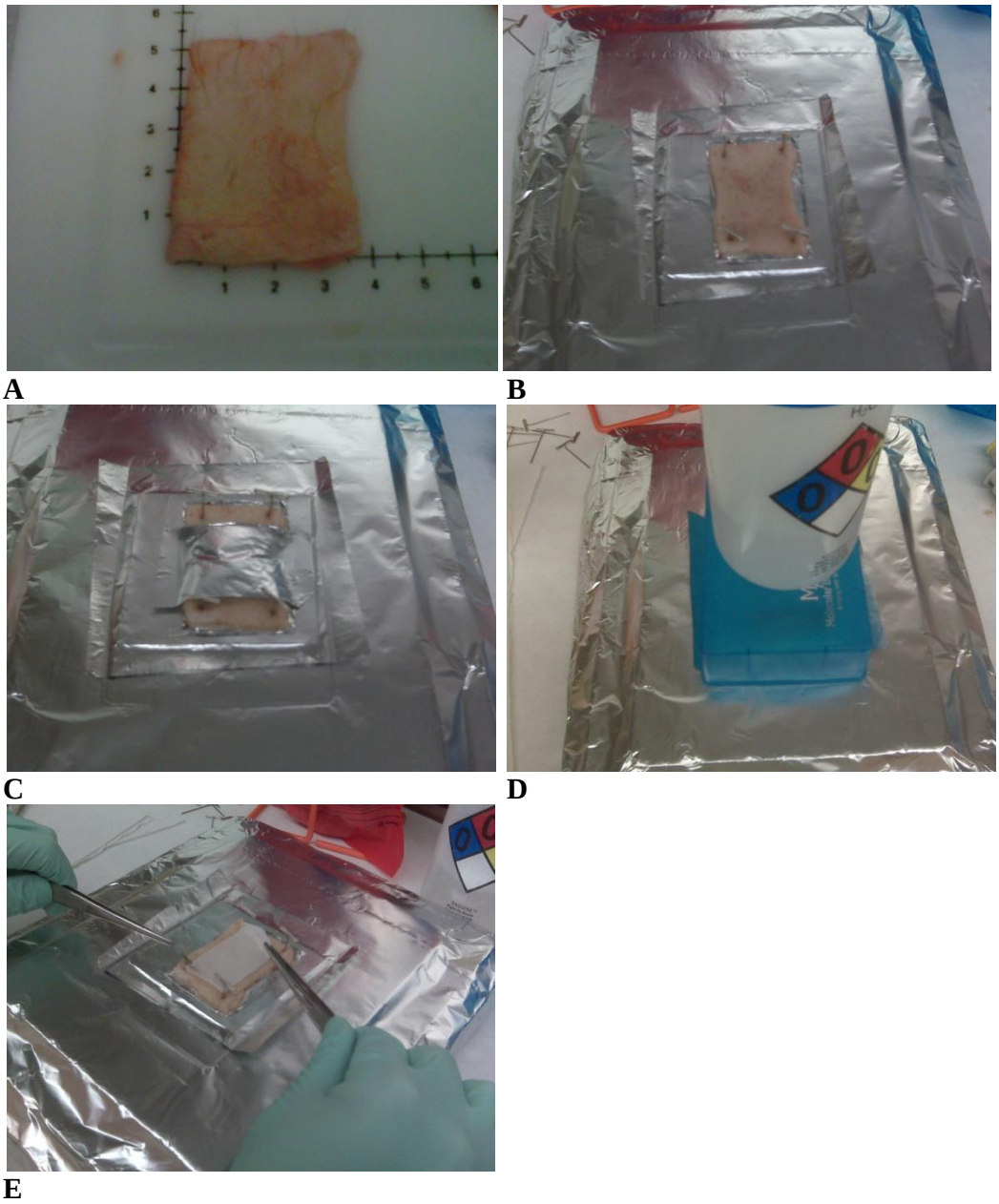

Figure 4.1. Experimental set-up: (A) skin is sectioned into 3.5 × 5 cm pieces, (B) skin is pinned to a Styrofoam™ board that is covered with foil and a piece of wax, (C) isocyanate is applied to the skin and occluded with foil, (D) an additional cover is placed over occluded skin, (E) skin is tape-stripped.

4.3.1 Experiments with HDI Monomer

4.3.1.1 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 [58]. EA was used because it is a common solvent for isocyanates, it evaporates quickly, it is non-reactive towards isocyanates, and has low toxicity [52]. Following the epicutaneous application of HDI, the skin was occluded with a piece of foil and covered with a plastic lid to minimize evaporation (Figure 4.1). 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) in acetonitrile (ACN, 41.05 g/mol)]. 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 x 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 –40ºC until processing and analysis of HDI monomer by LC-MS [47, 70].

4.3.1.2 Sample processing and analysis. HDI Neat: For analysis, samples were returned to room temperature. A white precipitate was observed in some of the samples. All samples were heated at 80ºC 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 urea derivative of 1,8-octamethylene diisocyanate; ODIU) was added (100 µl) to give an internal standard concentration of 1 pmol/µl. Samples were analyzed for HDI monomer using LC-MS [47].

HDI/EA: For analysis, 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 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. For all other samples (tape-strips, gauze, foil) after 15 min, internal standard (52 pmol/µl ODIU) was added (100 µl) to give an internal standard concentration of 1 pmol/µl. Samples were analyzed for HDI monomer using LC-MS [47].

Skin Tissues: For analysis, 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 ~4ºC. 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 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. 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 [47].

4.3.2 Experiments with Slow-drying and Fast-drying Clearcoat

4.3.2.1 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). Following the exposure period, the sample collection and processing were conducted as described for HDI monomer. 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 –40ºC until processing and analysis of HDI monomer, biuret, and isocyanurate by LC-MS [47].

4.3.2.2 Sample processing and analysis. For analysis, 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 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. 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 ODIU) was combined (1:1 v/v ratio) with the diluted aliquot to give an internal standard concentration of 1 pmol/µl. For the samples applied with the fast-drying clearcoat, the first 5 tape-strips were diluted by combining with internal standard (2 pmol/µl ODIU; 1:1 v/v ratio) to give an internal standard concentration of 1 pmol/µl. For the remaining tape-strip and foil samples from the experiments with both the slow- and fast-drying clearcoat, internal standard (52 pmol/µl ODIU; 100 µl) was added to give an internal standard concentration of 1 pmol/µl. Samples were analyzed for HDI monomer, biuret, and isocyanurate using LC-MS [47].

Skin Tissues: For analysis, 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 ODIU) was combined (1:1 v/v ratio) with the sample to give an internal standard concentration of 1 pmol/µl. Samples were analyzed for HDI monomer, biuret, and isocyanurate using LC-MS [47].

4.3.3 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 ($\mu g/cm^2h$) for 10 and 60 min exposures were calculated by dividing the sum of the total amount of isocyanate (HDI, biuret, 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 [95-97], 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.

