Quantitative monitoring and statistical modeling of dermal and inhalation exposure to monomeric and polymeric 1,6-hexamethylene diisocyanate during automotive spray-painting

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ABSTRACT

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Quantitative Monitoring and Statistical Modeling of Dermal and Inhalation Exposure to Monomeric and Polymeric 1,6-Hexamethylene Diisocyanate During Automotive Spray-painting
(Under the direction of Leena A. Nylander-French)

Dermal and inhalation exposures to both the monomeric and polymeric forms of 1,6-hexamethylene diisocyanate (HDI) are associated with respiratory sensitization and occupational asthma. However, limited research has been performed on the evaluation of dermal and inhalation exposure to individual monomeric and polymeric HDI in the automotive refinishing industry due to the lack of specific and sensitive analytical methods and measurement techniques. The objective of this research was to develop methodology to quantify dermal and inhalation exposure to HDI and its oligomers (uretidone, biuret, and isocyanurate), to use this methodology to obtain detailed exposure profiles for 47 automotive painters in North Carolina and Washington State, and to use linear mixed modeling to identify the primary determinants of analyte-specific breathing-zone concentrations (BZCs) and dermal concentrations. A highly sensitive and specific liquid chromatography/mass spectrometry method capable of quantifying monomeric and polymeric HDI in air, tape-stripped skin, and paint samples was developed and validated in the occupational setting. Isocyanurate represented the predominant species (*i.e.*, > 90%) of the HDI-based polyisocyanates in sampled paint, air, and skin. The tape-strip sampling methodology that we used had superior collection efficiency and specificity compared to other methods in the

literature, while our air measurements of HDI and isocyanurate depended on the type of sampler (*i.e.*, one- or two-stage) used to monitor the breathing-zone. The primary determinants of BZC and dermal concentration were unique to each analyte. As expected, for each of the measured polyisocyanate species, paint concentration was a significant predictor of BZC, and the product of BZC and paint time was a significant predictor of dermal concentration. The models developed in this study provided us with a better understanding of the processes leading to dermal and inhalation exposure to monomeric and polymeric HDI. This understanding was used to identify and quantitatively characterize control interventions for reducing polyisocyanate exposures for the ultimate goal of protecting automotive spray-painters from potential adverse health effects, such as occupational asthma.

To my loving wife, April.

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LIST OF ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
ATR-FTIR	Attenuated total reflectance-Fourier transform infrared
BU	Urea derivative of biuret
BZC	Breathing-zone concentration
DMF	*N*,*N'*-dimethylformamide
DMSO	Dimethyl sulfoxide
FL	Fluorescence
GFF	Glass-fiber filter
GM	Geometric mean
GSD	Geometric standard deviation
HDI	1,6-Hexamethylene diisocyanate
HDIU	Urea derivative of hexamethylene diisocyanate
HPLC	High performance liquid chromatography
HVLP	High-volume low-pressure
IPDI	Isophorone diisocyanate
IU	Urea derivative of isocyanurate
LC-MS	Liquid chromatography – mass spectrometry
LMM	Linear mixed modeling
LNI	Washington State Department of Labor and Industries
LOD	Limit of detection
LOQ	Limit of quantitation

MDI	Methylenebisphenyl diisocyanate
MMAD	Mass median aerodynamic diameter
MPP	1-(2-methoxyphenyl)piperazine
MSDS	Material Safety Data Sheet
NA	Not applicable
NC	North Carolina
NCO	Isocyanate function group
nd	non-detectable
NIOSH	National Institute for Occupational Safety and Health
ODIU	Octamethylene diisocyanate
OEL	Occupational exposure limit
OSHA	Occupational Safety and Health Administration
PPE	Personal protective equipment
PTFE	Polytetrafluoroethylene
REL	Recommended exposure limit
REML	Restricted maximum likelihood
SIM	Selective ion monitoring
STEL	Short-term exposure limits
TDI	Toluene diisocyanate
TLV	Threshold limit value
TRIG	Total reactive isocyanate groups
TWA	Time weighted average
UK-HSE	United Kingdom – Health and Safety Executive

UU	Urea derivative of uretidone
UV	Ultraviolet
WA	Washington State

CHAPTER 1

BACKGROUND AND SIGNIFICANCE

1.1. DIISOCYANATES

Diisocyanates are a group of highly reactive, low-molecular-weight aromatic and aliphatic compounds, characterized by two isocyanate functional groups (N=C=O). The most common diisocyanates (**Figure 1.1**) include the aliphatic compounds, 1,6-hexamethylene diisocyanate (HDI) and isophorone diisocyanate (IPDI), and the aromatic compounds, toluene diisocyanate (TDI) and methylenebisphenyl diisocyanate (MDI). In 2000, the world production of diisocyanates was estimated to be more than 6 billion tons annually, which is predicted to increase 10-15% per year (2000). Personal exposure to diisocyanates is estimated to be 280,000 workers each year in the United States (Dunn and Bradstreet 1983; NIOSH 1983).

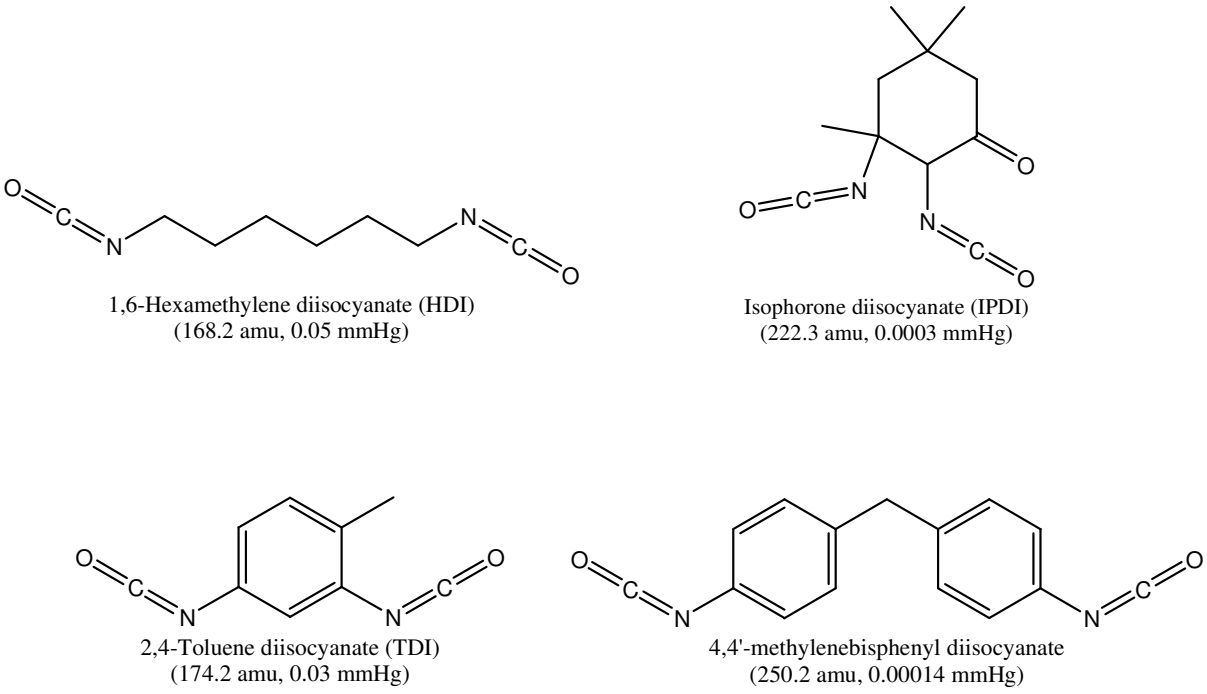


Figure 1.1. Molecular structures of the most commonly used diisocyanates in industry (includes molecular weights and vapor pressures at 25 °C).

Monomeric and polymeric diisocyanates are widely used in the production of polyurethane materials such as foams, elastomers, adhesives, and coatings. In industry, polyurethane is synthesized via polymer chemistry reaction between polyisocyanates and polyols (**Figure 1.2**). The properties of the resulting polyurethane (*i.e.*, density, flexibility, durability, *etc.*) depend on a number of factors, including functionality and molecular shape of the isocyanates and alcohols used in the reaction (Randall and Lee 2002; Saunders and Frisch 1962).

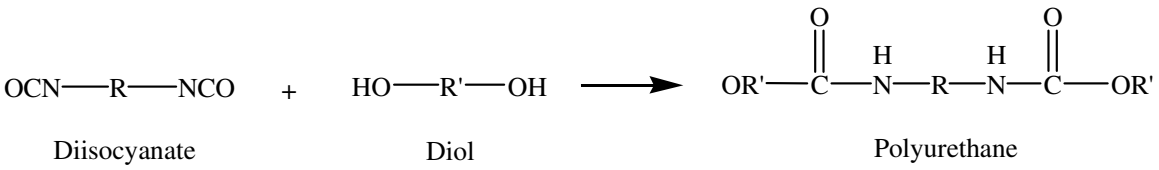


Figure 1.2. Formation of polyurethane via reaction between diisocyanate and diol.

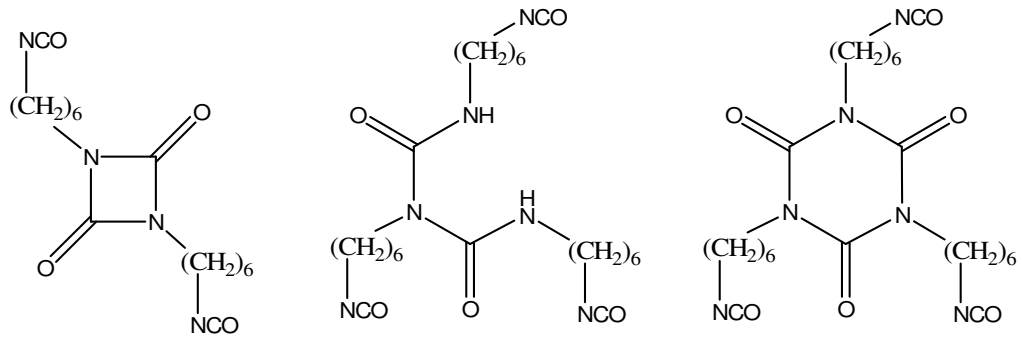
1.2. AUTOMOTIVE REFINISHING INDUSTRY

1.2.1. PAINTING PROCESS

In 1999, there were 35,000 automotive refinishing facilities in the U.S., employing approximately 270,000 workers (Census 1999). Paints used in the automotive refinishing industry contain aliphatic diisocyanates. Coatings based on aliphatic diisocyanates are more light-stable, more durable, and tend to retain their gloss longer than coatings based on aromatic diisocyanates (Randall and Lee 2002). Most automotive paints consist of polyisocyanates based on HDI, which contain trace amounts of HDI monomer (typically < 0.5%) and much higher amounts of HDI oligomers (2.5 – 20%) depending on the formulation (Bello *et al.* 2005; PPG 2007a; PPG 2007b). HDI oligomers commonly used in automotive paint (**Figure 1.3**) include the dimer, uretidone, and the trimers, biuret and isocyanurate. IPDI-based polyisocyanates may also be used in automotive coatings, but are typically present at lower levels than HDI-based polyisocyanates. For example, Woskie *et al.* (2004) observed that during spray-painting, median air concentrations of HDI-based polyisocyanates (N = 166) were more than five times the median air concentrations of IPDI-based polyisocyanates (N = 103).

Automotive paints are frequently applied using a two-stage system, where the first stage is the base coat and the second stage is the clearcoat. In this type of system, hardener

containing monomeric and polymeric HDI is added to the clearcoat. The polyisocyanates in the hardener react with polyols in the clearcoat solution to form polyurethane (Saunders and Frisch 1962). Drying and curing of polyurethane paint are two different processes. The drying rate depends mainly on the volatility of the solvent medium and temperature in the paint booth, while the curing rate depends on a number of factors, including the drying rate, efficacy of the catalyst, and the number and reactivity of isocyanate functional groups (Randall and Lee 2002).



Uretidone
(336.4 amu)

Biuret
(478.7 amu, 4.7E-7 mmHg)

Isocyanurate
(504.7 amu, 5.2E-9 mmHg)

Figure 1.3. Molecular structures of HDI oligomers commonly used in automotive paint (includes molecular weight and vapor pressure at 20 °C if known).

Automotive painting is generally accomplished using compressed-air spray guns inside ventilated booths (*i.e.*, crossdraft, downdraft, or semi-downdraft booths). Most of the paint droplets produced by the gun land on the surface of the automobile to form a polyurethane coating, but 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 polyisocyanates

(Carlton and England 2000). The overspray may be transported into the worker's personal space and result in potential dermal and inhalation exposure to polyisocyanates.

High-volume low-pressure (HVLP) spray guns have largely replaced conventional spray guns in paint-spray applications due to the high transfer efficiencies (65-75%) of HVLP guns. Conventional spray guns use nozzle pressures greater than 20 psig, while HVLP guns are operated at 10 psig or less (Carlton and Flynn 1997b). For HVLP guns, mass median aerodynamic diameters (MMAD) of over-spray paint mists have been measured in the breathing zone. Carlton and Flynn (1997b) reported an average MMAD of 18.9 µm, while Sabty-Daily *et al.* (2005) reported an average MMAD of 5.9 µm. Although different sampling methods were used, investigators in both of these studies measured aerosol size at a 90° orientation to the free-stream velocity. Regardless of which of these estimates is more accurate, conventional spray guns are expected to produce smaller aerosols than HVLP guns (Sabty-Daily *et al.* 2005).

1.2.2. DERMAL AND INHALATION EXPOSURES

The greatest potential for inhalation exposure exists during spray-painting when polyisocyanates are being aerosolized. Dermal exposure, on the other hand, may occur by one of three pathways: (1) immersion, (2) deposition of aerosol or uptake of vapor through the skin, or (3) surface contact (Fenske 1993). In the automobile refinishing industry, for example, HDI-containing paint can be deposited onto the skin during mixing or spraying applications, or after coming into direct contact with the paint, freshly painted products, or contaminated surfaces.

The extent of dermal and inhalation exposure depends largely on the use and efficiency of personal protective equipment (PPE). While respirators of some type (half-face, full-face, supplied air, *etc.*) are almost always worn by automotive refinishers, coveralls and gloves are worn with less frequency (Whittaker *et al.* 2005). Even when PPE is worn, polyisocyanates may breakthrough latex gloves (Liu *et al.* 2000) and half-face respirators (Liu *et al.* 2006).

Both dermal (Bello *et al.* 2008; Fent *et al.* 2006; Flynn *et al.* 2006; Liu *et al.* 2007; Pronk *et al.* 2006b) and inhalation (Janko *et al.* 1992; Lesage *et al.* 1992; Pronk *et al.* 2006a; Rudzinski *et al.* 1995; Sparer *et al.* 2004) exposures to polyisocyanates have been characterized in spray-painters in the automotive repair industry. The inhalation route has been considered the primary route of exposure leading to diisocyanate-induced asthma (NIOSH 1978). **Table 1.1** presents the current occupational exposure limits (OELs) for monomeric and polymeric HDI. Breathing-zone concentrations (BZCs) of HDI oligomers are more likely than HDI monomer to exceed the OELs. For instance, Janko *et al.* (1992) observed that only 6% of collected samples ($N = 562$) exceeded the National Institute for Occupational Safety and Health (NIOSH) ceiling limit of 140 $\mu g/m^3$ for HDI monomer, while 42% exceeded the Oregon short-term exposure limit (STEL) of 1000 $\mu g/m^3$ for HDI polyisocyanates.

The dermal route may play a significant role in the development of respiratory sensitization and occupational asthma. Several toxicological studies have demonstrated respiratory sensitization following dermal exposure. For example, Karol *et al.* (1981) reported that dermal exposure of guinea pigs to TDI induced pulmonary sensitization, and Rattray *et al.* (1994) observed that intradermal or topical exposure to MDI was substantially more effective than inhalation exposure at causing respiratory sensitization. These

observations were corroborated in a recent study in which mice were sensitized to HDI through the skin, resulting in both contact hypersensitivity and antibody response (*i.e.*, HDI-specific IgG and total serum IgE), and following inhaled antigen challenge, allergic type inflammation in the lung (Herrick *et al.* 2002). Furthermore, a growing number of case reports and epidemiological studies indicate that diisocyanate skin exposure occurs in the workplace and can increase the risk for asthma. For example, asthma and/or respiratory sensitization has been documented in workers who apply MDI-based orthopedic casts (Donnelly *et al.* 2004; Sommer *et al.* 2000) and in workers who directly handle MDI-containing glues (Valks *et al.* 2003) and resins (Petsonk *et al.* 2000).

Table 1.1. Occupational exposure limits ($\mu g/m^3$) for monomeric and polymeric HDI as work-shift[a] time weighted averages (TWA) and short-term exposure limits[b] (STEL)

Promulgating agency or institution[c]	Exposure limit name[d]	HDI monomer		Polyisocyanates as biuret and isocyanurate		Polyisocyanates as total NCO	
		TWA	STEL	TWA	STEL	TWA	STEL
NIOSH	REL	35	140	-	-	-	-
ACGIH	TLV	34	-	-	-	-	-
Bayer Corp.	OEL	-	-	500	1000	-	-
Oregon	OEL	-	-	500	1000	-	-
UK-HSE	OEL	-	-	-	-	20	70
Sweden	OEL	-	-	-	-	20	44

[a.] NIOSH considers a 10-hr work-shift, while the other institutions consider an 8-hr work-shift.

[b.] The STEL values represent a 15-min TWA for Bayer, Oregon, and ACGIH, a 5-min TWA for Sweden, and a 10-min ceiling limit for NIOSH and UK-HSE.

[c.] NIOSH = National Institute for Occupational Safety and Health; ACGIH = American Conference of Governmental Industrial Hygienists; UK-HSE = United Kingdom – Health and Safety Executive;

[d.] REL = Recommended Exposure Limit; TLV = Threshold Limit Value; OEL = Occupational Exposure Limit

1.3. HEALTH EFFECTS FROM DIISOCYANATE EXPOSURES

1.3.1. OVERVIEW OF HEALTH EFFECTS

Exposure to diisocyanates may cause adverse health effects, specifically to the respiratory tract and skin. Some symptoms of overexposure include cough, dyspnea, bronchitis, wheezing, pulmonary edema, and contact dermatitis (NIOSH 1978; NIOSH 1990), but the most common adverse health effect associated with diisocyanate exposure is asthma due to sensitization (Chan-Yeung and Malo 1995; NIOSH 1978; NIOSH 1986). The prevalence of diisocyanate-induced asthma in exposed workers is believed to be 5-10% (Bernstein 1996; Chan-Yeung and Malo 1995). A number of studies describe occupational asthma in diisocyanate exposed workers (Belin *et al.* 1981; Malo *et al.* 1983; Piirila *et al.* 2000). An exposed worker can become sensitized after a single acute exposure, but in most cases, sensitization takes a few months to several years of exposure (Chan-Yeung and Lam 1986; NIOSH 1978; NIOSH 1986; Weber 2004). Once sensitized, a worker can experience an asthmatic response even when exposed to levels below an occupational exposure limit (NIOSH 1978).