4.4 Results

4.4.1 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 4.1**. 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 (**Figure 4.2**). The average HDI amount ($\mu g/cm^2$) collected with the 30 tape-strips from the skins after epicutaneous application of HDI neat (**Figure 4.2A**) or HDI/EA (**Figure 4.2B**) 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 was detected in 90 and 85% of the skins applied with HDI neat and HDI/EA, respectively. However, the amount of breakthrough relative to the recovered amount of HDI was negligible (<1%).

4.4.2 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 4.2**. The results of the short-term absorption rates are presented in **Table 4.3**. 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 differences ($p = 0.25$) in the amount of isocyanurate for the slow- (3,464 μg) and fast-drying (4,118 μg) clearcoat was observed.

4.4.2.1 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 HDI, biuret, and isocyanaurate amount ($\mu g/cm^2$) 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 (**Figure 4.3**). 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.

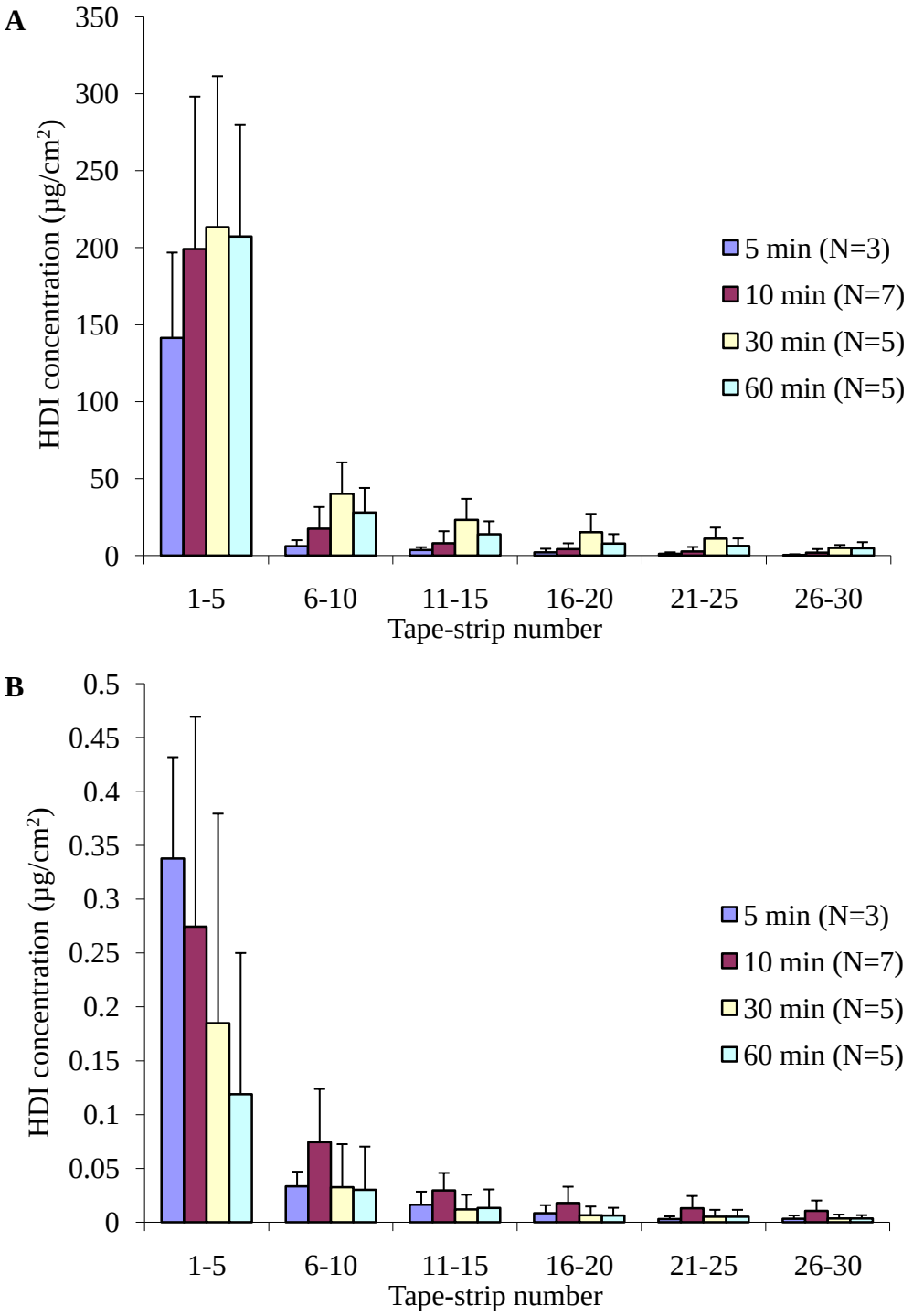

Figure 4.2. Amount of HDI measured in 30 sequential tape strips collected after epicutaneous application of (A) with 10 µl of HDI neat or (B) with 50 µl of 0.3 g/l HDI in ethyl acetate.

Table 4.1. Average percent recovery in each compartment after epicutaneous application of HDI neat (10 µl) or HDI/EA (50 µl of 0.3 g/l).

Spiking Agent	Compartment[A]	Percent Recovery (%)							
		5 min (N = 3)[B]		10 min (N = 7)[C]		30 min (N = 5)[D]		60 min (N = 5)[E]	
		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 – 79yr Female Caucasian lower leg
C. Donor 2 (N = 2) – 37yr Female African-American pannus; Donor 3 (N = 2) – 68yr Female Caucasian pannus; Donor 4 (N = 3) – 48yr Male Caucasian pannus
D. Donor 4 (N = 3); Donor 5 (N = 2) – 46yr 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.

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.

4.4.2.2 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 ($\mu g/cm^2$) 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 (**Figure 4.4**). 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.

Table 4.2. Average percent recovery for each compartment after epicutaneous application of slow- or fast-drying clearcoat.

Analyte	Spiking Agent	Compartment	Percent Recovery (%)					
			10 min (N = 3)[A]		30 min (N = 3)[A]		60 min (N = 3)[A]	
			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) – 47yr Female Caucasian pannus; Donor 7 (N = 1) – 72yr Male Caucasian thigh; Donor 8 (N = 1) – 53yr 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.

Table 4.3. 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)
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.

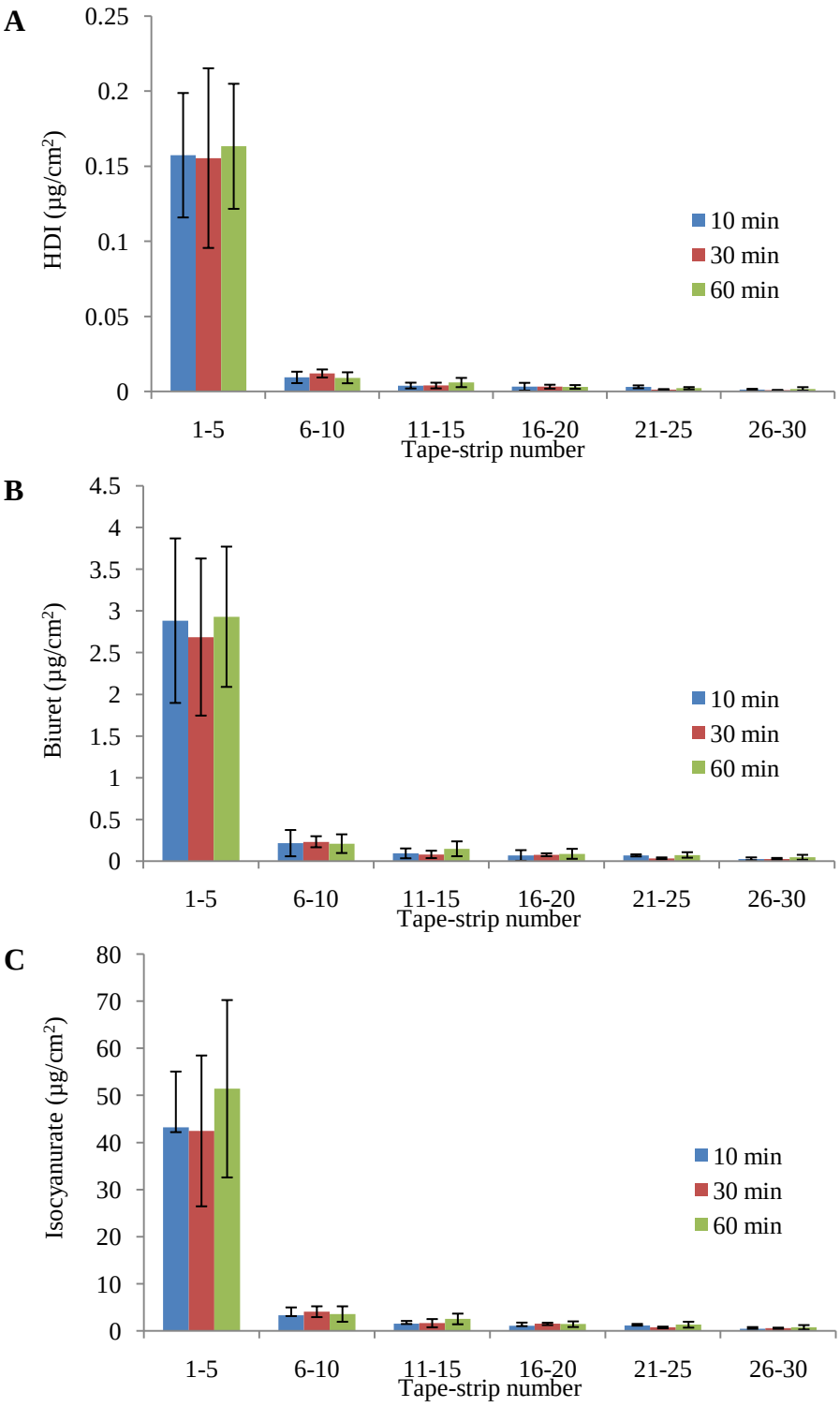

Figure 4.3. Amount of (A) HDI, (B) biuret, and (C) isocyanurate measured in 30 sequential tape-strips collected from the human skin after epicutaneous application of 50 µl of slow-drying clearcoat (N=3 for all time points)..

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.

4.5 Discussion

Dermal exposure to monomeric and polymeric HDI comprises a significant route for exposure to spray painters employed in the automotive refinishing industry [1, 40, 47, 68-71, 86, 89, 90]. Although the dermal exposure route has been established to be significant [71, 89, 90], 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.

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 [47, 70, 71]. This is clearly demonstrated by the fact that we recovered more HDI monomer in the deeper cell layers of the stratum corneum 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 [98, 99] of HDI and driving it faster into the deeper layers of the skin. This enhancement was further confirmed by the fact that at 5 min 81% of the total HDI applied in EA had penetrated the skin (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.