1.3.2. TOXICITY

In addition to the reactivity of the NCO functional group, properties affecting the toxicity of polyisocyanates include the volatility, electrophilicity, lipophilicity, and steric hindrance of the polyisocyanate compound and concomitant exposures (Bello *et al.* 2004). Such properties likely determine a molecule's permeability through biological barriers and ability to reach a target reaction site. For instance, monomeric HDI is more likely to reach the deeper part of the lungs than oligomeric HDI due to its higher vapor pressure. Recently, it

has been shown that more lipophilic isocyanates such as MDI and polymeric MDI may penetrate biological barriers faster than less lipophilic isocyanates such as isocyanurate (Pauluhn and Lewalter 2002). Consequently, one might expect a range of toxic effects caused by different polyisocyanates. Of particular interest is how diisocyanate monomers (*e.g.*, HDI) differ in toxicity from diisocyanate oligomers (*e.g.*, biuret and isocyanurate). Although additional research needs to be conducted to determine this, it has been observed that inhalation challenge with diisocyanate oligomers was more effective than the monomer at eliciting an asthmatic response in sensitized workers (Vandenplas *et al.* 1992).

1.3.3. MECHANISM OF DIISOCYANATE-INDUCED SENSITIZATION AND ASTHMA

The biological mechanism leading to diisocyanate-induced sensitization and asthma is unknown. Clinically, diisocyanate-induced asthma presents similar manifestations to those present in allergic asthma, suggesting common immunopathogenesis (Deschamps *et al.* 1998). Allergen-specific IgE is a key aspect of disease that involves type I hypersensitivity (*e.g.*, asthma) and often serves as a biomarker of sensitization to many common allergens. Allergen-specific IgG (or elevated IgG), on the other hand, is generally considered a marker of exposure (Wisnewski 2007). The presence of IgG that recognizes diisocyanate-albumin conjugates is almost never observed in unexposed individuals (Aul *et al.* 1999; Bernstein *et al.* 2006; Wisnewski *et al.* 2004; Ye *et al.* 2006), whereas diisocyanate-specific IgG often correlates well with diisocyanate inhalation exposures (Pronk *et al.* 2007; Wisnewski *et al.* 2004). Diisocyanate-specific IgE, however, has been found in less than 50% of diisocyanate-induced asthma sufferers (Wisnewski *et al.* 2004; Ye *et al.* 2006), and less than 5% of automotive spray-painters with respiratory symptoms (Pronk *et al.* 2007). The current

methods to measure diisocyanate-specific IgE may not be sensitive or specific enough to always quantify diisocyanate-specific IgE when present (Wisnewski 2007). Thus, levels of diisocyanate-specific IgE may be higher in persons with diisocyanate-induced asthma than what is reported in the literature. Nevertheless, the low presence of diisocyanate-specific IgE may suggest that other mechanisms, like cell-mediated allergic reactions or pulmonary irritation, are likely to be involved.

Regarding the cell mediated mechanism, it has been suggested that allergen-induced delayed asthma is analogous to allergen-induced delayed dermatitis (or type IV hypersensitivity) (Erjefalt and Persson 1992; Kimber 1996). According to this hypothesis, sensitizing chemicals penetrate into the viable epidermis and initiate the immunobiological processes that result in stimulation of T lymphocyte response. These processes involve Langerhans cells that transport the chemical allergen as a hapten carrier complex from the skin to draining lymph nodes where it is presented to naïve T-cells, which transform into memory T-cells (sensitization phase). Upon subsequent exposure, memory T-cells travel to the site of exposure and orchestrate an immune response to remove the hapten (elicitation phase), which, as a result, produces inflammation. If the lungs are the site of exposure during the elicitation phase, then asthmatic symptoms could result (Kimber 1996). This hypothesis is supported by a number of investigations showing that asthmatic responses may be elicited by inhalation challenge in guinea pigs or mice sensitized previously by topical or intradermal exposure to diisocyanates (Herrick *et al.* 2002; Karol *et al.* 1984; Rattray *et al.* 1994).

It is uncertain whether diisocyanate-induced asthma proceeds through type I or type IV hypersensitivity or a combination of the two. Because of this uncertainty, clinical and epidemiological investigations are needed to clarify the potential contribution of dermal

exposure to systemic immune response, including diisocyanate-specific IgE and IgG. Irrespective of the mechanism of diisocyanate-induced asthma, efforts should be taken to reduce both dermal and inhalation exposures to polyisocyanates in automotive spray-painters.

1.4. ANALYTICAL AND SAMPLING METHODOLOGY

1.4.1. ANALYTICAL METHODS

Most of the methods for analyzing diisocyanates were developed for air-sampling applications. The majority of the published analytical methods use high-performance liquid chromatography (HPLC) with an ultraviolet (UV), fluorescent (FL), or electrochemical detector to quantify oligomers as total reactive isocyanate groups (TRIG) (Bagon *et al.* 1984; Bello *et al.* 2002; Rando *et al.* 1995), which is the sum of free NCO groups found in all isocyanate species of a sample (Bello *et al.* 2002). A few methods have been published that use liquid chromatography – mass spectrometry (LC-MS) to quantify exposure to specific polyisocyanates (Karlsson *et al.* 1998; Vangronsveld and Mandel 2003). The specificity and sensitivity of LC-MS analysis provides exposure assessors with a tool to examine low levels of individual polyisocyanates. This is especially important in terms of correlating specific biomarkers or other endpoints to dermal and inhalation exposure indices.

1.4.2. AIR-SAMPLING METHODS

Measuring diisocyanates in air presents interesting sampling and analytical challenges. Diisocyanates can exist in air as vapor or aerosol. For example, HDI oligomers, with their low vapor pressures (*e.g.*, biuret ~ 4.7E-7 mmHg at 20 °C) will likely exist as aerosols in the

overspray, while the monomer with its higher vapor pressure (0.05 mmHg at 25 °C) may partially exist as vapor. Rando and Poovey (1999) observed that the HDI monomer was partitioned as approximately 80% vapor and 20% aerosol. Diisocyanates are also reactive with nucleophiles such as water, alcohols, and amines. Because of their reactivity, most air-sampling methods require immediate derivatization of collected diisocyanates. Most of the derivatizing agents are amines that react with diisocyanates to form chemically stable ureas.

In general, air sampling is performed by drawing workplace air through an impinger containing derivatizing solution or filter media impregnated with derivatizing agent. Impingers are efficient at collecting vapor and aerosols larger than 2 µm in diameter (Spanne *et al.* 1999); however, impingers are not practical for personal sampling as they are fragile and prone to spilling. Consequently, most exposure assessors use filter media to measure BZCs of diisocyanates.

Filter sampling may be performed using single-stage or dual-stage cassettes. Typically with dual-stage sampling for diisocyanates, the first stage is loaded with a polytetrafluoroethylene (PTFE) filter designed to collect aerosols and the second stage is loaded with an impregnated glass-fiber filter (GFF) designed to collect and derivatize vapor. 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 diisocyanates collected on the PTFE filter polymerize over time. This is especially likely when fast curing clearcoat is being sprayed. With single-stage sampling, a PTFE filter is not used. As a result, the impregnated GFF collects and derivatizes all phases of diisocyanates.

Both dual-stage and single-stage cassettes have been used in occupational sampling. The most common single-stage sampling method is the commercially available Occupational

Safety and Health Administration (OSHA) 42 cassette (OSHA 1983), which uses a GFF impregnated with 1-(2-pyridyl)piperazine. The most common dual-stage sampling method is the commercially available ISO-CHEK® sampler (Omega Specialty Instrument Co., Houston, TX), which employs a PTFE pre-filter and a GFF post-filter impregnated with 9-(*N*-methylaminomethyl)anthracene. While the OSHA 42 is designed to sample and quantify HDI monomer, the ISO-CHEK is capable of sampling and quantifying HDI polymers (*i.e.*, TRIG) as well as HDI monomer. 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 diisocyanates.

The ISO-CHEK sampler has performed well in occupational settings at measuring total HDI monomer and oligomers. For example, England *et al.* (2000) compared several of the most common air-sampling methods inside a paint booth during spraying and found that the ISO-CHEK collected significantly greater amounts of polymeric HDI (*i.e.*, TRIG) than did the impinger methods (*i.e.*, NIOSH 5521, NIOSH 5522, and proposed NIOSH 5525) and that all the methods, including OSHA 42, collected similar amounts of monomeric HDI. More recently, Ekman *et al.* (2002) investigated the performance of filter and impinger samplers that used the same derivatizing agent [1-(2-methoxyphenyl)piperazine] to quantify total isocyanates under a simulated spray-painting environment and found no significant difference ($\alpha = 0.05$) between single-stage filter sampling and impinger sampling.

1.4.3. DERMAL SAMPLING METHODS

Despite the high probability for dermal exposure in the automotive refinishing industry, the extent of dermal exposure to HDI monomer and oligomers has not been adequately

investigated in exposed workers, mainly due to insufficient quantitative methods for assessing dermal exposure. The most common dermal sampling methods can be classified into one of three groups: (1) surrogate skin techniques where patches, gloves, or whole-body suits are employed as collection media; (2) removal techniques where substances deposited on the skin are removed by washing or wiping; and (3) fluorescent tracer techniques where ultraviolet fluorescence is added to the chemical of interest and then detected on the skin using an imaging system (Fenske 1993).

The former two techniques have been used to measure dermal exposure to HDI in the automotive refinishing industry. Pronk *et al.* (2006b) used gloves to estimate exposure loading to the skin; Liu *et al.* (2000) used colorimetric wipes to qualitatively determine exposure on the skin; and recently, Bello *et al.* (2008) used wipe sampling to quantify isocyanates on the skin. These techniques provide valuable information on the amount of chemical present on the skin at the moment of sampling, but they fail to provide insight regarding the penetration of the chemical into the stratum corneum.

Recently, attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectrometry was used to measure dermal exposure to diisocyanate monomers and oligomers in the first few layers of the stratum corneum of guinea pig skin (Bello *et al.* 2006). The primary advantage of this technique is that it can provide real-time measurements of unbound diisocyanate exposure in the skin; the primary disadvantage of this technique is that it is relatively complex and requires expensive equipment in the field, which is also the main disadvantage of fluorescent-tracer techniques. This is probably why neither of these techniques has been used to measure dermal exposure to diisocyanates in workers.

Tape-strip sampling, on the other hand, is a relatively inexpensive, simple, and non-invasive method for measuring chemical exposure in the skin. Chemical penetration at different depths in the skin has been estimated using adhesive tape-strips to remove layers of stratum corneum for determination of chemical concentrations in the cell layers. For example, tape-strip sampling has been used to quantify dermal exposure to multifunctional acrylates (Nylander-French 2000), chemical components of jet fuel (*e.g.*, naphthalene) (Chao *et al.* 2005; Kim *et al.* 2006a; Kim *et al.* 2006b), and HDI monomer (Fent *et al.* 2006). Because allergenic compounds like HDI may trigger an immunological respiratory response through dermal absorption (Herrick *et al.* 2002; Karol *et al.* 1981; Rattray *et al.* 1994), it is important that dermal assays quantify the amount of HDI that has penetrated into the skin.

1.5. MODELING DIISOCYANATE EXPOSURES

1.5.1. OVERVIEW OF EXPOSURE MODELING

Exposure modeling is the process of constructing a representation of the underlying processes leading to exposure. In occupational studies, models can be used to aid in the understanding of exposure pathways, identify the primary determinants of different exposures, test the effectiveness of control interventions, explore exposure-biomarker relationships, and can even be used to predict exposures (*i.e.*, exposure reconstruction). Generally, three types of mathematical models are used for occupational exposure modeling: (1) deterministic models, (2) stochastic models, and (3) statistical models.

Deterministic models contain no random (stochastic) components; consequently, each component and input is determined exactly. Stochastic models, on the other hand, recognize that there could be a range of possible outcomes for a given set of inputs, and expresses the

likelihood of each one happening as a probability (Vogal 1999). A statistical model is a type of stochastic model and can be defined as a parameterized set of probability distributions. Although causality cannot be established by statistical analyses, associations among variables can be quantified. Statistical models take into account uncertainty by means of measurement error and individual variability and can predict an outcome based on a set of variables and associate a measure of variability with that prediction (Kleinbaum *et al.* 1998).

Both deterministic and stochastic (statistical) models have been used to describe and understand the processes leading to inhalation and dermal exposure to diisocyanates in the automotive refinishing industry. However, these models do have several limitations that need to be addressed.

1.5.2. INHALATION EXPOSURE MODELING

Flynn *et al.* (1999) developed a deterministic model for predicting BZCs of total aerosol mass during compressed-air spray-painting. The primary parameters of this model were generation rate, momentum flux of air from the gun, momentum flux of air to the worker's body, and worker orientation. The momentum flux ratio and worker orientation were found to be good predictors of the exposure in a controlled scale model wind-tunnel experiment. However, this model was limited in scope as it considered only cross-flow ventilation under high velocities. Furthermore, this study did not measure diisocyanates, which, due to their reactive nature, may behave differently than total aerosol mass.

Woskie *et al.* (2004) presented a statistical model for identifying the main determinants of polyisocyanate BZC during automotive spray-painting. Measured polyisocyanates included HDI monomer, total HDI-based polyisocyanates, and total IPDI-based

polyisocyanates; however, TRIG was used as the metric for the regression modeling. According to this model, the main determinants of BZC were volume of isocyanates applied, gallons of clear coat used per month, and type of paint booth where painting was performed.

There are several limitations to this study. This model was only able to describe 39% of the variability in the BZC. Also, multivariate regression modeling was used instead of mixed (multiple) regression modeling even though repeated measurements were performed on the workers. Mixed modeling may be a more appropriate approach since serial correlation is likely with repeated measurements. In addition, investigators decided to use TRIG rather than individual polyisocyanate species as the exposure metric and did not consider air velocity in the booth as a possible predictor. Air velocity is likely to be a major factor governing BZC.

1.5.3. DERMAL EXPOSURE MODELING

To our knowledge, no one has developed a statistical model for predicting dermal exposure to diisocyanates, most likely due to the lack of quantitative dermal exposure data. However, Flynn *et al.* (2006) developed a deterministic model for predicting dermal exposure to HDI resulting from automotive paint aerosol deposition on human forearm hair. Although this model tended to under-predict dermal exposure to unprotected arms, it demonstrated the potential for modeling exposures using variables collected in the field (*e.g.*, air velocity in the paint booth, air concentration, *etc.*) and the importance of quantitative monitoring (*i.e.*, tape-strip sampling) for model validation. A limitation of this model is that it did not consider exposure to polymeric HDI, which is likely to be more prevalent in the painting atmosphere than monomeric HDI.

Brouwer *et al.* (2001) developed a deterministic model for predicting dermal exposure to paint overspray (which did not contain diisocyanates). The primary parameters of this model were overspray mass generation rate, transmission of overspray, and aerosol deposition efficiency. The performance of this model was evaluated in the occupational setting by comparing dermal exposure predictions to actual levels in spray-painters. Dermal exposure was measured using a fluorescent tracer technique. The predicted levels of exposure showed reasonable rank correlation with the measured exposure, although the model tended to over-predict the actual level of exposure. This model was developed and evaluated for airless spray-painting and so may not apply directly to compressed-air spray-painting in the automotive refinishing industry. The other limitation of the model is that it did not consider exposure to diisocyanates. Diisocyanates may have different characteristics than general paint-aerosols due to their reactivity.

1.6. STUDY OBJECTIVES

Strides have been taken to protect automotive painters from inhalation exposures by reducing levels of HDI monomer in the hardener and requiring certain engineering controls (*i.e.*, paint booths) and personal protective equipment (*i.e.*, respirators) during spraying. Despite these efforts, inhalation and dermal exposures to monomeric and polymeric HDI are likely to occur in the spray-painting environment. Uptake of HDI monomer, uretidone, biuret, and isocyanurate through the skin is likely to vary due to their differences in reactivity, volatility, solubility, and molecular weight. Because the skin has active metabolic and immunological properties, the residence time of the different polyisocyanates in the skin may affect how

they are processed by the body, thereby leading to different levels of toxicity and even different health effects.

Modeling is an important step in understanding the underlying processes governing exposure. However, exposure modeling efforts have been limited by the lack of specific and sensitive methods for measuring and quantifying both dermal and inhalation exposures to HDI-based polyisocyanates. A complete and thorough characterization of painters' exposure (including in-depth statistical analyses) is necessary to achieve a better understanding of the fate and transport of polyisocyanates in the working environment and human body. The study objectives are as follows:

1. To develop unified methodology to measure HDI and its oligomers in the atmosphere, on the skin, and in bulk material and to use this methodology to characterize spray-painters' exposures in the automotive refinishing industry.
2. To develop a statistical model that uses the concentrations of the polyisocyanates in paint (*a priori*) as well as other workplace factors to describe the variability in the airborne concentrations of the polyisocyanates, to use this model to identify the primary determinants of inhalation exposure, and to explore the exposure pathways of the different polyisocyanates.
3. To develop a statistical model that uses the concentrations of the polyisocyanates in air (*a priori*) as well as other workplace factors to describe the variability in the dermal exposure levels of the polyisocyanates, to use this model to identify the primary determinants of dermal exposure, and to explore dermal exposure levels among the different polyisocyanates and exposed body parts.

CHAPTER 2

QUANTITATIVE MONITORING OF DERMAL AND INHALATION EXPOSURE TO 1,6-HEXAMETHYLENE DIISOCYANATE MONOMER AND OLIGOMERS

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2.1. ABSTRACT

Respiratory sensitization and occupational asthma are associated with exposure to 1,6-hexamethylene diisocyanate (HDI) in both monomeric and oligomeric forms. The monomer and polymers of diisocyanates differ significantly in their rates of absorption into tissue and their toxicity, and hence may differ in their contribution to sensitization. We have developed and evaluated a liquid chromatography – mass spectrometry (LC-MS) method capable of quantifying HDI and its oligomers (uretidone, biuret, and isocyanurate) in air, tape-stripped skin, and paint samples collected in the automotive refinishing industry. To generate analytical standards, urea derivatives of HDI, biuret, and isocyanurate were synthesized by reaction with 1-(2-methoxyphenyl)piperazine and purified. The urea derivatives were shown to degrade on average by less than 2% per week at –20 °C over a 2-month period in occupational samples. The average recovery of HDI and its oligomers from tape was 100% and the limits of detection were 2 and 8 fmol/µl, respectively. Exposure assessments were

performed on 13 automotive spray-painters to evaluate the LC-MS method and the sampling methods under field conditions. Isocyanurate was the most abundant component measured in paint tasks, with median air and skin concentrations of 2.4 mg/m^3 and 4.6 $\mu g/mm^3$, respectively. Log-transformed concentrations of HDI ($r = 0.79$, $P < 0.0001$) and of isocyanurate ($r = 0.71$, $P < 0.0001$) in the skin of workers were correlated with the log-transformed product of air concentration and painting time. The other polyisocyanates were detected on skin for less than 25% of the paint tasks. This LC-MS method provides a valuable tool to investigate inhalation and dermal exposures to specific polyisocyanates and to explore relative differences in the exposure pathways.