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

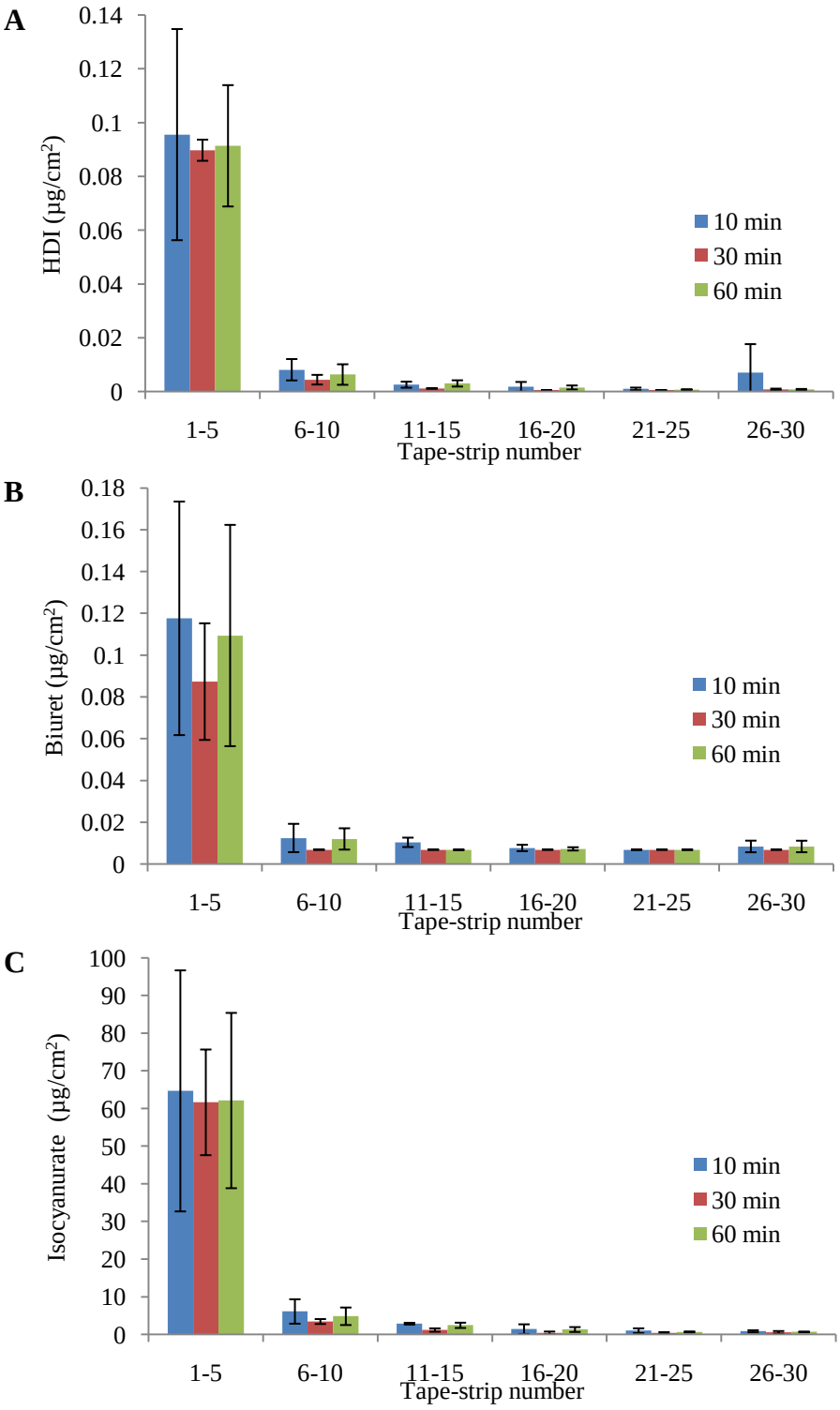

Figure 4.4. Amount of (A) HDI, (B) biuret, and (C) isocyanurate measured in 30 sequential tape-strips collected from the human skin after epicutaneous application of 50 µl of fast-drying clearcoat(N=3 for all time points).

significantly more HDI and biuret than fast-drying clearcoat). The data indicated initial rapid flux through 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. [52]. They investigated the residence time of model isocyanates [octyl isocyanate, polymeric HDI (pHDI), polymeric isophorone diisocyanate isocyanurate (pIPDI) and methylenediphenly 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. They 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. 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 [94]. 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 (Figures 4.2 – 4.4). 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.

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) 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 Fasano et al [100] was used to calculate the skin absorption time:

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

For example, if a worker were continuously exposed to HDI-containing clearcoat using a reasonable worst-case scenario, where the worker's lower arms, hands, and lower legs are exposed, the exposed skin area would be 5,280 cm^2 [71]. 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 [101] 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) and 60 min (0.219

μg/cm^{2}h), it would take approximately 3 and 18 min, respectively, to achieve a dose of HDI equivalent to the ACGIH TLV. Using the 10 (0.733 μg/cm^{2}h) and 60 min (0.109 μg/cm^{2}h) 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 5 and 35 min, respectively. For the fast-drying clearcoat the calculated skin absorption times [5 and 30 min for 10 (0.767 μg/cm^{2}h) and 60 min (0.131 μg/cm^{2}h) 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) [41] and the respective short-term absorption rates. For biuret, the skin absorption time was much shorter for the slow-drying clearcoat [2.3 and 13.6 min for the 10 (52.2 μg/cm^{2}h) and 60 min (4.17 μg/cm^{2}h) short-term absorption rates, respectively] compared to the fast-drying clearcoat [36 and 274 min for the 10 (1.58 μg/cm^{2}h) and 60 min 0.207 μg/cm^{2}h) short-term absorption rates, respectively]. Isocyanurate had the shortest skin absorption times regardless of clearcoat drying time (between 6 and 47 sec). 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.

Fasano et al. [100] 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 (5280 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 3.4 and 2 times the current TLV for slow-

drying clearcoat and fast-drying clearcoat, respectively. For biuret, the absorbed-absorbable dose at 10 and 60 min exposure would yield potential exposure approximately 4.5 times the current OEL for the slow-drying clearcoat and 28% for the fast-drying clearcoat. For isocyanurate, the absorbed-absorbable dose at 10 and 60 min exposures would yield potential exposure approximately 67-102 times the current OEL.

Typically, short-term exposure experiments would be conducted using diffusion cells with a receptor fluid. A finite does (i.e., $\mu l/cm^2$) 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 [52] 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.

4.6 Conclusions

Although this study is limited in size, we have demonstrated that monomeric and polymeric HDI rapidly penetrates 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.

CHAPTER 5

DISCUSSION AND CONCLUSIONS

5.1 Overview

Previously our laboratory has developed analytical methods [47] to measure air and dermal exposures to monomeric and polymeric HDI. Primary determinants of air [58] and dermal [71] exposures have been reported and relationships between air and dermal exposures explored. We have also developed analytical methods and measured HDI biomarkers in blood [89] and urine [90] and related these levels to air and dermal exposure measurements. Although, these research efforts significantly increased our understanding of isocyanate exposures, some important knowledge gaps remained to be elucidated.

In this dissertation, I have presented the evaluation and development of air- and dermal-sampling methodologies to assess HDI exposure in automotive spray painters (Chapters 2 – 4). In Chapter 2, I compared 13 different air samplers for their ability to monitor air exposures to monomeric and polymeric HDI. In Chapter 3, I presented the development and evaluation of a new patch sampler method to monitor dermal exposures to monomeric and polymeric HDI in three automotive spray painters. In Chapter 4, I describe the first *in vitro* studies of this kind using excised full-thickness human skin to determine the time-dependent penetration patterns of monomeric and polymeric HDI in human skin. I further estimated the short-term absorption rates and the time it would take for these compounds to be absorbed

through the skin and reach a body burden equal to the dose received by the inhalation exposure at the level of TLV or OEL. My findings indicate that the sampling devices commonly used by industrial hygienists and regulators do not accurately measure air exposure to isocyanates and that these methods may grossly underestimate spray painters' exposure. My work also highlights the importance of dermal exposure and its contribution to the total body burden in this exposed worker population. I demonstrated that these compounds are readily absorbed and penetrated into the skin and that the composition of the clearcoat mixture may affect the penetration rate of the individual isocyanate compounds (both monomeric and polymeric). I estimated that the dose received through dermal exposure to isocyanate-containing clearcoats in the occupational setting has a great potential to exceed established regulatory limits for inhalation exposure. In summary, a critical need exists to monitor both dermal and inhalation exposure quantitatively in isocyanate exposed worker populations and to re-evaluate regulatory exposure limits for isocyanate exposures. The use of proper personal protective equipment (PPE) to reduce dermal and inhalation exposures is imperative.

5.2 Air samplers

Previously, we observed dual-stage air samplers to underestimate breathing-zone concentrations of HDI and its oligomer isocyanurate compared to single-stage samplers [58]. This finding was further corroborated by our side-by-side comparison experiments of single- and dual-stage samplers, which indicated that single-stage samplers measured significantly higher levels of HDI monomer and isocyanurate [58]. These observations prompted us to

investigate the potential sampling biases associated with air-sampler types and analytical methods commonly used to quantitate monomeric and polymeric HDI levels.

Using both fast- and slow-drying clearcoat, we compared midget impingers with frit (reference samplers) with the following types of samplers: single- and dual-stage 37-mm polypropylene (PP) and polystyrene (PS) samplers (open- and closed-face), IOM (with plastic and stainless steel inserts), OSHA42, IsoChek®, and WA-DOSH samplers (Chapter 2). We observed significant differences in sampler performance between fast- and slow-drying clearcoat. We also observed open-face sampling to be the most effective when sampling for monomeric and polymeric HDI.

Overall, the single-stage open-face sampler was in best agreement with the impinger for measuring monomeric and polymeric HDI during spray-painting in the automotive refinishing industry. Of the three samplers analyzed by laboratories other than UNC (i.e., OSHA42, IsoChek®, and WA-DOSH), the WA-DOSH was in the best agreement with the impingers. It appears that the air samplers commonly used by regulators and industrial hygienists (OSHA42 and IsoChek®) do not accurately measure air exposures to monomeric and polymeric HDI. When selecting a sampling device for monomeric and polymeric HDI in the automotive refinishing industry, one must take into consideration the product being sampled, specifically the clearcoat drying time. Caution should be used when interpreting filter-cassette sampler results, especially when atmospheres containing fast-drying clearcoat aerosols are sampled. When fast-drying clearcoat was applied, almost all samplers used in this study underestimated HDI polyisocyanate concentrations. To our knowledge, this was the first study to investigate differences in sampler performance based on clearcoat formulation.

5.3 Dermal patch samplers

From the air sampling study, I learned that our current air sampling techniques do not accurately measure air levels of HDI. Because of this, I raised concerns about the performance of our tape-strip technique, which was used in our previous field study [71]. This led me to develop a new dermal patch sampler and to evaluate its performance to quantitatively measure dermal exposure to monomeric and polymeric HDI in the spray-painting environment (Chapter 3). When worn by painters during spray-painting tasks, impregnated patches measured more monomeric and polymeric HDI than non-impregnated patches and tape-strip samples. At most, the tape-strips measured 35% of HDI monomer that the impregnated patches measured. For biuret, all samplers had similar rates of detection. However, the tape-strips measured between 11 – 60% of the biuret measured with the impregnated patch. Isocyanurate was the predominant species measured by all samplers although the impregnated patch measured significantly ($\alpha = 0.05$) greater amounts than non-impregnated patches and tape-strips.

Findings from this study indicated that the tape-strips may not be effective at measuring dermal exposure of monomeric and polymeric HDI due to rapid penetration of these compounds into deeper layers of the skin. Impregnated patches are likely more accurate at measuring dermal isocyanate exposure than tape-strips because losses due to evaporation, reactivity with skin components, and/or penetration beyond the upper layers of the skin are minimized. Our ability to measure dermal isocyanate exposure accurately is critical for exposure and risk assessment in order to predict systemic exposure, develop sensitive and predictive models through multiple exposure routes, and ultimately protect the health of

workers. To our knowledge, this is the first dermal sampling patch that is capable of measuring monomeric and polymeric HDI.

5.4 Dermal penetration studies

Dermal patch sampling indicated that isocyanate exposure through the skin may be more significant than can be detected by tape-strip technique, and that we need to understand the uptake and penetration patterns of isocyanates in the human skin. Therefore, I designed a study to investigate the dermal penetration patterns and timelines, in order to estimate the true monomeric and polymeric HDI exposure received during a specific exposure time (Chapter 4). Towards this goal, I exposed excised full-thickness human skin to monomeric HDI (neat and mixed in ethyl acetate) or monomeric and polymeric HDI (slow- or fast-drying clearcoat) for a specific time course.

I demonstrated that monomeric and polymeric HDI rapidly penetrate into and beyond the stratum corneum of excised human full-thickness skin. Differences in the penetration patterns between slow- and fast-drying clearcoats indicated that the composition of the clearcoat mixture can affect the penetration rate of individual isocyanate compounds (both monomeric and polymeric). The tape-strip is capable of detecting monomeric and polymeric HDI in the human skin at least 30 tape-strips deep. Thus, this method was further validated for dermal exposure measurements of monomeric and polymeric HDI. In addition, I calculated the short-term absorption rates (Table 4.3) of monomeric and polymeric HDI for both slow- and fast-drying clearcoats for 10 and 60 min exposures. Using the short-term absorption rates, I demonstrated that the dose received through dermal exposure to HDI-containing clearcoats in an occupational setting has a great potential to exceed established

regulatory limits for inhalation exposure. These results highlight the potential large contribution of dermal exposure to the internal dose received during exposure to isocyanates. 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.

5.5 Limitations and suggestions for future research

We acknowledge that these studies were comprised of small sample sizes. However, we were able to ascertain information on the performance of air and dermal sampling methods as well as the important contribution of dermal exposure to the total internal dose received during exposure to isocyanates. Methods commonly used to measure air exposures to monomeric and polymeric HDI exposures are not accurate and may underestimate exposure. Additional studies comparing air sampling devices should be performed with larger sample sizes and where variables such as wind currents and the amount of clearcoat being applied are better controlled. In addition, further development and standardization of air samplers is warranted. Use of an appropriate sampling and/or analytical method is critical for accurate assessment of worker's exposure to isocyanates. The standardization of a sampling method will provide easier comparison and correlation of airborne isocyanate exposure and biomarker levels as well as health effects between different studies.

Our ability to measure air and dermal isocyanate exposure accurately is critical for exposure and risk assessment in order to predict systemic exposure, develop sensitive and predictive models through multiple exposure routes, and ultimately protect the health of workers. Although we only compared the performance of the patch samplers and the tape-

strip technique in three exposed workers, we observed that the tapes-strips may underestimate dermal exposure. Future studies involving a larger population are required to evaluate these and other dermal sampling methods (e.g., wipe sampling) to measure dermal exposure to isocyanates. To our knowledge, we are the first to report dermal penetration patterns of clearcoats in human skin. Although limited in size, our results emphasize the important role isocyanate dermal exposure may have to the internal dose received in the exposed workers. Future studies, with larger sample size, should be conducted to establish protective short-term exposure limits for isocyanates. Additionally, the use of proper PPE to reduce dermal and inhalation exposures is essential. Currently, research efforts are underway to determine what types of PPE are most effective at reducing isocyanate exposures.

The development of sensitive and specific biomarkers could have significant value in bridging the gap between exposure and internal dose. However, the mechanism for isocyanate induced hypersensitivity (including asthma) are not understood, therefore, a sensitive and specific biomarker may not increase our knowledge on health risks associated with isocyanate exposures. The relationship between biomarker and exposure levels (inhalation and dermal) need to be further studied. A useful biomarker for HDI exposure in the automotive refinishing industry would have to be an indicator of both monomeric and polymeric HDI exposure. The use of a biomarker may not replace air and dermal exposure measurements but ought to supplement them and provide a reliable estimate of the total internal dose. The improvement of air and dermal sampling as well as the development of useful biomarkers will provide us a better understanding of HDI exposures in the automotive refinishing industry and, ultimately, allow us to protect the health of these workers.