2.2. INTRODUCTION

Exposure to the monomeric and polymeric forms of 1,6-hexamethylene diisocyanate (HDI) may cause adverse health effects, specifically to the respiratory tract and skin. A number of studies describe occupational asthma (Belin *et al.* 1981; Malo *et al.* 1983; Piirila *et al.* 2000) and allergic contact dermatitis (Morgan and Haworth 2003; Wilkinson *et al.* 1991) associated with HDI exposure. Although the mechanistic pathway is unknown, there is increasing toxicological and epidemiological evidence that dermal exposure to diisocyanates plays a role in the development of respiratory sensitization and occupational asthma (Bello *et al.* 2007). Automotive paints based on HDI commonly include, in addition to the monomer, the HDI oligomers: uretidone, biuret, and isocyanurate.

Dermal (Fent *et al.* 2006; Flynn *et al.* 2006; Liu *et al.* 2007; Pronk *et al.* 2006b) and inhalation (Janko *et al.* 1992; Lesage *et al.* 1992; Pronk *et al.* 2006a; Rudzinski *et al.* 1995; Sparer *et al.* 2004) exposures to monomeric and polymeric HDI have been characterized in

spray-painters in the automotive repair industry. Methods have been published that use liquid chromatography – mass spectrometry (LC-MS) to quantify exposure to HDI oligomers (*i.e.*, biuret and isocyanurate) (Karlsson *et al.* 1998; Marand *et al.* 2005). However, these methods lack pure analytical standards for the oligomers. The most recent method (Marand *et al.* 2005) uses chemiluminescence nitrogen detection to characterize reference solutions for use as analytical standards. Although preparation of pure analytical standards is not trivial, once generated, such pure analytical standards provide a simpler, more efficient way of quantifying specific HDI oligomers.

Recently, investigators have reported inhalation exposure to polyisocyanates as total reactive isocyanate groups (TRIG) (Bagon *et al.* 1984; Bello *et al.* 2002; Rando *et al.* 1995) rather than specific isocyanate species. Measuring TRIG, which is the sum of free isocyanate (NCO) groups found on all isocyanate species in a sample, would be appropriate if all polyisocyanates behaved the same. However, rates of absorption into tissue and toxicity may vary between monomeric and polymeric diisocyanates because of differences in their physical and chemical properties, including molecular weight, lipid solubility, and reactivity.

Despite evidence suggesting that polyisocyanates may differ in absorption into tissue and toxicity, to our knowledge, quantitative analysis has not been used for identification of polyisocyanate species for both inhalation and dermal exposures. Our objective for this study was to develop an analytical method capable of quantifying HDI and its oligomers in air-filter, dermal tape-strip, and paint samples collected in occupational exposure settings. To meet this objective, our previously published tape-strip-LC-MS method for quantitation of dermal exposure to HDI (Fent *et al.* 2006) was modified to also quantify dermal exposure to the most common HDI oligomers in hardener (using purified analytical standards) and was

adapted for the analysis of air and paint samples. The specificity of the analytical method we describe provides investigators with a tool to quantify exposure to individual monomeric and polymeric diisocyanates and to explore quantitative relationships between the different routes of exposure for characterization of toxicity and adverse health effects.

2.3. METHODS

2.3.1. SYNTHESIS OF STANDARDS

All chemicals used in this study were obtained from Sigma Aldrich (St. Louis, MO), unless otherwise specified. Desmodur N 3200 and N 3300 A (Bayer Material Science, Pittsburgh, PA) were used as the sources of biuret and isocyanurate, respectively. It is important to note that the Desmodur products (Bayer) are not pure (*i.e.*, < 85%) and often contain significant amounts of other polyisocyanates (Bello *et al.* 2004).

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

The urea derivatives of HDI (HDIU) and 1,8-octamethylene diisocyanate (ODIU) were synthesized according to the National Institute for Occupational Safety and Health (NIOSH) Method 5521(NIOSH 1994). As reported previously (Fent *et al.* 2006), synthesized HDIU and ODIU were > 98% pure based on their total ion (m/z 100 to 2000) chromatograms. The urea derivatives of biuret (BU) and isocyanurate (IU) were synthesized as described below.

2.3.1.1. Urea derivative of biuret (BU)

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

2.3.1.2. Urea derivative of isocyanurate (IU)

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

2.3.1.3. Purification of BU and IU

BU and IU were purified by high performance liquid chromatography (HPLC) using a Varian Vista series HPLC and an Alltech C-8 column (22 × 250 mm, 10 µm particle size) (Nicholasville, KY) with an octadecylsilica packed pre-column (37 – 53 µm particles) eluted with methanol (A) and water (B) at 3 ml/min. Solvent composition was 85% A during the first 3 min, increasing to 100% A at 50 min. The UV absorbance of the eluate was monitored at 254 nm (PerkinElmer LC-85B spectrophotometer, Waltham, MA). BU and IU were each dissolved in methanol to 10 mg/ml and injected manually, 1 ml at a time. The most intense peaks, corresponding to BU and IU, were collected in separate vials. After collection,

methanol was evaporated by heating to 50 °C under a gentle stream of nitrogen. The remaining water was lyophilized to obtain ~10 mg each of BU and IU as dry white powder. LC-MS analysis of the HPLC-purified BU and IU dissolved in methanol (20 pmol/µl) showed that the products were > 97% pure (**Figure 2.1**).

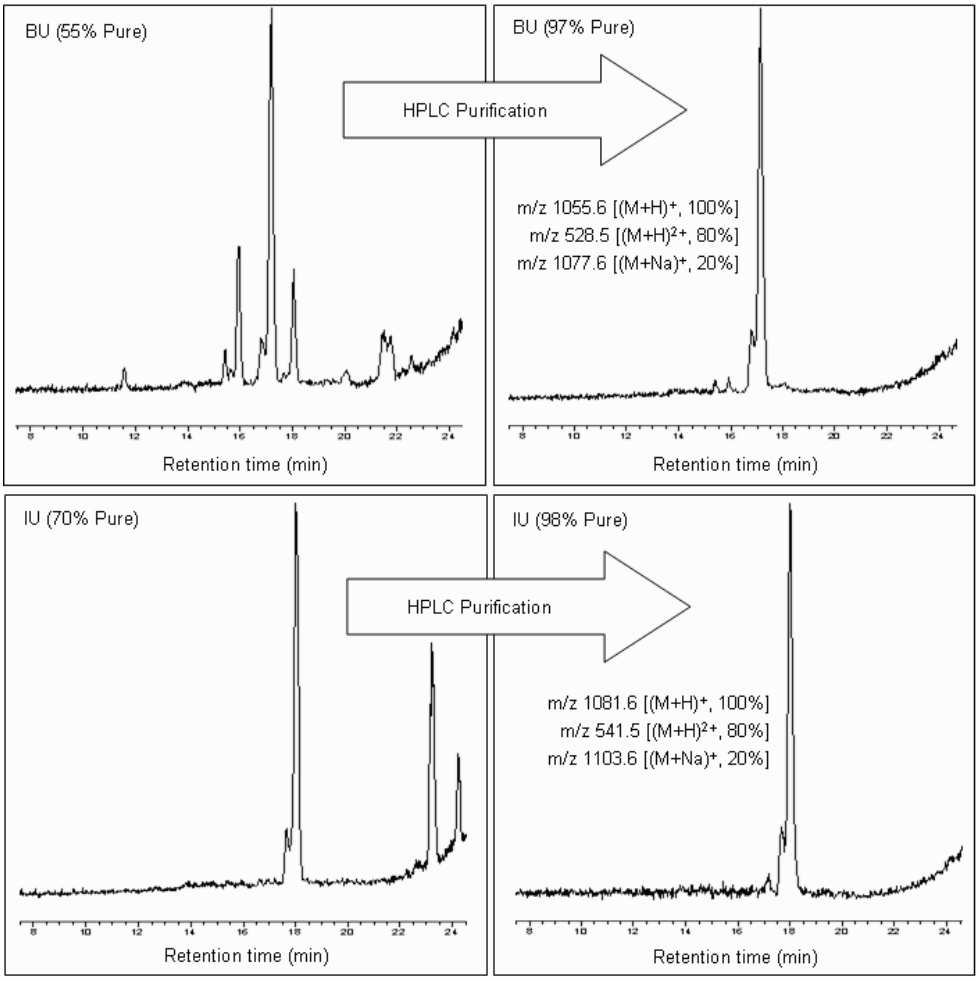

Figure 2.1. Total ion chromatograms (m/z 100-2000) from LC-MS analysis of 20 pmol/µl solutions of the synthesized urea derivatives of biuret (BU) and isocyanurate (IU) before and after HPLC purification.

2.3.2. PREPARATION OF STANDARD CURVES

The urea derivatives were dissolved in methanol to make stock solutions of each derivative (1 nmol/µl). Preliminary standards of each stock solution, except for ODIU, were prepared by diluting the stock solutions to 400, 200, 100, 20, 10, 4, 2, 0.4, 0.08, or 0.02 pmol/µl. Internal standard solution was made by diluting ODIU stock to 4 pmol/µl. The final standards were created by combining 200 µl of the internal standard solution with 200 µl each of the three preliminary standards for a total volume of 800 µl. Thus, the ten final standards were ¼ as concentrated as the preliminary standards, each with an internal standard concentration of 1 pmol/µl. Standard curves were generated by regressing the nominal concentration on the response ratio (*i.e.*, ratio of integrated analyte and internal standard peak) for each standard. The standards were analyzed in triplicate to account for instrument variability. The standard curve generated with the BU data was used to estimate concentrations of the urea derivative of uretidone (UU).

2.3.3. SAMPLE COLLECTION

2.3.3.1. Exposure monitoring in automotive spray-painters

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

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

Bulk samples of the clearcoat being sprayed by the painter were collected before each task. Samples (10 µl) of the mixed clearcoat were drawn into a 20 µl pipette and delivered to glass vials (I-Chem, New Castle, DE) filled with 15 ml of derivatizing solution. The pipette tip was also ejected into the solution to eliminate side-wall losses due to the viscosity of the clearcoat.

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

Tape-strip sampling was performed immediately after each task using a Cover-Roll® adhesive tape (Beiersdorf AG, Hamburg, Germany) cut into 4 × 2.5 cm^2 strips. Three successive tape-strips were collected on the dorsal side of each hand and on the dorsal and

volar side of each arm as described elsewhere (Fent *et al.* 2006). In order to prevent cross contamination, forceps cleaned with acetone were used to apply and remove the tape-strips and place them in 8 ml glass vials (Kimble, Vineland, NJ) containing 5 ml of derivatizing solution. For quality control, tape blanks were collected prior to paint application, which included tape-strip samples of each worker's arm (sample blanks) and samples of unused tape (field blanks).

2.3.3.2. Recovery of polyisocyanates from tape samples

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

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

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

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

2.3.4. SAMPLE PROCESSING

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

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

2.3.5. LC-MS ANALYSIS

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

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

2.3.6. STORAGE STABILITY

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

2.3.7. DATA ANALYSIS

The data were analyzed using SAS 9.1 statistical software (Cary, NC). Air, tape-strip, and paint samples containing levels of polyisocyanates below the limits of quantitation (LOQ) and detection (LOD) were assigned values determined by dividing the respective limits by the square root of two (Hornung and Reed 1990). Polyisocyanates collected with three successive tape-strips were summed together to estimate the dermal exposure to each sampled site of skin. However, subsequent tape-strips were excluded if the previous tape-strip collected levels below the LOD. These site-specific levels were averaged to determine the mean dermal exposure level for each task. Each tape-strip removes approximately one layer of corneocytes and any chemicals in that cell layer (Schwindt *et al.* 1998). According to Marks *et al.* (1981), corneocytes average 0.66 µm in thickness. Given the uncertainty and variability associated with tape-stripping, we assumed that triplicate tape-stripping would

collect approximately 1 mm^3 of skin (10 cm^2 area × 1 µm thickness). Thus, dermal exposure was reported as a concentration in the skin (ng/mm^3). Shapiro-Wilks tests for normality indicated that the dermal concentration data and the product of air concentration and paint time data were approximately log-normal for HDI (W = 0.81, 0.97, respectively) and isocyanurate (W = 0.95, 0.93, respectively). Therefore, regression analysis was performed on the natural log-transformed data.

2.4. RESULTS

2.4.1. PERFORMANCE OF THE ANALYTICAL METHOD

Different weighting factors ($w = x^{-1}, x^{-2}, y^{-1}, y^{-2}$) were evaluated for fitting standard curves. As specified in the literature (Almeida *et al.* 2002), the weighting factor that gave the smallest sum of the absolute relative error as a percentage of the nominal concentration was used for fitting the standard curve. The linear range of the standard curve was 0.005 to 1 pmol/µl for HDIU ($w = x^{-2}$, $R^2 = 0.968$) and 0.02 to 5 pmol/µl for BU ($w = y^{-2}$, $R^2 = 0.999$) and IU ($w = y^{-2}$, $R^2 = 0.992$). However, after analyzing samples, it was found that 47% of air samples and 94% of paint samples contained levels of isocyanurate greater than 5 pmol/µl. Levels of the other polyisocyanates in those samples were well within the dynamic range. To extend the upper limit of quantitation to 100 pmol/µl, the IU data were fit using a third order polynomial equation ($w = y^{-2}$, $R^2 = 0.997$). Polynomial fitting has proven useful for analyzing mixtures of highly variable compounds (Reilly *et al.* 2001). Thus, the polynomial curve was used to quantify isocyanurate in all occupational samples. None of the samples contained concentrations of isocyanurate exceeding 100 pmol/µl.

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

Analyzing three sets of quality control standards representing the low, middle, and high concentrations of the linear calibration curves allowed the evaluation of the precision and accuracy of the LC-MS assay. A set of isocyanurate standards representing the upper limit of the polynomial calibration curve was also used for the evaluation. Each set of quality control standards contained three replicates. In addition to the intra-day variation, analysis was performed one week later to evaluate the inter-day variation. The results are given in **Table 2.1**. The average quantified levels were within ±13% of the nominal concentrations for all the analyzed standards, each with a relative standard deviation less than 7%.

The LC-MS method was able to separate each of the HDI-based polyisocyanates in occupational samples. Tape samples are expected to provide the most complex matrix for analysis due to the presence of dissolved tape adhesive and skin components. **Figure 2.2** presents chromatograms from the LC-MS analysis of a tape sample, showing symmetrical peaks for each of the polyisocyanates of interest with no interfering peaks.

Table 2.1. Intra- and inter-assay accuracy and precision [relative standard deviation (RSD)] for quality control standards of HDI, biuret, and isocyanurate.

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

2.4.2. RECOVERY OF POLYISOCYANATES FROM TAPE SAMPLES

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

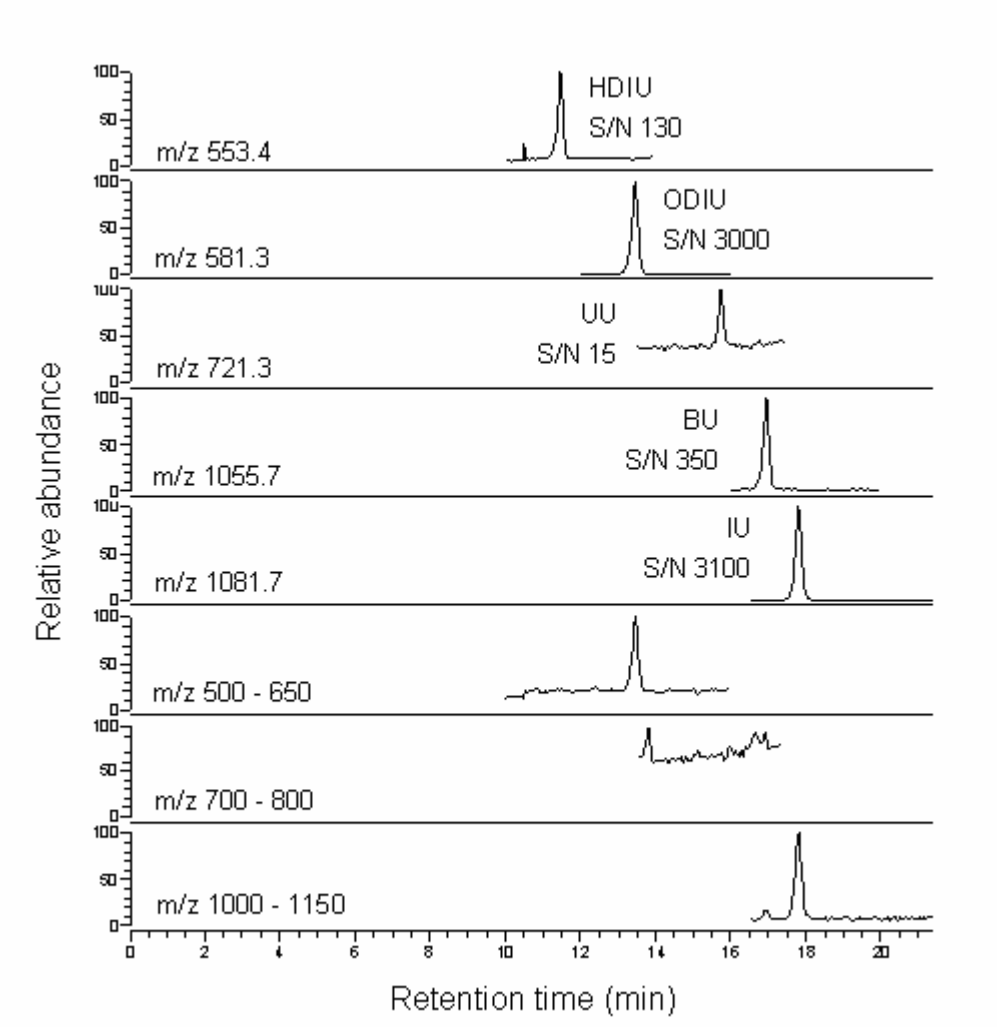

Figure 2.2. Chromatograms from LC-MS analysis of a tape sample collected from the arm of an automobile painter who did not wear protective clothing during paint application. Four different polyisocyanates were collected from the skin and quantitated as the urea derivatives of hexamethylene diisocyanate (HDIU), uretidone (UU), biuret (BU), and isocyanurate (IU). The urea derivative of octamethylene diisocyanate (ODIU) was added for the internal standard.

2.4.3. STABILITY OF DERIVATIZED POLYISOCYANATES IN TAPE SAMPLES

The tape samples used for the storage stability analysis did not contain uretidone, but did contain the other polyisocyanates of interest. Degradation was linear for all the urea derivatives of polyisocyanates tested. All three urea derivatives degraded at 6 – 7% per week at 4 °C. Degradation was less at lower temperature, to 2% per week for HDIU and IU, whereas BU losses were minimal at –40 ºC (**Table 2.2**) over a 2-month period.

Table 2.2. Degradation rates of urea derivatives of polyisocyanates collected from painter skin, stored at different temperatures over a two-month period.

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

[a.] Polyisocyanates were collected from painter's skin using tape-strips. Four tape-strips were derivatized, split into 3 storage groups, and then analyzed with LC-MS on a bimonthly basis.

[b.] Linear regression was used to estimate the percent change in concentration over time.

2.4.4. EXPOSURE MONITORING OF AUTOMOTIVE SPRAY-PAINTERS

Exposure assessments were carried out on workers performing 35 different paint tasks. **Table 2.3** presents a summary of the exposure-assessment results. Distributions of the exposure data are positively skewed. Thus, median values are the best measure of central tendency. Detectable levels of HDI and isocyanurate were found on the skin for 71 and 100% of the tasks, respectively. The other polyisocyanates were detected on skin for less

than 25% of the tasks. Therefore, statistical analysis was confined to the HDI and isocyanurate exposure data exclusively.