5.6 Cause for concern

We have documented that methods commonly used for regulatory purposes to monitor inhalation exposures to HDI may grossly underestimate exposure. We have also demonstrated that dermal exposure may contribute greatly to the internal dose received during spray-painting operations. Further, our findings indicate that the dose received through dermal exposure to HDI-containing clearcoats in the occupational setting has significant potential to exceed established regulatory limits when compared to that for inhalation exposure. A critical need exists to monitor dermal exposure quantitatively in exposed worker populations and to re-evaluate regulatory exposure limits for isocyanate exposures. This work also highlights the need for effective PPE and its proper use in this industry.

REFERENCES

1. Liu, Y., et al., *Qualitative assessment of isocyanate skin exposure in auto body shops: a pilot study.* Am J Ind Med, 2000. **37**(3): p. 265-74.

2. Tarlo, S.M., et al., *Outcome determinants for isocyanate induced occupational asthma among compensation claimants.* Occup Environ Med, 1997. **54**(10): p. 756-61.

3. NIOSH, *Preventing Asthma and Death from Diisocyanate Exposure*, in *U.S. Dept of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health*. 1996.

4. Bernstein, J.A., *Overview of diisocyante occupational asthma.* Toxicology, 1996. **111**: p. 181-189.

5. NIOSH, *Criteria for a recommended standard: occupational exposure to diisocyanates.* U.S. Dept of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, 1978.

6. NIOSH, *Pocket guide to chemical hazards.* U.S. Dept of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, 1990.

7. NIOSH, *Pocket guide to chemical hazards*, in *U.S. Dept of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health*. 2007.

8. Levy BS, W.D., *Occupational Health: Recognizing and preventing work-related diseases*. 1988, Boston/Toronto: Little, Brown and Company.

9. Chan-Yeung, M. and J.L. Malo, *Occupational asthma.* N Engl J Med, 1995. **333**(2): p. 107-12.

10. Wisnewski, A.V. and C.A. Redlich, *Recent developments in diisocyanate asthma.* Curr Opin Allergy Clin Immunol, 2001. **1**(2): p. 169-75.

11. NIOSH, *Occupational respiratory diseases.* U.S. Dept of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, 1986.

12. Chan-Yeung, M. and S. Lam, *Occupational asthma.* Am Rev Respir Dis, 1986. **133**(4): p. 686-703.

13. Weber, J., *A Summary of Health Hazard Evaluations: Issues Related to Occupational Exposure to Isocyanates, 1989 to 2002.* U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health., 2004: p. Available from www.cdc.gov/niosh/docs/2004-116/.

14. Fabbri, L.M., et al., *Fatal asthma in a subject sensitized to toluene diisocyanate.* Am Rev Respir Dis, 1988. **137**(6): p. 1494-8.

15. Adams, W.G., *Long-term effects on the health of men engaged in the manufacture of toluene di-isocyanate.* Br J Ind Med, 1975. **32**(1): p. 72-8.

16. Butcher, B.T., C.E. Mapp, and L.M. Fabbri, *Polyisocyanates and their prepolymers*. Asthma in the Workplace, ed. I.L. Bernstein, et al. 1993, New York, NY: Marcel Dekker, Inc.

17. Diem, J.E., et al., *Five-year longitudinal study of workers employed in a new toluene diisocyanate manufacturing plant.* Am Rev Respir Dis, 1982. **126**(3): p. 420-8.

18. Porter, C.V., R.L. Higgins, and L.D. Scheel, *A retrospective study of clinical, physiologic and immunologic changes in workers exposed to toluene diisocyanate.* Am Ind Hyg Assoc J, 1975. **36**(3): p. 159-68.

19. Weill, H., *Epidemiologic and medical-legal aspects of occupational asthma.* J Allergy Clin Immunol, 1979. **64**: p. 662-664.

20. White, W.G., et al., *Isocyanate-induced asthma in a car factory.* Lancet, 1988. **1**: p. 756-760.

21. Pronk, A., et al., *Respiratory symptoms, sensitization, and exposure response relationships in spray painters exposed to isocyanates.* Am J Respir Crit Care Med, 2007. **176**(11): p. 1090-7.

22. Wisnewski, A.V., et al., *Polyisocyanates and their prepolymers*, in *Asthma in the workplace*. 2006, Taylor & Francis Group: New York. p. 875.

23. Petsonk, E.L., et al., *Asthma-like symptoms in wood product plant workers exposed to methylene diphenyl diisocyanate.* Chest, 2000. **118**(4): p. 1183-93.

24. Simpson, C., et al., *Hypersensitivity pneumonitis-like reaction and occupational asthma associated with 1,3-bis(isocyanatomethyl) cyclohexane pre-polymer.* Am J Ind Med, 1996. **30**(1): p. 48-55.

25. Ulvestad, B., E. Melbostad, and P. Fuglerud, *Asthma in tunnel workers exposed to synthetic resins.* Scand J Work Environ Health, 1999. **25**(4): p. 335-41.

26. Welinder, H., et al., *IgG antibodies against polyisocyanates in car painters.* Clin Allergy, 1988. **18**(1): p. 85-93.

27. Bello, D., et al., *Polyisocyanates in occupational environments: a critical review of exposure limits and metrics.* Am J Ind Med, 2004. **46**(5): p. 480-91.

28. Vandenplas, O., et al., *Prepolymers of hexamethylene diisocyanate as a cause of occupational asthma.* J Allergy Clin Immunol, 1993. **91**(4): p. 850-61.

29. Vandenplas, O., et al., *Occupational asthma caused by a prepolymer but not the monomer of toluene diisocyanate (TDI).* J Allergy Clin Immunol, 1992. **89**(6): p. 1183-8.

30. McDonald, J.C., H.L. Keynes, and S.K. Meredith, *Reported incidence of occupational asthma in the United Kingdom, 1989-97.* Occup Environ Med, 2000. **57**(12): p. 823-9.

31. Karjalainen, A., et al., *Incidence of occupational asthma by occupation and industry in Finland.* Am J Ind Med, 2000. **37**(5): p. 451-8.

32. Ameille, J., et al., *Reported incidence of occupational asthma in France, 1996-99: the ONAP programme.* Occup Environ Med, 2003. **60**(2): p. 136-41.

33. Randall, D. and S. Lee, *The Polyurethanes Book*. 2002, New York, NY: Wiley.

34. Bello, D., et al., *A laboratory investigation of the effectiveness of various skin and surface decontaminants for aliphatic polyisocyanates.* J Environ Monit, 2005. **7**(7): p. 716-21.

35. Boutin, M., et al., *Determination of airborne isocyanates generated during the thermal degradation of car paint in body repair shops.* Ann Occup Hyg, 2006. **50**(4): p. 385-93.

36. Saunders, J. and K. Frisch, *Polyurethanes: Chemistry and Technology, Part I: Chemistry*. 1962, New York, NY: Interscience Publishers, a division of John Wiley & Sons, Inc.

37. Carlton, G.N. and E.C. England, *Exposures to 1,6-hexamethylene diisocyanate during polyurethane spray painting in the U.S. Air Force.* Appl Occup Environ Hyg, 2000. **15**(9): p. 705-12.