A relationship was expected between dermal concentration and the product of breathing-zone concentration (intensity of overspray surrounding the painter) and paint time (duration of time in which overspray can deposit on the skin). Log-transformed dermal concentration correlated with the log-transformed product of breathing-zone concentration and paint time for HDI ($r = 0.79$, SE = 0.94, $P < 0.0001$, **Figure 2.3.A**) and isocyanurate ($r = 0.71$, SE = 1.14, $P < 0.0001$, **Figure 2.3.B**), respectively. A test for coincident lines ($\alpha = 0.05$) revealed that the two lines in Figure 4 do not have significantly different slopes ($P = 0.580$), but do have significantly different intercepts ($P < 0.0001$).

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

Table 2.3. Assessments of exposure to HDI and related oligomers conducted on automotive spray-painters.[a]

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

a. Samples were obtained from 13 workers performing 35 separate paint tasks. One air sample was excluded due to pump malfunction and one paint sample was lost in transport.

b. Levels below the limits of detection and quantitation were assigned values by dividing the respective limits by $\sqrt{2}$.

c. Levels below the limit of detection (non-detects) are represented by the symbol “nd”

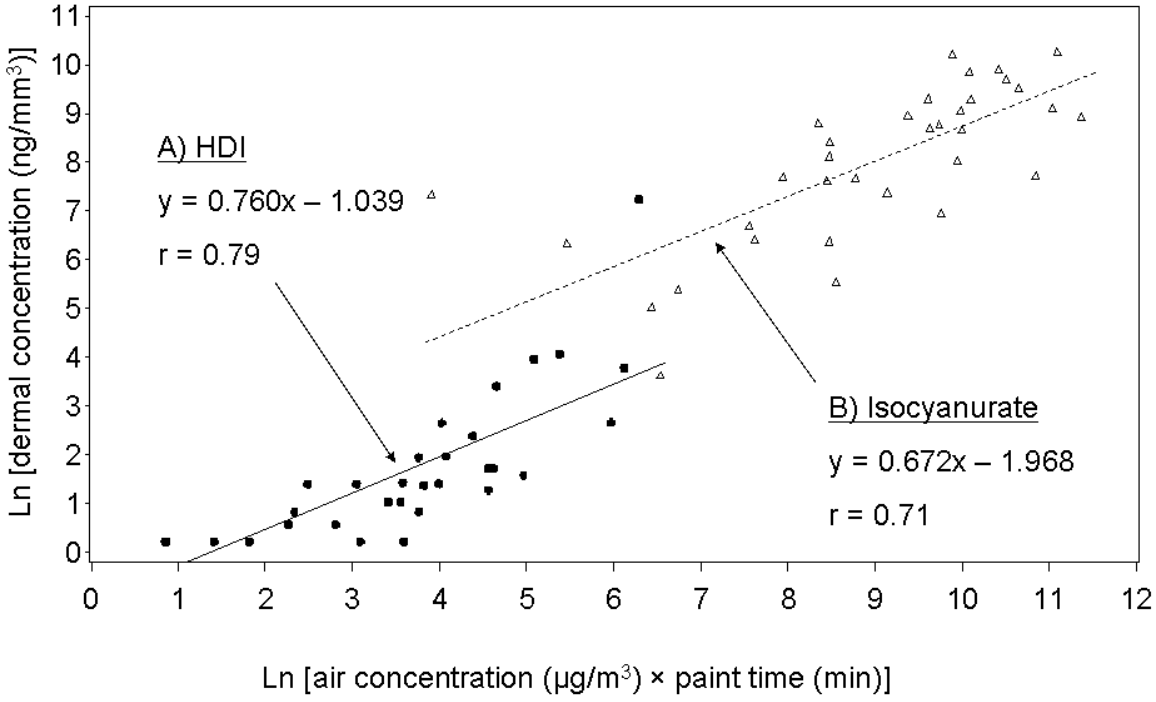

Figure 2.3. Regression of log-transformed dermal concentration of (A) HDI and (B) isocyanurate on the log-transformed product of the respective air concentration and paint time for workers not wearing protective clothing or gloves.

2.5. DISCUSSION

The analytical method described in this study is specific (employing SIM to identify individual monomeric and polymeric HDI) and sensitive (capable of detecting trace amounts of diisocyanates in different media). By synthesizing and purifying urea derivatives of biuret and isocyanurate for use as standards, we were able to confidently quantify the mass of individual polyisocyanates. The response ratios were linear over 2.3 orders of magnitude for each polyisocyanate, while a third-order polynomial equation was able to explain the response ratio for isocyanurate over 3.7 orders of magnitude. These dynamic ranges cover 100% of the levels in the occupational samples collected in this study. The linear and polynomial calibration curves were used to quantify levels of each polyisocyanate in quality control standards, demonstrating high precision and accuracy and consistency over a one-week time period.

The two-stage air-sampling method used in this study is similar in design to the commercially available ISO-CHEK® method. The ISO-CHEK® method has been shown to perform similarly to other commonly used air-sampling methods (*i.e.*, NIOSH 5521) during automotive spray-painting operations (England *et al.* 2000). One advantage of a two-stage sampler is that it attempts to separate the vapor and aerosol portion of monomeric diisocyanates (*i.e.*, HDI). The fraction of HDI aerosol measured with the two-stage sampler averaged 57 ± 9.4%. In contrast, Rando and Poovey (1999) used denuder sampling in conjunction with impaction/filter sampling to estimate the aerosol fraction of HDI monomer in automotive paint overspray at 20%. These conflicting observations may reflect actual differences in sampled conditions or could be due to measurement bias.

Because of the uncertainty associated with measuring aerosol/vapor partitioning, the HDI air concentrations were reported as total HDI. The median breathing-zone concentrations of HDI, uretidone, biuret, and isocyanurate were 7.24, 5.06, 4.58, and 2,370 $\mu g/m^3$, respectively. Because the breathing-zone concentrations represent task-based (20 min or less) time-weighted averages (TWAs), short-term exposure limits (STELs) are appropriate for comparison. The sensitivity of the analytical method allows detection of HDI and its oligomers in air (sampling at 1 l/min for 15 min) at concentrations that are over 700 times lower than the NIOSH ceiling limit for HDI (140 $\mu g/m^3$) or the Oregon STEL for biuret and isocyanurate (1 mg/m^3). Oregon is the only government entity in the United States to promulgate an STEL for HDI-based polyisocyanates. The Oregon STEL was exceeded in 65% of the samples, with the highest isocyanurate air concentration (17,800 $\mu g/m^3$) being over 15 times greater than the recommended limit.

The workers in this study were protected by half-face respirators equipped with organic vapor cartridges. A recent study found that the average workplace protection factor for such respirators was 388 for polymeric HDI (Liu *et al.* 2006). Such protection would reduce the inhaled portion of the highest isocyanurate concentration to approximately 45 $\mu g/m^3$. This level of protection, however, can be achieved only when the respirator is worn and maintained properly, which is not always the case.

Likewise, personal protective clothing and gloves may be used to protect worker skin from exposure. Workers in this study, however, did not wear protective clothing or gloves. As a result, median dermal concentration levels of HDI, uretidone, biuret, and isocyanurate were 3.92, 9.51, 13.5, and 4,590 ng/mm^3, respectively. Log-transformed dermal concentration was correlated with the log-transformed product of breathing-zone

concentration and paint time for workers exposed to both isocyanurate and HDI. We did not find a significant correlation between dermal concentration and the product of paint concentration and paint time for HDI ($P = 0.0917$) or isocyanurate ($P = 0.308$). This underscores the important role played by factors other than the concentration in the paint, such as airflow in the booth and painter positioning, in determining both breathing-zone concentration and dermal concentration.

The regression models (Figure 2.3) demonstrate the potential for using the product of breathing-zone concentration and paint time as a predictor for dermal concentration in unprotected workers. The similar slopes of the regression lines suggest that the effect of air concentration and paint time on dermal concentration is the same for HDI and isocyanurate. However, because the regression lines have significantly different intercepts ($P < 0.0001$), one would expect lower dermal concentration levels for HDI than for isocyanurate (on the order of about 92%) at the same level of predictor. Assuming the regression lines describe actual differences in the exposure pathways, there are several possible explanations for these differences. Firstly, because HDI exists partially as vapor in overspray, HDI may supply less exposure to the skin than isocyanurate, which exists solely as aerosol in overspray. Secondly, once on the skin, body temperature and air currents may cause HDI, with its high vapor pressure (0.05 mm Hg at 25 °C), to evaporate off the skin. Lastly, HDI may absorb into the skin and/or react with macromolecules in the skin or with alcohols in the paint more rapidly than isocyanurate.

Determining the cause of the differences between predicted dermal concentrations of HDI and isocyanurate is complicated by the high vapor pressure of HDI. Oligomers of HDI, on the other hand, have relatively low vapor pressures. Thus, any differences between

predicted dermal exposure levels among HDI oligomers are likely due to different rates of skin absorption or chemical reactivity. According to Marzulli *et al.* (1981), compounds less than 400 amu are more likely than larger molecules to penetrate the skin. Thus, we would expect uretidone to penetrate the skin more rapidly than the other oligomers we measured. However, neither uretidone nor biuret was quantified in enough tape samples to perform regression modeling in this study.

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

CHAPTER 3

QUANTIFICATION AND STATISTICAL MODELING OF BREATHING-ZONE CONCENTRATIONS OF MONOMERIC AND POLYMERIC 1,6-HEXAMETHYLENE DIISOCYANATE

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3.1. ABSTRACT

We conducted a repeated exposure-assessment survey for task-based breathing-zone concentrations (BZCs) of monomeric and polymeric 1,6-hexamethylene diisocyanate (HDI) during spray-painting on 47 automotive spray-painters from North Carolina and Washington State. We report here the use of linear mixed modeling (LMM) to identify the primary determinants of the measured BZCs. Both one-stage ($N = 98$) and two-stage ($N = 198$) filter samplers were used to measure concentrations of HDI, uretidone, biuret, and isocyanurate. The geometric mean (GM) level of isocyanurate (1440 $\mu g/m^3$) was higher than all other analytes (*i.e.*, GM < 14 $\mu g/m^3$). The mixed models were unique to each analyte and included factors such as analyte-specific paint concentration, airflow in the paint booth, and sampler type. The effect of sampler type was corroborated by side-by-side one- and two-stage personal air sampling ($N = 16$). According to paired *t*-tests, significantly ($\alpha = 0.05$) higher

concentrations of HDI ($P = 0.0270$) and isocyanurate ($P = 0.0016$) were measured using one-stage samplers. Marginal R^2 statistics were calculated for each model; significant fixed effects were able to describe 28, 55, 58, and 21% of the variability in BZCs of HDI, uretidone, biuret, and isocyanurate, respectively. Mixed models developed in this study characterize the processes governing polyisocyanate BZCs and the data suggest that these processes may differ among the different polyisocyanates. In addition, the mixed models identify ways to reduce polyisocyanate BZCs and, hence, protect painters from potential adverse health effects.

3.2. INTRODUCTION

Automotive coatings such as primers, sealers, and clearcoats are often based on polyisocyanates of 1,6-hexamethylene diisocyanate (HDI). These formulations consist of trace amounts of HDI monomer and higher amounts of HDI oligomers (*e.g.*, uretidone, biuret, and isocyanurate) (Fent *et al.* 2008b; Janko *et al.* 1992; Sparer *et al.* 2004). During spray-painting, polyisocyanates react with polyols to form polyurethane. However, because this reaction is not immediate, overspray in the breathing-zone is likely to contain unreacted polyisocyanates. Diisocyanates are considered a major cause of occupational asthma (Bernstein 1996; Chan-Yeung and Malo 1995). Efforts undertaken in the automotive refinishing industry to protect workers from inhalation exposures include replacing semi-volatile diisocyanate monomers in the hardener with less volatile diisocyanate oligomers and prepolymers. In addition, workplace health and safety regulations require the use of ventilated booths and respirators during spray-painting (Pronk *et al.* 2006a; Sparer *et al.* 2004). Despite these efforts, painters may still inhale polyisocyanates because of the high

levels of diisocyanate oligomers in the painting atmosphere (Janko *et al.* 1992; Lesage *et al.* 1992; Pronk *et al.* 2006a; Rudzinski *et al.* 1995; Sparer *et al.* 2004). Inadequate protection from respirators due to improper fit, poor maintenance, or insufficient efficiency may also lead to inhalation exposure (Liu *et al.* 2006).

Differences in exposure pathways, biological uptakes, and toxicities among individual polyisocyanates may be expected due to differences in their reactivity, volatility, solubility, and molecular weight. Consequently, exposure assessments aiming to understand these differences should characterize exposures to individual polyisocyanates rather than total reactive isocyanate groups (TRIG). Mathematical modeling may then be used to characterize the processes that govern individual polyisocyanate exposures. An increase in our knowledge and understanding of exposure pathways will help inform strategies to evaluate control technologies and prevent adverse health effects within the occupational environment.

Several deterministic models have been developed for understanding exposures during compressed air spray-painting (Carlton and Flynn 1997a; Carlton and Flynn 1997c; Flynn *et al.* 1999). However, to our knowledge, only once (Woskie *et al.* 2004) have statistical methods (*i.e.*, multiple regression) been used to investigate the effects of general process-related variables (*i.e.*, shop size, cars painted per month, *etc*.) on air concentrations of TRIG. Greater insight may be achieved by using linear mixed modeling (LMM) (Laird and Ware 1982) to examine the effects of more specific process-related variables (*i.e.*, airflow in the paint booth, volume of the paint booth, *etc.*) and task-related variables (*i.e.*, paint concentration, paint time, *etc*.) on air concentrations of individual polyisocyanates. This approach also accounts for serial-correlation of repeated measures and estimating within- and between-worker variability.

The objectives of this study were (1) to measure breathing-zone concentrations (BZCs) of HDI monomer and oligomers (*i.e.*, uretidone, biuret, and isocyanurate) during automotive spray-painting using a previously published method (Fent *et al.* 2008b) and (2) to use worker and work environment information to predict exposures and, hence, identify the primary determinants of exposures. To achieve these goals, LMM was applied to evaluate the fixed effects of booth type and covariates upon BZCs of monomeric and polymeric HDI. This work enhances our understanding of the pathways leading to monomeric and polymeric HDI exposures during spray-painting and helps identify the most effective control interventions for reducing those exposures. Furthermore, these models may serve useful for future studies attempting to assign exposures to unsampled workers and/or studies exploring biological uptake and toxicity.

3.3. MATERIALS AND METHODS

3.3.1. RECRUITMENT OF PAINTERS

Automotive spray-painters in central North Carolina (NC) and the Puget Sound area of Washington State (WA) were recruited to participate in an exposure-assessment study, consisting of air sampling, dermal tape-strip sampling, and biological monitoring (*i.e.*, collection of blood and urine). Letters explaining the study, including potential hazards associated with study participation, were mailed to automotive repair shops in both geographical locations. After approximately two weeks, phone calls were made to the managers of each shop to gauge interest in participation. If both the manager and painter(s) expressed interest in the study, visits were made to the respective shops at which time the study was verbally explained and consent forms were provided to the manager and painter(s).

On the first exposure-assessment visit, the consent form was read to the study subjects and then signed by the participants prior to data collection. Signatures were obtained on subsequent visits if any changes were made to the consent form, but only after thorough explanation of those changes. A total of 15 painters in NC and 32 painters in WA participated in the study. In order to assess their exposures, painters were visited on three separate occasions over a one year period, with visits at least one month apart. Due to attrition, 14 of the 47 painters were visited twice and 6 painters were visited once.

3.3.2. DATA COLLECTION AND ANALYSIS

We attempted to sample exposures during each paint task in which diisocyanate-containing paint was applied (*e.g.*, primer, sealer, clearcoat, single-stage, *etc.*). The majority (92%) of the sampled paint tasks involved the application of clearcoat, which is expected to contain the highest levels of polyisocyanates (Sparer *et al.* 2004). Personal breathing-zone measurements of each paint task were made using one-stage sampling or two-stage sampling described elsewhere (Fent *et al.* 2008b). Both one-stage (*i.e.*, OSHA-42, OSHA 1983) and two-stage (*i.e.*, ISO-CHEK, Omega Specialty Instrument Co., Houston, TX) samplers are commonly used to monitor atmospheres containing diisocyanates (England *et al.* 2000). The two-stage samplers used in this study contained an untreated polytetrafluoroethylene pre-filter (designed to collect diisocyanate aerosols) and a glass-fiber filter impregnated with derivatizing agent (designed to collect and derivatize diisocyanate vapors). The one-stage samplers were identical to the two-stage samplers except that the pre-filter was not included in the cassettes. Two-stage sampling was performed primarily during the first and second visits while one-stage sampling or side-by-side one- and two-stage sampling was performed

primarily during the third visit. Directly following the completion of a paint task, one- and two-stage filters were processed by placing the filters in vials containing derivatizing solution.

More than one air sample was collected if the paint task took longer than 20-30 minutes to prevent overloading of the filters. Results were adjusted to time weighted averages (TWAs) over the painting time for each paint task. A total of 98 one-stage and 198 two-stage personal air samples were collected. Of these, 16 represented side-by-side sets of samples. Most painters only painted in one type of paint booth; however, 4 painters did paint in multiple paint booths. On average, 2.4 personal air samples were collected from each painter during a visit. More than one air sample was collected from all but 2 painters, both of whom painted inside crossdraft booths.

Data were collected on the painters and their work environments for use as potential covariates. Prior to each paint task, samples of the mixed paint were collected for polyisocyanate analysis as described elsewhere (Fent *et al.* 2008b). Airflow inside the paint booth was measured using a rotating vane anemometer (VelociCalc®, TSI, Shoreview, MN) at a perpendicular distance of 10 cm from the return duct. Data on location-specific outdoor relative humidity and temperature were retrieved from a historical database at www.wunderground.com. All paint booths were temperature controlled to approximately 24 – 27 °C. Making the assumption that the majority of painting took place during the hottest part of the day (12:00 pm – 4:00 pm), temperature during painting was estimated using the maximum outdoor temperature, unless the maximum outdoor temperature was less than 24 °C, in which case a temperature of 23.9 °C was assigned to the paint booth. **Table 3.1** summarizes all the variables that were considered in statistical analysis. The selection of

these variables was based on the general dilution equation (Burgess *et al.* 2004), which states that air concentration at time *t* can be approximated by:

$$C_t = \frac{KG}{Q}\left[1 - \exp\left(\frac{-Qt}{KV}\right)\right],$$

where *K* is a mixing factor, *G* is the contaminant generation rate, *Q* is the ventilation flow rate, and *V* is the volume in the paint booth. This model assumes the generation rate is constant and the contaminant is removed solely by the ventilation system (*i.e.*, ignores particle settling) (Burgess *et al.* 2004). Among the variables not directly represented in this conceptual model, gun type and analyte-specific paint concentration may explain variability in *G*, and booth type and booth enclosure may explain variability in *K*. Since polyisocyanates can react with moisture and/or polymerize in the atmosphere, temperature and relative humidity may explain variability in BZC. Further, because measured BZC is likely to vary from actual BZC (where actual BZC ~ C_t) due to differences in sampling efficiency (*i.e.*, filter breakthrough, polymerization on filters, *etc.*), sampler type and total time may explain variability in sampling efficiency.