38. Burgess, W., *Recognition of Health Hazards in Industry*. 1995, New York, NY: John Wiley & Sons, Inc.

39. Redlich, C.A. and C.A. Herrick, *Lung/skin connections in occupational lung disease.* Curr Opin Allergy Clin Immunol, 2008. **8**(2): p. 115-9.

40. Bello, D., et al., *Skin exposure to isocyanates: reasons for concern.* Environ Health Perspect, 2007. **115**(3): p. 328-35.

41. Janko, M., et al., *Occupational exposure to 1,6-hexamethylene diisocyanate-based polyisocyanates in the state of Oregon, 1980-1990.* Am Ind Hyg Assoc J, 1992. **53**(5): p. 331-8.

42. Lesage, J., et al., *Workers' exposure to isocyanates.* Am Ind Hyg Assoc J, 1992. **53**(2): p. 146-53.

43. Myer, H.E., S.T. O'Block, and V. Dharmarajan, *A survey of airborne HDI, HDI-based polyisocyanate and solvent concentrations in the manufacture and application of polyurethane coatings.* Am Ind Hyg Assoc J, 1993. **54**(11): p. 663-70.

44. Pisaniello, D.L. and L. Muriale, *The use of isocyanate paints in auto refinishing--a survey of isocyanate exposures and related work practices in South Australia.* Ann Occup Hyg, 1989. **33**(4): p. 563-72.

45. Goyer, N., *Performance of Painting Booths Equipped with Down-Draft Ventilation.* American Industrial Hygiene Association Journal, 1995. **56**(3): p. 258-265.

46. Rudzinski, W.E., et al., *Sampling and Analysis of Isocyanates in Spray-Painting Operations.* American Industrial Hygiene Association Journal, 1995. **56**(3): p. 284-289.

47. Fent, K.W., et al., *Quantitative monitoring of dermal and inhalation exposure to 1,6-hexamethylene diisocyanate monomer and oligomers.* J Environ Monit, 2008. **10**(4): p. 500-7.

48. Erjefalt, I. and C.G. Persson, *Increased sensitivity to toluene diisocyanate (TDI) in airways previously exposed to low doses of TDI.* Clin Exp Allergy, 1992. **22**(9): p. 854-62.

49. Rattray, N.J., et al., *Induction of respiratory hypersensitivity to diphenylmethane-4,4'-diisocyanate (MDI) in guinea pigs. Influence of route of exposure.* Toxicology, 1994. **88**(1-3): p. 15-30.

50. Herrick, C.A., et al., *A novel mouse model of diisocyanate-induced asthma showing allergic-type inflammation in the lung after inhaled antigen challenge.* Journal of Allergy and Clinical Immunology, 2002. **109**(5): p. 873-878.

51. Karol, M.H., et al., *Dermal contact with toluene diisocyanate (TDI) produces respiratory tract hypersensitivity in guinea pigs.* Toxicol Appl Pharmacol, 1981. **58**(2): p. 221-30.

52. Bello, D., et al., *An FTIR investigation of isocyanate skin absorption using in vitro guinea pig skin.* J Environ Monit, 2006. **8**(5): p. 523-9.

53. Streicher, R.P., et al., *Selecting isocyanate sampling and analytical methods.* Appl Occup Environ Hyg, 2002. **17**(3): p. 157-62.

54. England, E., et al., *Comparison of sampling methods for monomer and polyisocyanates of 1,6-hexamethylene diisocyanate during spray finishing operations.* Appl Occup Environ Hyg, 2000. **15**(6): p. 472-8.

55. Streicher, R.P., E.R. Kennedy, and C.D. Lorberau, *Strategies for the simultaneous collection of vapours and aerosols with emphasis on isocyanate sampling.* Analyst, 1994. **119**(1): p. 89-97.

56. Nordqvist, Y., et al., *A chemosorptive cylindrical denuder designed for personal exposure measurements of isocyanates-evaluation on generated aerosols of 4,4'-methylenediphenyl diisocyanate.* J Environ Monit, 2005. **7**(5): p. 469-74.

57. Spanne, M., P. Grzybowski, and M. Bohgard, *Collection efficiency for submicron particles of a commonly used impinter.* AIHA J (Fairfax, Va), 1999. **60**(4): p. 540-544.

58. Fent, K.W., et al., *Quantification and Statistical Modeling--Part I: Breathing-Zone Concentrations of Monomeric and Polymeric 1,6-Hexamethylene Diisocyanate.* Ann Occup Hyg, 2009.

59. OSHA, *OSHA Methods Manual*. 2nd ed. 1994, Salt Lake City: OSHA.

60. Bello, D., R.P. Streicher, and S.R. Woskie, *Evaluation of the NIOSH draft method 5525 for determination of the total reactive isocyanate group (TRIG) for aliphatic isocyanates in autobody repair shops.* . J Environ Monit, 2002. **4**(3): p. 351-60.

61. NIOSH, *NIOSH Manual of Analytical Methods*. 4th ed. NIOSH Manual of Analytical Methods. 1994, Cincinnati, OH: NIOSH.

62. Rando, R.J., H.G. Poovey, and D.R. Mokadam, *Laboratory Comparison of Sampling Methods for Reactive Isocyanate Vapors and Aerosols*, in *Isocyanates: Sampling, Analysis, and Health Effects, ASTM STP 1408*. 2002, American Society for Testing and Materials: West Conshohocken, PA.

63. Podolak, G.E., et al., *Collection and analysis of airborne hexamethylene diisocyanate by a modified OSHA method*, in *Sampling and Calibration for Atmospheric Measurements, ASTM STP 957*. 1987, American Society for Testing and Materials: West Conshohocken, PA. p. 203-214.

64. Dharmarajan, V., Lingg, R.D., Booth, K.S., and Hackathorn, D.R., *Recent Developments in the Sampling and Analysis of Isocyanates in Air*, in *Sampling and Calibration for Atmospheric Measurements*, J.K. Taylor, Editor. 1987, American Society for Testing and Materials: Philadelphia. p. 190-202.

65. Sennbro, C.J., et al., *Determination of isocyanates in air using 1-(2-methoxyphenyl)piperazine-impregnated filters: long-term sampling performance and field comparison with impingers with dibutylamine.* Ann Occup Hyg, 2004. **48**(5): p. 415-24.

66. Ekman, J., et al., *Comparison of sampling methods for 1,6-hexamethylene diisocyanate, (HDI) in a commercial spray box.* Analyst, 2002. **127**(1): p. 169-173.

67. Bello, D., et al., *Field comparison of impingers and treated filters for sampling of total aliphatic isocyanates with the MAP reagent.* AIHA J (Fairfax, Va), 2002. **63**(6): p. 790-6.

68. Liu, Y., et al., *Skin exposure to aliphatic polyisocyanates in the auto body repair and refinishing industry: a qualitative assessment.* Ann Occup Hyg, 2007. **51**(5): p. 429-39.