Table 3.1. Summary of variables used to model concentrations of HDI, uretidone, biuret, and isocyanurate in the breathing-zone of automotive spray-painters.

Type	Name	Description	Range of values	Mean value	Median value
Classification	Booth type[a]	Type of ventilated paint booth	Downdraft, semi-downdraft, crossdraft	NA	NA
Continuous	Airflow	Airflow inside the paint booth (m^3/min)	0 - 469	221	238
	Booth volume	Volume of the paint booth (m^3)	55.3 - 684	101	95.2
	Experience	Experience spray-painting cars (yrs)	0.25 - 40	13.4	12
	Humidity	Average relative humidity (%)	39.0 - 96.0	72.6	74.0
	Paint concentration (HDI)[b]	Concentration of HDI in paint (mg/l)	0.72 - 1060	280	262
	Paint concentration (uretidone)[b]	Concentration of uretidone in paint (mg/l)	5.71 - 20,900	882	68.2
	Paint concentration (biuret)[b]	Concentration of biuret in paint (mg/l)	8.12 - 23,800	2050	838
	Paint concentration (isocyanurate)[b]	Concentration of isocyanurate in paint (mg/l)	8.56 - 357,000	95,800	94,400
	Paint time	Time spent inside the booth painting (min)	1.0 - 56.0	8.49	6.50
	Temperature	Estimate temperature during spraying (°C)	23.9 - 33.9	25.0	23.9
	Total time	Total operating time of the sampling pumps (min)	3.0 - 105	21.4	17.0
Dichotomous	Enclosure	Type of enclosure surrounding the paint booth	1: curtain 0: wall	0.063	0
	Gun type	Type of spray-gun used for applying paint	1: HVLP 0: conventional	0.92	1
	Paint type	Type of paint applied to the surface of the vehicle	1: clearcoat 0: other	0.92	1
	Sampler type	Type of sampler used to monitor air concentration	1: two-stage 0: one-stage	0.66	1

a. Because booth type is a character variable use for classification, mean and median values could not be calculated (NA = non-applicable).

b. HDI, uretidone, biuret, and isocyanurate were non-detectable in 3.1, 36, 17, and 1.0% of all paint samples, respectively. Non-detectable and non-quantifiable levels of the different polyisocyanates in paint were assigned values by dividing the respective limits by $\sqrt{2}$. Thus, minimum values (*i.e.*, 0.72, 5.71, 8.12, and 8.56) represent assigned values for non-detectable levels.

3.3.3. STATISTICAL ANALYSIS

The data were analyzed using SAS 9.1 statistical software (Cary, NC). Paint concentrations of HDI and biuret were approximately normally distributed (Shapiro Wilks $W = 0.93$). Log transformations were made to paint concentrations of uretidone and biuret, as well as to air concentrations of each polyisocyanate to satisfy normality assumptions ($W > 0.85$) prior to statistical analysis. Concentrations below detection and quantitation limits were assigned values by dividing the respective limits by $\sqrt{2}$. The covariates were evaluated for potential collinearity by examining the Spearman correlation coefficients among pairs of covariates. Paint time and total time ($r = 0.78$) were the only variables to exceed our criterion for high correlation (*i.e.*, $r > 0.70$) and will only be included together in the models if there is evidence they describe separate variability.

LMM (SAS PROC MIXED) was used to investigate the relative influences of fixed effects representing booth type and covariates on BZCs of HDI, uretidone, biuret, and isocyanurate, while estimating within- and between-worker variability via the use of random effects. The general form of the model is provided below:

$$Y_{hij} = \ln(X_{hij}) = \mu_y + \alpha_h + \sum_{u=1}^{U} \delta_u C_{uhij} + \beta_{hi} + \varepsilon_{hij}$$

for $h = 1, 2,\ldots, H$ booth types, $i = 1, 2,\ldots, k_h$ painters using booth type h, $j = 1, 2,\ldots, n_i$ measurements from painter i in booth type h, and $u = 1, 2,\ldots, U$ covariates in booth type h, where

X_{hij} = polyisocyanate concentration of the j-th measurement of the i-th painter in the h-th booth type,

Y_{hij} = natural log-transformed value of X_{hij},

μ_y = intercept,

α_h = fixed effect for the *h*-th booth type,

C_{uhij} = covariates (or interaction of covariates) for the *j*-th measurement of the *i*-th painter in the *h*-th booth type,

δ_u = regression coefficients representing the fixed effects of the *u* covariates,

β_{hi} = random effect of the *i*-th painter in the *h*-th booth type; four painters had multiple random effects due to painting in more than one booth type, and

ε_{hij} = random error of the *j*-th measurement for the *i*-th painter in the *h*-th booth type.

It is assumed under this model that β_{hi} and ε_{hij} are mutually independent and normally distributed with means of zero and respective variances $\sigma^2_{B,h}$ and $\sigma^2_{W,h}$ representing the between and within-worker variance components for *h*-th booth type, where total variance $\sigma^2_{y,h} = \sigma^2_{B,h} + \sigma^2_{W,h}$ for the *h*-th booth type. It is also assumed that Y_{hij} is normally distributed with mean $\mu_{y,h} = (\mu_y + \alpha_h + \sum_{u=1}^{U} \delta_u C_{uhij})$ and variance $\sigma^2_{y,h}$.

The effect of pooling $\sigma^2_{W,h}$ or pooling both $\sigma^2_{B,h}$ and $\sigma^2_{W,h}$ was evaluated using likelihood ratio tests as described by Rappaport *et al.* (1999). According to these tests, $\sigma^2_{W,h}$ may be pooled among the various booth types for the analyte biuret. For the rest of the analytes, $\sigma^2_{B,h}$ and $\sigma^2_{W,h}$ are distinct for each booth type.

Candidate covariates were selected by running separate models that considered individual terms and the interaction terms between analyte-specific paint concentration and airflow. From these models, those variables with *P*-values of less than 0.15 were used to obtain final models. Final models were built using a backwards elimination procedure in which the least

significant variables ($P > 0.10$) were eliminated one-at-a-time. Insignificant main effects were always retained if their respective interaction terms were significant. To allow for separate parameter estimates for each booth type, interactions between the classification variable booth type and each of the significant variables were evaluated one-at-a-time and retained if the 95% confidence intervals of any two of the parameter estimates did not overlap. To assess model fit, transformed residuals and Malhalanobis distance were examined. These diagnostic measures did not identify excessive outliers or problematic observations.

Several R^2 statistics have been proposed for assessing the goodness-of-fit of fixed effects (Orelien and Edwards 2008; Xu 2003). Marginal R^2 statistics are more appropriate than conditional R^2 statistics for estimating explained variability from fixed effects because marginal R^2 statistics do not use random effects in the computation of predicted means that lead to residuals (Orelien and Edwards 2008). In this study, a marginal R^2 statistic proposed by Vonesh and Chinchilli (1997) was used. Orelien and Edwards (2008) found this statistic to perform extremely well at differentiating between full and reduced models and not diverging when models were over-fitted. This marginal R^2 statistic was calculated using simplified mixed-models that pooled the within- and between-worker variability among the different booth types and used two dichotomous variables for booth type to account for the fixed effect of booth type.

3.4. RESULTS

3.4.1. SUMMARY STATISTICS

A summary of BZCs measured in NC and WA is provided in **Table 3.2**. Because the exposure data were positively skewed, the measures of central tendency and scatter were best described using the geometric mean (GM) and geometric standard deviation (GSD), respectively. Although greater variability was observed in the NC samples, higher GM values were observed in the WA samples for all analytes except uretidone. According to two sample (Satterthwaite) *t*-tests of the log-transformed data, significant differences ($\alpha = 0.05$) were observed between the NC and WA measurements for HDI ($P = 0.0220$), biuret ($P < 0.0001$) and isocyanurate ($P = 0.0093$). It is important to note that location (NC *vs.* WA) was not a significant predictor in any of the final mixed models. Thus, differences in BZCs between NC and WA were adequately explained by the significant fixed effects.

Table 3.2. Breathing-zone concentrations ($\mu g/m^3$) of monomeric and polymeric HDI[a] for samples collected in North Carolina and Washington State.

Analyte	North Carolina (N = 88)[b]			Washington State (N = 200)[b]		
	GM[c]	GSD[d]	Range	GM[c]	GSD[d]	Range
HDI	3.16	5.16	0.14 - 179	5.00	3.97	0.06 - 65.5
Uretidone	5.42	8.41	0.48 - 1430	5.05	5.53	0.36 - 613
Biuret	5.58	6.36	0.68 - 7720	19.69	6.30	0.66 - 1020
Isocyanurate	953	6.11	5.04 - 17,800	1686	3.82	2.40 - 18,700

[a.] Non-detectable and non-quantifiable levels of the different polyisocyanates collected on air-filters were assigned values by dividing the respective limits of detection by $\sqrt{2}$.

[b.] Number of measurements representing the time weighted average recorded for each paint task.

[c.] Geometric mean.

[d.] Geometric standard deviation.

A summary of the air-sampling results by booth type, including restricted maximum likelihood (REML) estimates of the within- and between-worker variability, is presented in **Table 3.3**. Between-worker variability was greater than within-worker variability for BZCs of uretidone and biuret for all booth types combined. Similarly, greater between-worker variability than within-worker variability was observed in BZCs of all polyisocyanates measured in crossdraft booths. The GM levels of isocyanurate (1440 $\mu g/m^3$) were higher than all other analytes (*i.e.*, GM < 14 $\mu g/m^3$). For all the measured polyisocyanates, GM levels varied considerably among the different booth types with the lowest levels being observed in downdraft booths. Crossdraft booths had the highest GM levels of HDI, uretidone, and biuret, while the semi-downdraft booths had the highest GM level of isocyanurate. These differences may be due in part to differences in the airflows among the booth types as downdraft, semi-downdraft, and crossdraft booths had average airflows of 250, 190, and 102 m^3/min, respectively. However, after adjusting for airflow in the multivariate models, we observed, on average, higher BZCs in crossdraft or semi-downdraft booths than in downdraft booths for all the measured polyisocyanates (data not shown).

3.4.2. STATISTICAL MODELING

The mixed models developed for each analyte and booth type are described in **Table 3.4**. According to marginal R^2 statistics, significant fixed effects were able to describe an estimated 28, 55, 58, and 21% of the overall variability in the BZCs of HDI, uretidone, biuret, and isocyanurate, respectively. Analyte-specific paint concentration and airflow were the only variables that were significant in three or more of the models. For this reason, the effect of changing paint concentration and airflow was evaluated by comparing model

predictions where all other variables in the models were assigned median values (**Table 3.5**). These evaluations were performed using models specific to downdraft booths since these booths were the most commonly used booths in this study. As expected, the models predicted increasing BZCs with increasing paint concentrations and decreasing airflow. For example, doubling airflow from 200 m^3/min (just below the mean) to 400 m^3/min (just below the maximum) resulted in approximately 35% lower BZC predictions of HDI, biuret, and isocyanurate. However, given the same paint concentration (*e.g.*, 500 mg/l), the models predicted higher levels of isocyanurate (2,310 µg/m^3) than any of the other analytes (*e.g.*, uretidone = 22.2 µg/m^3).

Because sampler type was a significant predictor of BZC in the HDI and isocyanurate models, paired *t*-tests were conducted on the results of side-by-side one- and two-stage sampling ($N = 16$). In comparison to two-stage samplers, one-stage samplers measured significantly ($\alpha = 0.05$) higher levels of HDI (mean difference = 1.28 µg/m^3, $P = 0.0270$) and isocyanurate (mean difference = 739 µg/m^3, $P = 0.0016$). Insignificant differences between one- and two-stage samplers were observed for the analytes biuret (mean difference = 4.40 µg/m^3, $P = 0.0798$) and uretidone (mean difference = –34.0 µg/m^3, $P = 0.1327$).

Table 3.3. Breathing-zone concentrations ($\mu g/m^3$) of monomeric and polymeric HDI[a] by type of paint booth.

Analyte	Booth type	No. workers[b]	No. measurements[c]	No. non-detects	Summary statistics			REML[f] estimates (logged data)	
					GM[d]	GSD[e]	Range	Within-worker variance[g]	Between-worker variance
HDI	Downdraft	31	197	26	2.94	4.35	0.06 - 48.0	1.54	0.58
	Semi-downdraft	10	60	1	9.97	2.30	0.17 - 65.5	0.57	0.20
	Crossdraft	10	31	1	10.2	4.39	0.21 - 179	0.61	3.05
	All booths	47	288	28	4.31	4.39	0.06 - 179	1.27	0.96
Uretidone	Downdraft	31	197	146	3.86	5.64	0.46 - 1,430	1.93	0.92
	Semi-downdraft	10	60	18	9.7	6.11	0.94 - 613	0.51	4.49
	Crossdraft	10	31	13	10.3	8.76	0.36- 521	1.81	3.63
	All booths	47	288	177	5.16	6.30	0.36 - 1,430	1.61	1.92
Biuret	Downdraft	31	197	115	6.96	4.66	0.66 - 798	1.23	1.17
	Semi-downdraft	10	60	10	53.0	5.47	1.33 - 734	1.23	2.68
	Crossdraft	10	31	5	63.4	10.1	0.68 - 7,720	1.23	6.51
	All booths	47	288	130	13.5	6.89	0.66 - 7,720	1.25	2.85
Isocyanurate	Downdraft	31	197	2	1220	4.81	2.40 - 17,800	1.91	0.58
	Semi-downdraft	10	60	0	2190	2.08	269 - 8,920	0.29	0.36
	Crossdraft	10	31	1	1690	7.69	2.54 - 18,700	0.64	7.05
	All booths	47	288	3	1440	4.53	2.54 - 18,700	1.59	0.83

a. Non-detectable and non-quantifiable levels of the different polyisocyanates collected on air-filters were assigned values by dividing the respective limits by $\sqrt{2}$.

b. A total of four painters painted in more than one booth type; two painted in both crossdraft and semi-downdraft booths, one painted in both crossdraft and downdraft booths, and one painted in both semi-downdraft and downdraft booths.

c. Represents the time weighted average recorded for each paint task. Out of 296 measurements, 8 were excluded due to sampling pump malfunction.

d. Geometric mean.

e. Geometric standard deviation.

f. Restricted maximum likelihood.

g. Within worker variability was pooled among the various booth types for the biuret data as justified using likelihood ratio tests.

Table 3.4. Linear mixed models for predicting breathing-zone concentrations[a] of HDI, uretidone, biuret, and isocyanurate in automotive spray-painters.

Covariates[b]	HDI (R^2 = 0.28)[c]		Uretidone (R^2 = 0.55)[c]		Biuret (R^2 = 0.58)[c]		Isocyanurate (R^2 = 0.21)[c]	
	Parameter estimates (downdraft, semi-downdraft, crossdraft)[d]	*P*-values[e]	Parameter estimates (downdraft, semi-downdraft, crossdraft)[d]	*P*-values[e]	Parameter estimates (downdraft, semi-downdraft, crossdraft)[d]	*P*-values[e]	Parameter estimates (downdraft, semi-downdraft, crossdraft)[d]	*P*-values[e]
Intercept	(1.59, 2.34, 1.44)	0.131	(2.13, 3.97, 1.33)	0.3003	(0.194, 0.291, 3.56)	< 0.0001	(7.19, 7.70, 7.14)	0.0586
Paint concentration (mg/l)	(0.00166, 0.00240, 0.00532)	< 0.0001	(0.446, 0.264, 0.787)	< 0.0001	0.510	< 0.0001	0.000010	0.0007
Sampler type (1 = two-stage, 0 = one-stage)	-0.599	< 0.0001					-0.219	0.0891
Airflow (m^3/min)	-0.00193	0.0155			(0.00160, 0.00788, -0.0138)	< 0.0001	0.000833	0.5615
Temperature (°C)			-0.107	0.0047				
Paint concentration (mg/l) x airflow (m^3/min)					-0.00064	0.0461	-2.94E-08	0.0135
Experience (years painting)					-0.0516	< 0.0001	-0.0371	0.0039

a. N = 277 (11 of 288 observations were excluded due to missing covariate data).

b. Breathing-zone concentrations of all analytes and paint concentrations of dimer and biuret were log-transformed prior to statistical analysis.

c. Marginal R^2 statistics proposed by Vonesh and Chinchilli (1997).

d. Separate intercepts were determined for each booth type as specified in the mixed model. Separate covariate parameter estimates were provided for the different booth types if the 95% confidence intervals for any two of the parameter estimates did not overlap.

e. *P*-values are based on approximate F-tests of fixed effects.

Table 3.5. Effect of changing the analyte-specific paint concentrations[a] on predicted mean breathing-zone concentrations of each measured polyisocyanate[b] in downdraft booths.

Paint conc. (mg/l)	Predicted mean breathing-zone concentration (µg/m^3)			
	HDI	Uretidone	Biuret	Isocyanurate
10	4.40	3.88	4.48	2310
25	4.51	5.84	6.21	2310
50	4.70	8.0	7.96	2310
100	5.11	10.8	10.2	2311
250	6.55	16.3	14.2	2312
500	9.92	22.2	18.1	2313
1000	22.7	30.3	23.3	2317
2500		45.6	32.3	2327
5000		62.1	41.3	2345
10000		84.5	53.0	2380
25000				2490
50000				2684
100000				3119
250000				4893

a. Predictions were not made for paint concentrations exceeding the maximum measured paint concentrations of individual monomeric and polymeric HDI.

b. Linear mixed models (Table 3.4) specific to downdraft booths were used to predict mean breathing-zone concentrations of the logged exposure data ($\mu_{\ln}$). Median values (Table 3.1) were used for all other covariates in the models. Marginal R^2 statistics and total variance estimates ($\sigma^2_{\ln}$) for the analytes measured in downdraft booths were used to compute arithmetic means (μ_x) with the following formula:

$$\mu_x = \exp\left(\mu_{\ln} + \frac{\sigma^2_{\ln}(1-R^2)}{2}\right).$$

3.5. DISCUSSION

Personal air samples were collected from 47 automotive spray-painters in this study, thereby providing estimates of BZCs of monomeric and polymeric HDI in the automotive refinishing industry. Although isophorone diisocyanate (IPDI) may be an important constituent of some automotive coatings, we did not analyze for IPDI or its oligomers in this study. Using quantitative inhalation exposure and covariate data in LMM, we identified the primary determinants of BZCs of HDI, uretidone, biuret and isocyanurate. The mixed models developed in this study described more than half the variability in BZCs of uretidone and biuret ($R^2 > 50\%$) and lesser variability in BZCs of HDI and isocyanurate ($R^2 > 20\%$). Marginal R^2 statistics calculated for models specific to each booth type and analyte (data not shown) were highly variable, ranging from 0.08 for isocyanurate exposure in semi-downdraft booths to 0.74 for uretidone exposure in crossdraft booths. Low R^2 values (*i.e.*, < 0.20) may partially reflect the lack of between-worker variability in the respective exposure distributions (between-worker variability is generally easier to characterize than within-worker variability). Nevertheless, the large range of marginal R^2 values among the different booth types for analyte-specific models suggests that the processes governing BZCs are different for the different booth types. Thus, classification of the mixed models by analyte and booth type is appropriate.

Although unique models were built for each measured polyisocyanate, analyte-specific paint concentration and airflow were significant predictors in three or more of the models. In addition, the interaction between paint concentration and airflow was significant in the biuret and isocyanurate models, suggesting that the relationship between paint concentration and BZC depends on the airflow in the booth. Expectations are that higher analyte-specific paint

concentrations will lead to higher BZCs while increased airflow will lead to lower BZCs of each polyisocyanate. This was observed in the model predictions in which changing analyte-specific paint concentration and airflow were evaluated. Unexpectedly, using the same analyte-specific paint concentration, the models predicted higher BZCs of isocyanurate than any of the other analytes (Table 3.5).

It is possible that the isocyanurate model simply over-predicts lower levels of BZCs. In fact, significant differences between predicted means and actual values were observed when the actual values were below the 5% quantile (~ 50 $\mu g/m^3$), and it was evident that paint concentration below 2,500 mg/l had a negligible effect on isocyanurate BZC (Table 3.5). It is important to recognize, however, that the isocyanurate model performed well in terms of prediction (*i.e.*, 90% of the predictions within ± 2 scaled residuals) and, therefore, provides reasonable estimates of central tendency.

Another possibility is that the analysis of paint samples underestimated the true concentration of isocyanurate in the paint. The interquartile range of isocyanurate paint concentration was 45,000 to 135,000 mg/l, representing approximately 3.0 to 8.5% of the paint formulation. According to material safety data sheets (PPG 2006; PPG 2007a; PPG 2007b; PPG 2007c) of some of the most common hardeners used in the workplace and assuming a hardener to coating ratio of 4:1, the proportion of polymeric HDI is expected to range from 2.5 to 20%. Thus, measurements of isocyanurate in paint are within the expected range.

Assuming that the mixed models accurately represent conditions in the atmosphere, differences in reactivity could explain the differences in the predicted mean BZCs (Table 3.5). For example, HDI, uretidone, and biuret may polymerize more rapidly in the

atmosphere than isocyanurate. In addition, isocyanurate may be formed during the polymerization process as other polyisocyanates react with each other. The significant effect of temperature in the uretidone model may be indicative of increasing reactivity with increasing temperature. In fact, uretidone may be the most reactive polyisocyanate measured in this study due to the unstable structure of its four-member ring.

Reactivity of polyisocyanates is probably the reason why the effect of sampler-type was significant in the HDI and isocyanurate models. Two-stage samplers may underestimate BZCs of reactive polyisocyanates due to polymerization of polyisocyanates on the untreated pre-filter of the sampler. This problem may be avoided by using one-stage samplers onto which all polyisocyanates are simultaneously collected and derivatized on one filter. Thus, higher BZCs may be measured when one-stage samplers are used instead of two-stage samplers. The significant effect of sampler type was corroborated by paired two-sample *t*-tests comparing side-by-side sets of one- and two-stage samplers in which significant differences ($\alpha = 0.05$) were observed for the analytes HDI ($P = 0.0270$) and isocyanurate ($P = 0.0016$). Significant differences were not observed between one- and two-stage sampling for uretidone and biuret, demonstrating that individual polyisocyanates may differ in their reactivity. Further investigation is needed to evaluate the reactivity of the different polyisocyanates in the painting atmosphere and implications of this reactivity on human health endpoints such as tissue absorption and respiratory sensitization.

Experience (*i.e.*, years painting) was a significant variable in both the biuret and isocyanurate models in which more experience was associated with lesser exposure. Interestingly, in both models the effect of experience was greatest in crossdraft booths. Flynn *et al.* (1999) found that the painter orientation relative to the direction of the airflow

played a significant role in affecting BZCs in crossdraft booths. It is likely that more experienced painters received less exposure to biuret and isocyanurate because they produce less overspray or position their bodies to avoid overspray. Consequently, training automotive spray-painters on the best techniques for applying paint may help reduce personal exposures.

In comparison to our mixed models, Woskie *et al*. (2004) developed a multiple regression model to predict BZCs of TRIG. Significant covariates in this model included: volume of polyisocyanates applied, volume of clearcoat used per month, and type of paint booth. These general process-related variables described an estimated 39% of the variability in the BZCs of TRIG, which is within the range of variability (21 – 58%) described by our analyte-specific models. Because the models generated in our study used specific process- and task-related variables, it is difficult to compare our models to the model developed by Woskie *et al*. (2004). Nevertheless, our models may be more practical in terms of identifying practices and control technologies to reduce personal exposures.

In addition to statistical models, deterministic models have been used to understand exposures during spray-painting. Among the most notable in the literature is the model developed by Flynn *et al*. (1999) for predicting BZCs of general aerosols during spray-painting in crossdraft booths. In addition to painter orientation, the most important parameters of this model were generation rate, momentum flux of air from gun, and momentum flux of air to worker's body. Although these parameters were not directly measured in our study, generation rate may depend on the concentrations of polyisocyanates in paint, momentum flux of air from gun likely depends on the type of spray gun being used, and momentum flux of air to painter's body may depend on the airflow in the booth. These variables, except for gun type, were significant in three or more of the mixed models, and it

is probable that gun type would have been significant had there been more variability in gun type (*i.e.*, HVLP guns were used in 92% of the paint tasks). It is important to note, however, that airflow had a protective effect even in crossdraft booths. Thus, airflow in this study generally functioned to draw overspray away from the painter's body rather than towards the painter's body.

The BZCs reported in this paper (Table 3.2) represent task-based (generally 30 min or less) time-weighted averages (TWAs). Thus, ceiling limits or short-term exposure limits (STELs) are more appropriate for comparison than work-shift (*i.e.*, 8-hr TWA) exposure limits. The National Institute for Occupational Safety and Health (NIOSH) ceiling limit for HDI (*i.e.*, 140 $\mu g/m^3$) was exceeded only once (*i.e.*, 179 $\mu g/m^3$) during this exposure-assessment study. This is not surprising since HDI represented less than 1% of all polyisocyanates in the automotive paint. Oregon is the only government entity in the United States to promulgate a STEL for HDI-based polyisocyanates biuret and isocyanurate (*i.e.*, 1 mg/m^3). The BZCs measured in this study are not directly comparable to the Oregon STEL because they were not time-weighted over 15 min. Nevertheless, it is interesting to note that the Oregon STEL was exceeded by 71% of the task-based BZCs, with the highest isocyanurate BZC (18,700 $\mu g/m^3$) being over 18 times greater than the recommended limit.

In a 1980 to 1990 survey of Oregon automotive repair shops, Janko *et al.* (1992) measured a GM of 14 $\mu g/m^3$ for HDI and 1,600 $\mu g/m^3$ for HDI-based polyisocyanates, with respective peak concentrations of 340 and 18,400 $\mu g/m^3$. Similar levels of biuret and isocyanurate combined (GM = 1,380, peak = 18,700 $\mu g/m^3$) and lower levels of HDI (GM = 4.7, peak = 179 $\mu g/m^3$) were measured in our study. It is important to note that painters in this study were protected by respirators of various types (*i.e.*, half face, powered air

purifying, supplied air, *etc.*). Over 70% of the painters wore half-face respirators equipped with organic vapor cartridges. The Occupational Safety and Health Administration (OSHA) assigned protection factor for half-face respirators is 10 (OSHA 2006). After accounting for the OSHA protection factor (*i.e.*, dividing the BZCs by 10), we observed that more than 5% of the adjusted BZCs exceeded the Oregon STEL. Liu *et al.* (2006) found that the average workplace protection factor for half-face respirators equipped with organic vapor cartridges was 388 for polymeric HDI. Such protection would reduce the inhaled portion of the highest isocyanurate concentration to ~50 $\mu g/m^3$. Although well below the Oregon STEL, this level of exposure could still pose health risks to susceptible or sensitized individuals. This underscores the importance of reducing air concentrations inside the paint booths.

Based on the mixed models developed (Table 3.4), different approaches may be required to reduce airborne concentrations of different polyisocyanates. Two variables (*i.e.*, analyte-specific paint concentration and airflow) were common to three or more of the mixed models. According to the model predictions, reducing analyte-specific paint concentrations and/or increasing airflow results in lower BZCs of polyisocyanates. In addition, lower BZCs of all polyisocyanates were measured in downdraft booths than crossdraft or semi-downdraft booths, which is consistent with previous findings of particulate levels in paint booths (Heitbrink *et al.* 1995). Although painters and shop managers have limited control over polyisocyanate concentrations in the paint and the type of paint booth installed in the workplace, airflow inside the paint booth can be maximized by changing supply and return air-filters on a regular basis and ensuring that plastic sheeting and masking tape are not obstructing the return ducts. These simple acts of maintenance and prevention could have tremendous implications on the health and safety of automotive spray-painters.

The mixed models may provide a reasonable way of estimating worker exposure in retrospective studies where air-sampling data is lacking but where the other covariates can be adequately estimated. However, validation of these models is necessary to confirm their usefulness for exposure reconstruction. A significant finding from this study was the effect of sampler type on measured BZCs of HDI and isocyanurate. Because two-stage samplers appear to underestimate air concentrations of HDI and isocyanurate, investigators should carefully consider the type of sampler to use when designing an exposure-assessment study for reactive compounds like polyisocyanates.

CHAPTER 4

QUANTIFICATION AND STATISTICAL MODELING OF DERMAL EXPOSURE TO MONOMERIC AND POLYMERIC 1,6-HEXAMETHYLENE DIISOCYANATE

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4.1. ABSTRACT

We conducted a quantitative dermal and inhalation exposure assessment of monomeric and polymeric 1,6-hexamethylene diisocyanates (HDI) in 47 automotive spray-painters from North Carolina and Washington State. We report here the use of linear mixed modeling (LMM) to identify the primary determinants of dermal exposure. Dermal concentrations of HDI, uretidone, biuret, and isocyanurate were significantly higher ($\alpha = 0.05$) in 15 painters who did not wear coveralls or gloves ($N = 50$ paint tasks) than in 32 painters who did wear coveralls and gloves ($N = 200$ paint tasks) during spray-painting. Regardless of whether protective clothing was worn, isocyanurate was the predominant species measured in the skin (GM = 41.7 ng/mm^3), with a 95% detection rate. Other polyisocyanates (GM $\leq$ 2.00 ng/mm^3) were detected in skin during less than 23% of the paint tasks. According to marginal R^2 statistics, mixed models generated in this study described no less than 40% of

the variability in dermal concentrations of the different polyisocyanates measured in painters who did not wear protective clothing. These models also described greater than 55% of the variability in dermal concentrations of isocyanurate measured in all painters (N = 288 paint tasks). The product of analyte-specific breathing-zone concentration (BZC) and paint time was the most significant variable in all the models. Through LMM, a better understanding of the exposure pathways governing individual polyisocyanate exposures may be achieved. In particular, we were able to establish a link between BZC and dermal concentration, which may be useful for exposure reconstruction and quantitatively characterizing the protective effect of coveralls and gloves. This information can be used to reduce dermal exposures and better protect automotive spray-painters from potential adverse health effects.

4.2. INTRODUCTION

Automotive coatings are often based on polyisocyanates of 1,6-hexamethylene diisocyanate (HDI), consisting of trace amounts of HDI monomer and higher amounts of HDI oligomers (*e.g.*, uretidone, biuret, and isocyanurate) (Fent *et al.* 2008b; Janko *et al.* 1992; Sparer *et al.* 2004). During spray-painting, most 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. This "overspray" forms a paint mist that is likely to contain unreacted polyisocyanates. In addition to inhalation exposure to HDI, the potential for dermal exposure exists as polyisocyanate particles and vapor in the overspray may contact the skin via deposition or absorption, respectively. Even when protective clothing (*i.e.*, coveralls and gloves) is worn, polyisocyanates may break

through the protective barriers and contact the skin (Bello *et al.* 2008; Liu *et al.* 2007; Pronk *et al.* 2006b).

Diisocyanates are considered a leading cause of occupational asthma in exposed workers (Bernstein 1996; Chan-Yeung and Malo 1995). Although the mechanism of diisocyanate-induced asthma is not well understood, the inhalation route has been considered the primary route of exposure leading to respiratory sensitization. Hence, most investigations have focused on studying inhalation exposure to polyisocyanates (Janko *et al.* 1992; Lesage *et al.* 1992; Pronk *et al.* 2006a; Rudzinski *et al.* 1995; Sparer *et al.* 2004). However, the dermal route has received increased attention. Animal studies have shown that topical exposure to diisocyanates can result in respiratory sensitization (Herrick *et al.* 2002; Karol *et al.* 1981; Rattray *et al.* 1994), while case studies and epidemiology studies have shown associations between dermal exposure and occupational asthma (Donnelly *et al.* 2004; Petsonk *et al.* 2000).

Despite increasing evidence that dermal exposure to diisocyanates may play a role in the development of respiratory sensitization and occupational asthma, very few studies have been conducted to measure dermal exposure to diisocyanates. Of the exposure assessments that have been performed, some used colormetric wipes to determine exposure qualitatively (Liu *et al.* 2007; Liu *et al.* 2000) or used gloves as a surrogate measure for exposure loading on the skin (Pronk *et al.* 2006b). Recently, a few methods have been developed to measure unbound polyisocyanates in the skin. Bello *et al.* (2008) developed a wipe-sampling method for quantifying dermal exposure to total reactive isocyanate groups (TRIG) and Fent *et al.* (2008b) developed a tape-strip sampling method for quantifying dermal concentrations of individual polyisocyanates (*i.e.*, HDI monomer, uretidone, biuret, and isocyanurate).

Although both methods were shown to effectively estimate dermal exposure to polyisocyanates in automotive spray-painters, the specificity of the tape-strip method allows investigators to also explore differences among the various polyisocyanates species present in automotive paint formulations. This specificity is important because polyisocyanates may differ in their toxicities (Vandenplas *et al.* 1993; Vandenplas *et al.* 1992) and abilities to penetrate biological barriers (Bello *et al.* 2006; Pauluhn and Lewalter 2002).

Because of the limited amount of reliable quantitative data, there have been few efforts to model dermal exposure to polyisocyanates. When quantitative data are available, statistical methods can be used to identify the primary determinants of dermal exposure in a given occupational setting. For example, Fent *et al.* (2008b) demonstrated that the product of breathing-zone concentration (BZC) and paint time can be used to describe the variability of dermal concentration in painters who did not wear protective clothing.

The objectives of this study were to (1) quantify dermal concentrations of HDI monomer and oligomers (*i.e.*, uretidone, biuret, and isocyanurate) during automotive spray-painting using a tape-strip method (Fent *et al.* 2008b) and (2) use linear mixed modeling (LMM) (Laird and Ware 1982) to describe the variability in the dermal concentration estimates. Previously, we demonstrated the usefulness of LMM for evaluating the fixed effects of covariates on BZCs of individual polyisocyanates (Fent *et al.* 2008a). The LMM models we developed in this study aid in (1) understanding the dermal exposure pathways, (2) identifying the most effective control interventions for reducing dermal concentrations, and (3) reconstructing dermal concentrations in unsampled automotive painter populations.

4.3. MATERIALS AND METHODS

4.3.1. RECRUITMENT OF PAINTERS

The protocol used to recruit automotive painters for study participation in central North Carolina (NC) and the Puget Sound area of Washington State (WA) has been described previously (Fent *et al.* 2008a). A total of 47 painters (15 from NC and 32 from WA) participated in the study. In order to assess their exposures, painters were visited three times over a one year period, with visits at least one month apart. Due to attrition, 20 of the 47 painters were visited fewer than three times.

4.3.2. DATA COLLECTION AND ANALYSIS

An effort was made to sample exposures for every paint task in which diisocyanate-containing paint was applied. The majority (92%) of these paint tasks involved the application of clearcoat, which is expected to contain the highest levels of polyisocyanates (Sparer *et al.* 2004). Levels of monomeric and polymeric HDI in the skin were measured after each paint task using a previously described tape-strip sampling method (Fent *et al.* 2008b). The tape-strip samples (4×2.5 cm^2) were collected from six different sites of the skin. Generally, if the painter did not wear coveralls, tape-strippings were performed on the right and left volar and dorsal arm ($N = 332$ measurements) and the right and left dorsal hand ($N = 142$). If the painter wore coveralls, tape-strippings were performed on the right and left volar arm ($N = 487$), right and left dorsal hand ($N = 286$), and right and left neck ($N = 266$). Occasionally, samples were collected from the wrist ($N = 179$) or face ($N = 36$). Only 8 skin samples were collected from the legs.

A total of three successive tape-strip samples were collected from each site of the skin to ensure adequate collection of exposed corneocytes. Dermal exposure (ng/cm^2) to each sampled site was estimated by summing the polyisocyanate levels measured in three successive tape-strips. However, subsequent tape-strips were excluded if the previous tape-strip collected levels below the limit of detection. For each subject, the regional surface areas were estimated using the Haycock's formula (Haycock *et al.* 1978) in conjunction with the Berkow chart (Deitch 2008). The Haycock's formula calculates the total body surface area of skin based on the weight and height of the person while the Berkow chart provides estimates of the surface area contribution from each body part. Because values were not given in the Berkow chart for the wrist and face, the surface area contribution from these body parts were estimated by measuring the surface areas of the investigators' wrists and faces and then comparing them to the surface areas of the lower arms (for which the Berkow chart does provide a value). According to this procedure, the wrists and face contribute to approximately 1% and 2% of the total surface area of the skin, respectively.

The total mass of exposure collected from each body part was calculated by multiplying the point measurements (ng/cm^2) and regional surface area estimates (cm^2) from the sampled body parts. Unsampled regions were assumed to have received no exposure. Measurements taken from the legs were not included in this calculation due to the limited number of measurements. The mass of exposure to each body part was then summed and divided by the total body surface area of the skin (ng/cm^2). Lastly, because three successive tape-strip samples are considered to remove a volume of skin approximately 1 µm in thickness (Fent *et al.* 2008b), dermal exposure was reported as a concentration (ng/mm^3) in the skin, which is consistent with proposed nomenclature (Zartarian *et al.* 2005).

Personal one-stage (N = 98) and two-stage (N = 198) sampling was performed in the breathing-zone of spray-painters during each paint task as previously described (Fent *et al.* 2008a). It is important to note that both one-stage (*i.e.*, OSHA 42, OSHA 1983) and two-stage (*i.e.*, ISO-CHEK, Omega Specialty Instrument Co., Houston, TX) cassettes are commonly used to monitor atmospheres containing diisocyanates (England *et al.* 2000). Greater than one air sample and one set of corresponding tape-strip samples were collected from all but two painters, both of whom painted inside crossdraft booths.

Data were collected from the painters and their work environments for use as potential covariates in LMM. Methods used to measure airflow, temperature, and humidity have been previously published (Fent *et al.* 2008a). Variables considered in the statistical analysis are described in **Table 4.1**. Other variables were collected and evaluated as fixed effects in the mixed models, including booth volume, total sampling time, and worker experience. However, these variables were not included in Table 4.1 because they were not significant in the models and were not meaningful in terms of understanding the exposure processes.