69. Bello, D., et al., *Skin exposure to aliphatic polyisocyanates in the auto body repair and refinishing industry: II. A quantitative assessment.* Ann Occup Hyg, 2008. **52**(2): p. 117-24.

70. Fent, K.W., et al., *Tape-strip sampling for measuring dermal exposure to 1,6-hexamethylene diisocyanate.* Scand J Work Environ Health, 2006. **32**(3): p. 225-40.

71. Fent, K.W., et al., *Quantification and Statistical Modeling--Part II: Dermal Concentrations of Monomeric and Polymeric 1,6-Hexamethylene Diisocyanate.* Ann Occup Hyg, 2009.

72. Pronk, A., et al., *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, 2006. **63**(9): p. 624-31.

73. NIOSH, *Criteria for a recommended standard: occupational exposure to diisocyanates*, in *U.S. Dept of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health*. 1978.

74. NIOSH. *National Occupational Research Agenda Priority Areas: Asthma & Chronic Obstructive Pulmonary Disease*. 1996; Available from: http://www.cdc.gov/niosh/docs/96-115/diseas.html.

75. OSHA, *Diisocyanates: Method 42*, U.D.o. Labor, Editor. 1983, OSHA: Salt Lake City, UT.

76. Levine, S.P., *Critical review of methods for sampling, analysis, and monitoring of vapor-phase toluene diisocyanate.* Appl Occup Environ Hyg, 2002. **17**(12): p. 878-90.

77. Streicher, R.P., et al., *Determination of airborne isocyanate exposure: considerations in method selection.* AIHAJ, 2000. **61**(4): p. 544-56.

78. Huynh, C.K., T. Vu-Duc, and H. Savolainen, *Design and evaluation of a solid sampler for the monitoring of airborne 1,6-hexamethylene diisocyanate (HDI) and its prepolymers in two-component spray painting.* Am Ind Hyg Assoc J, 1992. **53**(3): p. 157-62.

79. WA State Division of Occupational Safety and Health, *L&I Method 0050: HDI, IPDI, and MDI (Vapor) MAMA Derivatives*, 2005. Available upon request: 805 Plum Street, Olympia, WA, 98501.

80. WA State Division of Occupational Safety and Health, *L&I Method 0067: HDI and IPDI Polyisocyanate Aerosol*,. 2008. Available upon request: 805 Plum Street, Olympia, WA, 98501.

81. Hornung, R.W. and L.D. Reed, *Estimation of average concentration in presence of nondetectable values.* Appl Occup Environ Hyg, 1990. **5**: p. 46-51.

82. Pronk, A., et al., *Inhalation exposure to isocyanates of car body repair shop workers and industrial spray painters.* Ann Occup Hyg, 2006. **50**(1): p. 1-14.

83. Carlton, G.N. and M.R. Flynn, *The influence of spray painting parameters on breathng zone particle size distributions.* Appl Occup Env Hyg, 1997. **12**: p. 744-750.

84. Sabty-Daily, R.A., W.C. Hinds, and J.R. Froines, *Size distribution of chromate paint aerosol generated in a bench-scale spray booth.* Ann Occup Hyg, 2005. **49**(1): p. 33-45.

85. Liu, Y., et al., *Skin exposure to aliphatic polyisocyanates in the auto body repair and refinishing industry: III. A personal exposure algorithm.* Ann Occup Hyg, 2009. **53**(1): p. 33-40.

86. Bello, D., et al., *Slow curing of aliphatic polyisocyanate paints in automotive refinishing: a potential source for skin exposure.* J Occup Environ Hyg, 2007. **4**(6): p. 406-11.

87. Thomasen, J.M., et al., *Field comparison of air sampling methods for monomeric and polymeric 1,6-hexamethylene diisocyanate.* J Occup Environ Hyg, 2011. **8**(3): p. 161-78.

88. Nylander-French, L.A., *A tape-stripping method for measuring dermal exposure to multifunctional acrylates.* Ann Occup Hyg, 2000. **44**(8): p. 645-51.

89. Flack, S.L., et al., *Quantitative plasma biomarker analysis in HDI exposure assessment.* Ann Occup Hyg, 2009. **54**(1): p. 41-54.

90. Gaines, L.G., et al., *Urine 1,6-hexamethylene diamine (HDA) levels among workers exposed to 1,6-hexamethylene diisocyanate (HDI).* Ann Occup Hyg, 2010. **54**(6): p. 678-91.

91. Creely, K.S., et al., *Assessing isocyanate exposures in polyurethane industry sectors using biological and air monitoring methods.* Ann Occup Hyg, 2006. **50**(6): p. 609-21.

92. Kaaria, K., et al., *Exposure to 2,4- and 2,6-toluene diisocyanate (TDI) during production of flexible foam: determination of airborne TDI and urinary 2,4- and 2,6-toluenediamine (TDA).* Analyst, 2001. **126**(7): p. 1025-31.

93. Woskie, S.R., et al., *Determinants of isocyanate exposures in auto body repair and refinishing shops.* Ann Occup Hyg, 2004. **48**(5): p. 393-403.

94. Thomasen, J.M., K.W. Fent, and L.A. Nylander-French, *Development of a Sampling Patch to Measure Dermal Exposures to Monomeric and Polymeric 1,6-Hexamethylene Diisocyanate: A Pilot Study.* Submitted.

95. Kim, D., M.E. Andersen, and L.A. Nylander-French, *Dermal absorption and penetration of jet fuel components in humans.* Toxicol Lett, 2006. **165**(1): p. 11-21.

96. Pershing, L.K., et al., *Comparison of skin stripping, in vitro release, and skin blanching response methods to measure dose response and similarity of triamcinolone acetonide cream strengths from two manufactured sources.* J Pharm Sci, 2002. **91**(5): p. 1312-23.

97. Pershing, L.K., et al., *Assessment of dermatopharmacokinetic approach in the bioequivalence determination of topical tretinoin gel products.* J Am Acad Dermatol, 2003. **48**(5): p. 740-51.

98. Catz, P., Friend, DR, *Transdermal delivery of levonorgestrel. VIII. Effect of enhancers on rat skin, hairless mouse skin, hairless guinea pig skin, and human skin.* International Journal of Pharmaceutics, 1990. **58**(2): p. 93-102.

99. Magnusson, B.M., K.A. Walters, and M.S. Roberts, *Veterinary drug delivery: potential for skin penetration enhancement.* Adv Drug Deliv Rev, 2001. **50**(3): p. 205-27.

100. Fasano, W.J. and K.N. Baer, *The in vitro permeability coefficient and short-term absorption rates for vinyl toluene using human cadaver skin mounted in a static diffusion cell model.* Drug Chem Toxicol, 2006. **29**(1): p. 39-55.

101. ACGIH, *Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices*. 2010, Cincinnati, OH: American Conference of Governmental Industrial Hygienists (ACGIH).