Table 4.1. Summary of variables used to model dermal concentrations of monomeric and polymeric HDI.

Type	Name	Description	Range of values	Mean value	Median value
Classification	Booth type[a]	Type of ventilated paint booth	Downdraft, semi-downdraft, crossdraft	NA	NA
Continuous	Airflow	Airflow inside the paint booth (m^3/min)	0 - 469	222	238
	BZC (HDI) × paint time[b]	Product of HDI breathing-zone concentration (µg/m^3) and paint time (min)	1.07 - 1480	88.7	33.7
	BZC (uretidone) × paint time[b]	Product of uretidone breathing-zone concentration (µg/m^3) and paint time (min)	8.59 - 9740	376	9.56
	BZC (biuret) × paint time[b]	Product of biuret breathing-zone concentration (µg/m^3) and paint time (min)	12.2 - 26,400	1,060	33.8
	BZC (isocyanurate) × paint time[b]	Product of isocyanurate breathing-zone concentration (µg/m^3) and paint time (min)	12.9 - 582,000	26,300	12,100
	Humidity	Average relative humidity (%)	39.0 - 96.0	72.5	74.0
	Temperature	Estimate temperature during spraying (°C)	23.9 - 33.9	25.0	23.9
Dichotomous	Coveralls	Were coveralls worn during spray painting?	1: yes 0: no	0.70	1
	Coveralls old	Were the coveralls used for more than 8 weeks?	1: yes 0: no	0.25	0
	Coveralls nylpoly	Was the coverall material a nylon / polyester blend?	1: yes 0: no	0.39	0
	Coveralls poly	Was the coverall material polyester?	1: yes 0: no	0.071	0
	Coveralls polycot	Was the coverall material a polyester / cotton blend?	1: yes 0: no	0.16	0
	Gloves	Were gloves worn during spray painting?	1: yes 0: no	0.78	1
	Gloves nitrile	Was the glove material nitrile (as opposed to latex)?	1: yes 0: no	0.38	0
	Gloves thick	Were the gloves thick (i.e., > 0.13 mm)?	1: yes 0: no	0.38	0
	Gun type[c]	Type of spray-gun used for applying paint	1: HVLP 0: conventional	0.92	1
	Hood	Was a hood or neck covering worn during spray painting?	1: yes 0: no	0.28	0
	Sampler type	Type of sampler used to monitor air concentration	1: two-stage 0: one-stage	0.67	1
	Wrists covered	Were the wrists covered by gloves or coveralls?	1: yes 0: no	0.51	1

a. Because booth type is a character variable used for classification, mean and median values could not be calculated (NA = non-applicable).

b. Selected based on a previous finding relating dermal concentrations of HDI and isocyanurate in painters not wearing protective clothing to the products of respective breathing-zone concentrations (BZC) and paint time. Note that HDI, uretidone, biuret, and isocyanurate were non-detectable in 9.7, 61, 45, and 1.0% of all air samples, respectively. Non-detectable and non-quantifiable levels of the different polyisocyanates on air filters were assigned values by dividing the respective limits by $\sqrt{2}$.

c. HVLP = high-volume low-pressure.

Selection of variables was based on our previous finding that the variability of dermal concentration in painters not wearing coveralls and gloves can be described using the product of BZC and paint time, where both the outcome and predictor are log-transformed (Fent *et al.* 2008b). In the following conceptual model, dermal concentration represents the exposure outcome, BZC represents the intensity of exposure surrounding the painter, and paint time represents the duration of exposure:

$$Exposure = Intensity \times Duration.$$

Using this conceptual model as the framework, protective clothing would provide a barrier to aerosol deposition or vapor absorption. The protective effect of coveralls, gloves, and hood (*i.e.*, protective neck covering) may depend on a number of factors, including material type, age, and thickness. Consequently, statistical modeling may provide a way to estimate the effectiveness of protective clothing commonly used in the automotive refinishing industry.

4.3.3. STATISTICAL ANALYSIS

The data were analyzed using SAS 9.1 statistical software (Cary, NC). Prior to statistical analysis, concentrations below detection and quantitation limits were assigned values by dividing the respective limits by $\sqrt{2}$. Log-transformations were made to the products of BZC and paint time for each polyisocyanate to satisfy normality assumptions (Shapiro Wilks $W >$ 0.80). Dermal concentrations were also log-transformed. However, due to the high percentage of non-detectable values, log-transformed dermal concentrations of HDI ($W =$ 0.68), uretidone ($W = 0.60$), and biuret ($W = 0.41$) fit the normal distribution poorly. The covariates used for LMM were evaluated for potential collinearity by examining the

Spearman correlation coefficients among pairs of covariates. None of the variables exceeded our criterion for high correlation (*i.e.*, $r > 0.70$).

Linear regression (SAS PROC REG) was used to evaluate the effect of the product of analyte-specific BZC and paint time on dermal concentrations of each measured polyisocyanate in painters who did not wear coveralls and gloves. LMM (SAS PROC MIXED) were used to investigate the relative influences of fixed effects representing booth type and covariates on dermal concentrations of monomeric and polymeric HDI, while estimating within- and between-worker variability via the use of random effects. The general form of the mixed model and assumptions for these data are provided in Fent *et al.* (2008a). The effect of pooling within-worker variance or pooling both within- and between-worker variance among the different booth types was evaluated using likelihood ratio tests as described by Rappaport *et al.* (1999). According to these tests, both within- and between-worker variance may be pooled among the various booth types for isocyanurate, but should remain distinct for all other analytes.

Candidate covariates were selected by analyzing separate models that considered individual covariates and the products of BZC and paint time. From these models, those variables with *P*-values of less than 0.15 were used to obtain final models. Final models were built using a backwards elimination procedure in which the least significant variables ($P > 0.10$) were eliminated one-at-a-time. To allow for separate parameter estimates for each booth type, interactions between the classification variable booth type and each of the significant variables were evaluated one-at-a-time and retained if the 95% confidence intervals of any two of the parameter estimates did not overlap.

As in Fent *et al.* (2008a), transformed residuals and Malhalanobis distance were examined to assess model fit and a marginal R^2 statistic proposed by Vonesh and Chinchilli (1997) was used to estimate explained variation from the fixed effects. The diagnostic measures did not identify excessive outliers or problematic observations.

4.4. RESULTS

4.4.1. SUMMARY STATISTICS

Body region estimates (ng/cm^2) of dermal exposure to monomeric and polymeric HDI are summarized in **Table 4.2** for each sampled region. Because the data are positively skewed and contain a large percentage of non-detects, geometric mean (GM) and geometric standard deviation (GSD) best describe central tendency and scatter, respectively. The highest GM levels were collected from the legs for all the analytes except uretidone. However, due to the limited number of measurements, exposure to the legs was not included in the whole-body exposure calculations.

Whole-body dermal concentration estimates of the polyisocyanates measured in spray-painters from NC and WA are provided in **Table 4.3**. According to two-sample (Satterthwaite) *t*-tests of the log-transformed data, significant differences ($\alpha = 0.05$) were observed between the NC and WA measurements for HDI ($P = 0.0010$), uretidone ($P = 0.0011$), and isocyanurate ($P < 0.0001$). These differences may be related to differences in protective clothing use; gloves, coveralls, and hood were worn 47, 40, and 17% of the time in NC versus 94, 88, and 37% of the time in WA. However, the effect of location (*i.e.*, NC *vs.* WA) was significant even after adjustment for other significant fixed effects (*i.e.*, gloves and coveralls) in the multivariate model for predicting dermal concentrations of isocyanurate.

Descriptive statistics are presented in **Table 4.4** for the whole-body dermal concentration estimates, including restricted maximum likelihood (REML) estimates of the within- and between-worker variance. With the exception of uretidone, the GM dermal concentrations of all polyisocyanates varied among the different booth types, with the lowest levels observed in downdraft booths. For uretidone, the highest levels were observed in crossdraft booths. In addition to having the highest dermal concentrations, painters who sprayed in crossdraft booths also had the greatest exposure variability. Isocyanurate was the predominant species measured in skin with GM levels 20 times greater than all other analytes. While isocyanurate was detectable in skin for 95% of the paint tasks, the other polyisocyanates were detectable in skin for less than 23% of the paint tasks.

Table 4.5 presents a comparison of polyisocyanate dermal concentrations by whether painters wore protective clothing. According to two-sample (Satterthwaite) *t*-tests, significant differences ($\alpha = 0.05$) were observed between dermal concentrations in painters wearing coveralls and gloves and in painters who did not wear protective clothing for all the measured polyisocyanates ($P \leq 0.0180$). The 32 painters who wore coveralls and gloves had considerably less variable dermal concentrations compared to the 15 painters who did not wear coveralls and gloves. Therefore, much of the variability in dermal concentrations measured in this study may be attributable to painters who did not wear coveralls or gloves.

Table 4.2. Dermal exposure[a] to monomeric and polymeric HDI (ng/cm^2) by the sampled body region.

Sampled region	Contribution to total body surface area[b] (%)	No. measurements[c]	HDI		Uretidone		Biuret		Isocyanurate	
			GM[d]	GSD[e]	GM[d]	GSD[e]	GM[d]	GSD[e]	GM[d]	GSD[e]
Lower arms	6	819	0.19	2.80	1.31	2.53	1.65	2.61	30.3	7.69
Hands	5	428	0.16	2.25	1.06	1.75	1.77	2.94	21.3	8.76
Neck	2	304	0.15	1.80	0.98	1.30	1.48	1.65	18.2	4.76
Wrists	1	179	0.16	2.08	1.08	1.68	1.72	2.01	35.5	5.93
Face	2	36	0.16	2.08	1.07	1.67	1.49	1.54	20.7	4.62
Lower legs	14	8	0.35	1.39	1.30	1.88	2.25	2.75	287	2.94

a. Tape-strip samples collecting levels below the limits of detection and quantitation were assigned values by dividing the respective limits by √2.

b. Based on the Berkow chart (Deitch 2008).

c. Summation of levels collected with 3 successive tape-strip samples corresponds to one measurement.

d. Geometric mean.

e. Geometric standard deviation.

Table 4.3. Whole-body dermal concentrations[a] (ng/mm^3) of monomeric and polymeric HDI measured in 15 painters from North Carolina and 32 painters from Washington State.

Analyte	North Carolina (N = 95 paint tasks)			Washington State (N = 201 paint tasks)		
	GM[b]	GSD[c]	Range	GM[b]	GSD[c]	Range
HDI	0.31	4.01	0.083 - 121	0.19	1.70	0.083 - 1.76
Uretidone	1.99	3.22	0.57 - 55.9	1.31	1.66	0.67 - 22.8
Biuret	2.51	4.95	0.81 - 2830	1.80	1.48	0.81 - 15.6
Isocyanurate	150	7.46	2.14 - 7880	22.9	4.39	1.00 - 997

[a.] In calculating whole-body dermal concentration, tape-strip samples collecting levels below the limits of detection and quantitation were assigned values by dividing the respective limits by √2.

[b.] Geometric mean.

[c.] Geometric standard deviation.

Table 4.4. Whole-body dermal concentrations[a] (ng/mm^3) of monomeric and polymeric HDI measured in painters using different booth types.

Analyte	Booth type	No. workers[b]	No. paint tasks	No. non-detects[c]	Summary statistics			REML[f] estimates (logged data)	
					GM[d]	GSD[e]	Range	Within-worker variance[g]	Between-worker variance[g]
HDI	Downdraft	31	204	154	0.19	1.93	0.083 - 3.45	0.29	0.12
	Semi-downdraft	10	61	29	0.24	2.04	0.083 - 4.63	0.37	0.2
	Crossdraft	10	31	7	0.55	6.36	0.12 - 121	1.94	1.58
	All booths	47	296	190	0.22	2.52	0.083 - 121	0.46	0.56
Uretidone	Downdraft	31	204	177	1.46	2.16	0.57 - 55.9	0.34	0.20
	Semi-downdraft	10	61	52	1.37	1.97	0.57 - 22.8	0.22	0.49
	Crossdraft	10	31	22	2.10	3.22	0.95 - 34.9	1.10	0.29
	All booths	47	296	251	1.50	2.25	0.57 - 55.9	0.40	0.27
Biuret	Downdraft	31	204	190	1.68	1.39	0.81 - 15.6	0.10	0.015
	Semi-downdraft	10	61	43	2.14	2.42	0.81 - 550	0.83	0.032
	Crossdraft	10	31	20	5.53	10.80	1.35 - 2830	1.82	4.11
	All booths	47	296	253	2.00	2.64	0.81 - 2830	0.40	0.97
Isocyanurate	Downdraft	31	204	11	31.8	6.42	1.00 - 509	1.35	2.20
	Semi-downdraft	10	61	2	61.6	4.81	1.57 - 2670	1.35	2.20
	Crossdraft	10	31	2	123	8.17	1.57 - 7880	1.35	2.20
	All booths	47	296	15	41.7	6.55	1.00 - 7880	1.33	2.54

a. In calculating whole-body dermal concentration, tape-strip samples collecting levels below the limits of detection and quantitation were assigned values by dividing the respective limits by $\sqrt{2}$.

b. A total of four painters painted in more than one booth type; two painted in both crossdraft and semi-downdraft booths, one painted in both crossdraft and downdraft booths, and one painted in both semi-downdraft and downdraft booths.

c. Based on whether or not detectable levels of the respective polyisocyanates were recovered with tape-strip samples from any region of the skin following the completion of a paint task.

d. Geometric mean.

e. Geometric standard deviation.

f. Restricted maximum likelihood.

g. Within- and between-worker variance was pooled among the booth types for isocyanurate exposure as justified by likelihood ratio tests.

Table 4.5. Whole-body dermal concentrations[a] (ng/mm^3) of monomeric and polymeric HDI measured in 32 painters who wore coveralls and gloves and 15 painters who did not wear coveralls and gloves during spray-painting.

Analyte	Coveralls and gloves worn (N = 192 paint tasks)				Coveralls and gloves not worn (N = 50 paint tasks)			
	No. non-detects[b]	GM[c]	GSD[d]	Range	No. non-detects[b]	GM[c]	GSD[d]	Range
HDI	149	0.17	1.58	0.083 - 1.76	20	0.44	5.03	0.12 - 121
Uretidone	185	1.17	1.26	0.66 - 4.40	41	1.68	2.84	0.57 - 34.9
Biuret	169	1.80	1.47	0.81 - 15.6	38	3.80	8.08	0.81 - 2830
Isocyanurate	13	17.6	3.90	1.00 - 540	1	287	5.93	1.57 - 7880

a. In calculating whole-body dermal exposure, tape-strip samples collecting levels below the limits of detection and quantitation were assigned values by dividing the respective limits by √2.

b. Based on whether or not detectable levels of the respective polyisocyanates were recovered with tape-strip samples from any region of the skin following the completion of a paint task.

c. Geometric mean.

d. Geometric standard deviation.

4.4.2. STATISTICAL MODELING

Because increased variability in dermal concentrations occurred in painters who did not wear protective clothing, LMM was used to evaluate the fixed effects of the covariates on dermal concentrations of each measured polyisocyanate in painters who did not wear coveralls or gloves. Booth type was not used as a classification variable in these models due to the limited number of measurements from painters in crossdraft booths (*i.e.*, 4 painters, 10 paint tasks) and semi-downdraft booths (*i.e.*, 1 painter, 2 paint tasks). The products of BZC and paint time were highly significant ($P < 0.0010$) in all the models. **Figure 4.1** presents the mixed-effects regression of dermal concentration on the product of BZC and paint time for each analyte. In addition to the product of analyte-specific BZC and paint time, gun type was a significant variable in the models for HDI ($P = 0.0173$), uretidone ($P = 0.0366$), and isocyanurate ($P = 0.0804$), and airflow was a significant variable in the models for HDI ($P =$

0.0181) and biuret ($P = 0.0105$). According to marginal R^2 statistics, the full mixed models described 55, 40, 58, and 51% of the variability in dermal concentrations of HDI, uretidone, biuret, and isocyanurate, respectively.

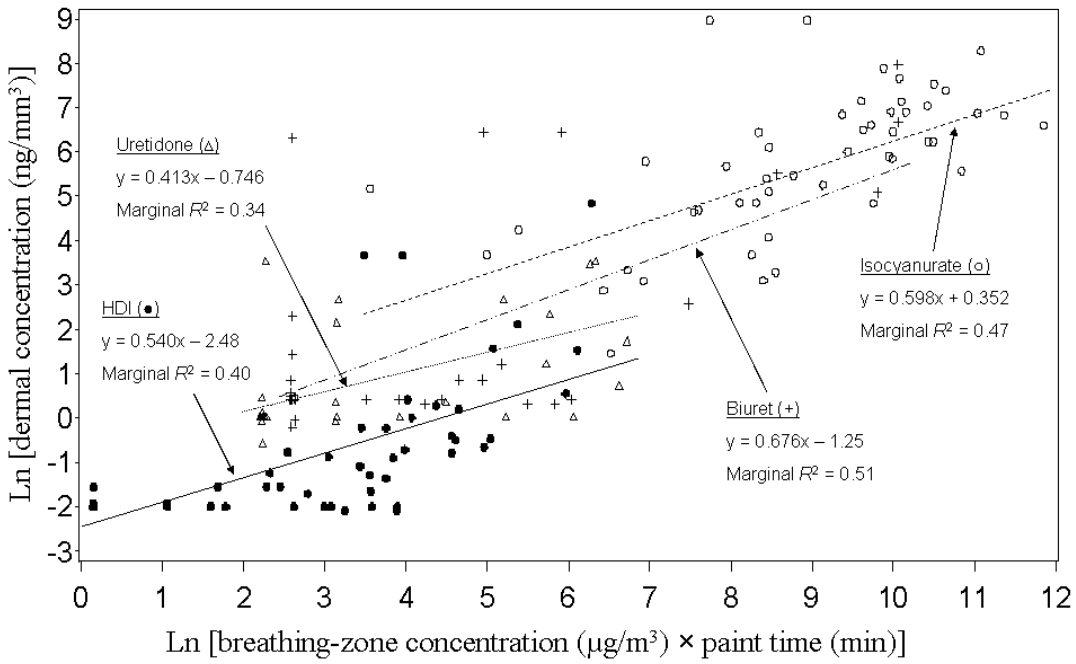

Figure 4.1. Mixed-effect regressions of log-transformed dermal concentrations of HDI, uretidone, biuret, and isocyanurate on the products of respective breathing-zone concentrations and paint times in 15 unprotected workers (no coveralls or gloves worn) performing 50 separate paint tasks.

Because dermal concentrations of isocyanurate were detectable in 95% of the paint tasks and varied considerably even in painters with protected arm and hand skin, LMM was performed for isocyanurate using the unrestricted dataset (*i.e.*, 47 painters, 288 paint tasks). This unrestricted dataset was not used for LMM of dermal concentrations of HDI, uretidone, and biuret due to high number of non-detects and limited variability in painters wearing

coveralls and gloves. The mixed model developed for predicting dermal concentrations of isocyanurate is described in **Table 4.6**. According to the marginal R^2 statistics, significant fixed effects were able to describe 57% of the variability in dermal concentrations of isocyanurate. Although coveralls and gloves were significant predictors in the model, material type, age, and thickness were not significant. The relative effectiveness of coveralls and gloves can be estimated by comparing model predictions calculated with and without the fixed effect of protective clothing. According to this procedure, use of protective clothing was associated with a 93% reduction of isocyanurate concentration in the skin.

Table 4.6. Linear mixed model[a] for predicting dermal concentrations of isocyanurate[b] in automotive spray-painters.

Covariates[c]	Parameter estimates (downdraft, semi-downdraft, crossdraft)[d]	*P*-values[e]
Intercept	(2.87, 3.38, 3.10)	< 0.0001
Isocyanurate BZC × paint time (μg·min/m^3)	0.401	< 0.0001
Gloves (1 = yes, 0 = no)	-1.62	< 0.0001
Coveralls (1 = yes, 0 = no)	-1.01	< 0.0001
Sampler type (1 = two-stage, 0 = one-stage)	-0.400	0.0454
Gun type (1 = HVLP, 0 = conventional)	-0.815	0.0032

[a.] According to the marginal R^2 statistic, the model described 57% of the variability in the dermal concentrations of isocyanurate.

[b.] N = 288 (8 of 296 observations were excluded due to missing air-sampling data).

[c.] Dermal concentrations and the products of breathing-zone concentration (BZC) and paint time were log-transformed prior to statistical analysis.

[d.] Separate intercepts were determined for each booth type as specified in the mixed model.

[e.] *P*-values are based on approximate F-tests of fixed effects.

4.5. DISCUSSION

In this study, a previously published tape-strip sampling method (Fent *et al.* 2008b) was used to quantify dermal concentrations of individual polyisocyanates in automotive spray-painters. Because quantitative dermal concentration and covariate data were obtained, LMM could be used to evaluate the fixed effects of covariates on dermal concentration while estimating within- and between-worker variance components via random effects. The mixed models developed in this study described a considerable amount of variability ($R^2 \geq 0.40$) in dermal concentrations of isocyanurate in all 47 painters as well as dermal concentrations of HDI, uretidone, biuret, and isocyanurate in 15 painters who did not wear coveralls or gloves during spraying.

The product of analyte-specific BZC and paint time was the most significant variable in all the mixed models. The effect of this variable on dermal concentrations of polyisocyanates in painters who did not wear protective clothing can be seen in Figure 4.1. Using the same product of analyte-specific BZC and paint time (*e.g.*, 5.0 μg·min/m^3), the models in Figure 4.1 predicted ~40% lower dermal concentrations of HDI than uretidone, ~80% lower dermal concentrations of uretidone than biuret, and ~55% lower dermal concentrations of biuret than isocyanurate. Because HDI (0.05 mm Hg at 25 °C) exists partially as vapor in overspray, HDI may supply less exposure to the skin or evaporate off the skin. The oligomers, on the other hand, have relatively low vapor pressures (*e.g.*, biuret 4.7×10^{-7} mm Hg at 20 °C). Therefore, any differences between predicted dermal concentrations of individual HDI oligomers are likely due to the different rates of skin absorption or chemical reactivity. Further investigation into dermal absorption and reactivity differences among polyisocyanates is warranted.

Although the products of BZC and paint time were able to describe much of the variability (34 – 52%) in dermal concentrations in painters who did not wear protective clothing, other variables (*i.e.*, gun type and airflow), when included in the mixed models, were able to increase the explained variability (40 – 58%). While these models were developed to describe the variability in unprotected painters, the mixed model described in Table 4.6 was developed primarily to identify additional determinants of dermal concentration, as well as to evaluate the effectiveness of protective clothing used by painters in this study.

As expected, gloves and coveralls were significant predictors in the mixed model (Table 4.6). However, wrist protection and hood were not significant, possibly owing to the relatively small surface area of the wrist, inadequacy of loose-fitting hoods for protection, and/or less intense overspray surrounding the face and neck compared to the arms and hands during painting. The variables related to material type, age, and thickness were not significant in the model, which may suggest that similar protection was achieved for the different types of protective clothing used by painters. However, the effects of material type, age, and thickness are likely to be subtle compared to the major protective effects of wearing coveralls and gloves, and as such, would be difficult to identify with LMM. Therefore, more controlled experiments are needed to fully evaluate the effectiveness of protective materials. In addition, sampler type and gun type were significant variables in the model. The effect of sampler type and gun type on BZCs of the monomeric and polymeric HDI has been discussed previously (Fent *et al.* 2008a). Briefly, two-stage sampling underestimates BZCs compared to one-stage sampling for the analytes HDI and isocyanurate, most likely due to polymerization of these polyisocyanates on the untreated pre-filter, while high-volume low-

pressure (HVLP) spray guns have greater transfer efficiencies and produce less overspray and, generally, larger particles than conventional spray guns. It is probable that these same effects are described by sampler type and gun type in this model.

Previously, we observed significantly higher ($\alpha = 0.05$) BZCs of HDI, biuret and isocyanurate in WA than in NC (Fent *et al.* 2008a). However, in this study, we observed that painters in NC had significantly higher dermal concentrations of HDI, uretidone, and isocyanurate than painters in WA (Table 4.3). The mixed model for predicting dermal concentrations of isocyanurate in all painters (Table 4.6), which included the protective effect of coveralls and gloves, could not explain this difference (*i.e.*, the effect of location was significant when added to the model). It is possible that climatic differences could be the cause of these differences, but temperature and humidity were not significant variables in the mixed model (Table 4.6). Clearly, there is a location-specific effect on dermal concentration estimates of isocyanurate that could not be explained by differences in protective clothing use or differences in temperature and humidity between NC and WA, or any other variables in our model.

To our knowledge, statistical modeling has not been used to investigate dermal exposure to polyisocyanates in the automotive refinishing industry. However, Brouwer *et al.* (2001) developed a deterministic model for predicting dermal exposure to overspray in airless spray-painters. The primary factors of this model were overspray generation rate, transmission of overspray, and aerosol deposition efficiency. These factors could not be measured directly in our study, but may be estimated by the variables in Table 4.1. For example, BZC may be representative of the overspray generation rate, airflow and booth type may be important

factors in the transmission of overspray, and gun type, which influences the size of overspray particles, may affect the aerosol deposition efficiency.

All of these variables were significant in one or more of the models. The effect of gun type has already been discussed. Increasing airflow was associated with decreasing dermal concentration, most likely due to the increased capture and removal of overspray from the painters' personal space at higher airflows. Transmission of overspray, therefore, may be influenced by factors other than the airflow, which may not have been characterized in this study. However, it is possible that BZCs were measured in such close proximity to the painters' skin that, in effect, transmission of overspray had occurred. Under this scenario, instantaneous BZC would be related to instantaneous dermal concentration by a factor related to aerosol deposition. Consequently, the product of BZC and paint time would be related to cumulative dermal concentration for the paint task, which is essentially what was estimated in this study.

Isocyanurate was the most abundant polyisocyanate collected from the skin whether or not coveralls and gloves were worn (Table 4.4). The reason for the higher levels and detection rate of isocyanurate in skin may simply be due to the greater abundance of isocyanurate in the atmosphere (GM = 1440 $\mu g/m^3$) compared to the other analytes (GM < 14 $\mu g/m^3$).

For all the measured polyisocyanates, the highest dermal concentrations were in painters who sprayed in crossdraft booths. The isocyanurate model generated in this study predicted higher dermal concentrations for workers painting in semi-downdraft and crossdraft booths than for workers painting in downdraft booths. According to our previously published study (Fent *et al.* 2008a), painters who sprayed in downdraft booths had lower BZCs than painters

who sprayed in the other booths for all the measured polyisocyanates. Flynn *et al.* (1999) observed that, depending on worker orientation, crossdraft booths may actually draw overspray across the painter's body. It is conceivable that this effect may also occur in semi-downdraft booths. Thus, the higher concentrations of polyisocyanates in the air coupled with the inability of the ventilation system to draw air away from the painter's personal space may have led to higher dermal concentrations in painters who used crossdraft and semi-downdraft booths.

The results reported here are consistent with the tape-strip validation measurements previously reported (Fent *et al.* 2008b). Few investigators have quantified and reported exposure to polyisocyanates in human skin. Bello *et al.* (2008) used wipe sampling to quantify dermal exposure (ng/cm^2) to TRIG in painters who did not wear protective clothing (GM = 1.9, GSD = 10.9, $N = 49$ measurements) and in painters who wore coveralls (GM = 1.0, GSD = 3.2, $N = 3$) and gloves (GM = 1.0, GSD = 5.2, $N = 17$). After converting regional dermal exposure estimates (ng/cm^2) of individual polyisocyanates into estimates of TRIG for this study, it became clear that we measured considerably higher levels of polyisocyanates in the skin of painters who did not wear protective clothing (GM = 66, GSD = 3.0, $N = 300$) and in the skin of painters who wore coveralls (GM = 4.5, GSD = 3.3, $N = 487$) and gloves (GM = 3.2, GSD = 3.0, $N = 314$). Given the specificity of the analytical method, the polyisocyanates measured and reported here do not necessarily represent all the possible polyisocyanate species in automotive paint. For example, monomeric and polymeric isophorone diisocyanate, which is sometimes present, and polymers of HDI larger than isocyanurate were not quantified. Therefore, the actual TRIG concentrations are most likely to be higher than what we were able to measure with our analyte-specific LC-MS method.

Nevertheless, compared to other methods for measuring dermal exposure to polyisocyanates, it appears the tape-strip method we describe has superior collection and quantification efficiency. Furthermore, the specificity of the tape-strip method provides a means to investigate individual monomeric and polymeric HDI concentrations in the skin. It is also the only method available to quantitatively measure polyisocyanate species in the non-viable skin layer, thus, providing an estimate of the absorbed dose.

This study provides a significant contribution to the characterization of the processes governing dermal exposures to individual polyisocyanates (HDI monomer and its oligomers) in automotive spray-painters. Through LMM, we were able to identify the primary determinants of dermal exposure to monomeric and polymeric HDI. The mixed models developed related dermal concentration to the product of BZC and paint time. As a result, these models may be particularly useful for exposure reconstruction studies where information on BZC and paint time is readily available or can be estimated. However, further validation is necessary to determine the accuracy of these models. Although this study was able to demonstrate the effectiveness of the use of coveralls and gloves to reduce exposure, isocyanurate was detected in the skin of painters wearing coveralls and gloves for 93% of the paint tasks. This underscores the importance of reducing BZCs in the painting atmosphere. By reducing the BZCs, the amount of overspray available for deposition will be reduced, thus providing less loading onto protective clothing and exposed skin. Moreover, this study describes exposure-assessment tools to estimate the doses of individual polyisocyanates to the skin and lungs. This information may be used to investigate the roles of both monomeric and polymeric HDI, as well as dermal and inhalation routes of exposure, in the development of respiratory sensitization and occupational asthma.

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1. OVERVIEW

In this dissertation, I have presented three related papers (Chapters 2-4). Each manuscript builds upon the previous body of work and makes significant contributions to exposure and risk assessment. The first manuscript (Chapter 2) describes the methodology for sampling and analyzing skin, air, and paint samples for individual monomeric and polymeric HDI. I used this methodology during a longitudinal repeated-measures study to estimate polyisocyanate paint concentrations, BZCs, and dermal concentrations in a cohort of 47 automotive spray-painters. The second manuscript (Chapter 3) presented the paint and air-sampling results from the longitudinal study as well as generated mixed models that used paint concentrations and other information collected from the painters and their work environments to describe the variability in BZCs of HDI, uretidone, biuret, and isocyanurate. The third manuscript (Chapter 4) presented the dermal sampling results from the longitudinal study as well as generated mixed models that used the product of analyte-specific BZC and

paint time and other process- and task-related variables to describe the variability in dermal concentrations of HDI, uretidone, biuret, and isocyanurate. In the following sections, I discuss the strengths and limitations of the air sampling (5.2), tape-strip sampling (5.3), and paint sampling (5.4) methodology used in this study. I also discuss the implications of the longitudinal repeated-measures study (5.5) on the generalization of the results and the implications of the BZC modeling (5.6) and dermal exposure modeling (5.7) on the health and safety of automotive spray-painters.

5.2. AIR SAMPLING

The one- and two-stage air-sampling methods we used are similar in design, but dissimilar in terms of analysis, to the OSHA-42 (OSHA 1983) and ISO-CHEK® methods (Omega Specialty Instrument Co., Houston, TX), which are two of the most commonly used methods for sampling atmospheres containing diisocyanates. The OSHA-42 method uses HPLC with UV detection to quantify HDI, while the ISO-CHEK® method uses HPLC with UV and fluorescence (FL) detection to quantify both HDI and TRIG. In this study, I developed and used LC-MS to quantify specific polyisocyanate species (*i.e.*, HDI, uretidone, biuret, and isocyanurate) collected on the one- and two-stage samplers. Thus, the method I used is more specific than the OSHA-42 and ISO-CHEK® methods. The specificity of our air-sampling methods provides a means to investigate exposures to individual polyisocyanate species, which is important since polyisocyanates may have different exposure pathways in the painting environment due to their differences in volatility and reactivity.

Using LMM, I observed that air-sampler type was a significant variable for predicting BZCs of HDI and isocyanurate. The parameter estimate for the fixed effect of sampler type

was negative in both models indicating that the two-stage sampler underestimates BZCs compared to the one-stage sampler. I corroborated this finding by conducting paired *t*-tests of side-by-side one- and two-stage sampling results. These findings suggested that HDI and isocyanurate polymerized on the surface of the untreated PTFE filter in the two-stage sampler. It is expected that the same phenomena would also bias the ISO-CHEK® sampling results. However, because the ISO-CHEK® method is non-specific (*i.e.*, HPLC-UV / FL), it may be able to quantify polyisocyanates as TRIG even after they polymerize if the resulting compounds contain an isocyanate functional group. As a result, the finding that two-stage samplers have lower sampling efficiency compared to one-stage samplers cannot be generalized to the ISO-CHEK® method. Further investigation of the sampling biases associated with the type of sampler (*i.e.*, one- *vs.* two-stage) and analytical method (*i.e.*, LC-MS *vs.* HPLC-UV / FL) is warranted.

In addition to the potential for polymerization of polyisocyanates on untreated filters, there are a few other limitations to these air-sampling methods. Only short-term monitoring (*i.e.*, < 30 min) can be performed due to the potential for overloading the filters, and polyisocyanates may break through the impregnated filters if they are not immediately derivatized. All these limitations should be considered when choosing air-sampling methodology for polyisocyanates.

5.3. TAPE-STRIP SAMPLING

Few quantitative methods for measuring polyisocyanate exposure in the skin are presented in the literature. Bello *et al.* (2008) developed a wipe-sampling method for quantifying dermal exposure to TRIG. Compared to this method, the tape-strip sampling method I used is more

specific, capable of quantifying individual polyisocyanates in the skin rather than TRIG. The ability to measure individual polyisocyanates in skin is important because polyisocyanates may differ in their ability to penetrate skin (Bello *et al.* 2006; Pauluhn and Lewalter 2002) or react with macromolecules in skin. In addition, I was able to measure exponentially greater levels of polyisocyanates in the skin of painters who did not wear protective clothing and gloves than Bello *et al.* (2008). Thus, the tape-strip sampling method I developed appears to have superior collection and quantification efficiency compared to the method developed by Bello *et al.* (2008).

There are limitations to the tape-strip sampling method we used. Firstly, sweat and body hair may interfere with the ability of tape-strips to remove corneocytes from the skin and, hence, polyisocyanates. Prevalence of body hair differs from person to person and is generally most prevalent on the dorsal arms. Sweat may have interfered with the dermal concentration measurements in NC painters during the summer months due to the hot and humid climate of the Southeast. Secondly, polyisocyanate levels on tape-strips may not represent the actual dose to the skin due to the reactivity and absorption of polyisocyanates in the skin. Lastly, point estimates (ng/cm^2) for the different body parts may not be representative of the actual total exposure to that body part due to the spatial variability in overspray distribution across the skin. These limitations should be considered when designing a dermal exposure-assessment study that involves tape-strip sampling.

Additional research is needed to standardize the tape-strip sampling measurements. This standardization would aid in the investigation of the inter-individual variability in the dermal exposure estimates. One promising development in this area is the method by Chao *et al.* (2004) to quantify the mass of keratin on tape-strip samples, where keratin levels are related

to the amount of collected corneocytes. This method could be adapted and applied to the tape-strip samples collected in future studies exploring polyisocyanate exposures.

5.4. PAINT SAMPLING

To my knowledge, chemical analysis of paint samples from the automotive refinishing industry has not been previously performed. Instead, air-sampling results and information reported in material safety data sheets (MSDSs) have been used to estimate the proportions of polyisocyanate species in paint (Pronk *et al.* 2006a; Rudzinski *et al.* 1995; Sparer *et al.* 2004; Woskie *et al.* 2004). Median paint concentrations measured in this study (Table 3.1) show that isocyanurate represents the majority of all polyisocyanates in the paint mixtures (> 98%), followed by biuret (< 1%), HDI (< 0.3%), and uretidone (< 0.08%). It should be noted, however, that I did not attempt to quantify polyisocyanates larger than isocyanurate.

Polyisocyanate paint concentrations estimated in this study were critical to the development of analyte-specific BZC models. There are, however, a few limitations to the paint sampling method that should be noted. Clearcoat is rather viscous and, as a result, may have adhered to the pipet tips used to deliver the clearcoat into the derivatizing solution. Thus, concentrations of polyisocyanate species in clearcoat may have been overestimated. However, because clearcoat is highly reactive and congeals rapidly when it is submerged into solution, polyisocyanates may have reacted before being derivatized. Thus, concentrations of polyisocyanates in clearcoat may have been underestimated. Depending on which of these sampling biases dominated, paint concentrations may have been either under- or overestimated.

5.5. LONGITUDINAL EXPOSURE-ASSESSMENT STUDY

We visited 47 automotive spray-painters on three separate occasions for the purpose of quantifying their task-specific exposures and collecting information from them and their work environments for use as covariates in subsequent modeling. There are several considerations related to this study design that have implications on the generalization of the results.

The recruitment process involved mail and telephone solicitation. That solicitation was performed by graduate students in NC and associates from the WA Department of Labor and Industries (LNI). As a result, the study population is not a random subset of automotive spray-painters and, thus, may not be representative of the automotive refinishing industry in general. Participation of automotive repair shops may have been influenced by the background of the solicitors. For example, because the LNI is a regulatory agency, automotive repair shops with better health and safety practices may have been more likely to participate in the study.

A working day of a spray-painter consists of cycles of short tasks. We made an effort to sample exposures during each task, particularly if that task involved spraying clearcoat. Occasionally, we did not monitor paint tasks involving either primer or sealer because the paint did not contain a large proportion of hardener (*i.e.*, > 20%) and/or the areas to be painted were relatively small (*i.e.*, < 2 min paint tasks). Thus, cumulative exposure measured in these instances may underestimate the true exposure from spray-painting.

We collected air samples during each applicable paint task. We conducted two-stage sampling primarily during the first and second visits and one-stage sampling primarily during the third visit. As a result, the one- and two-stage sampling results are not directly

comparable. Therefore, the significant effect of sampler type in the HDI and isocyanurate BZC models could be due to painters having greater exposures during the third visit. Paired *t*-tests of side-by-side one- and two-stage sampling results, however, corroborate the significant effect of sampler type. The fixed effect of sampler type in the models, therefore, is most likely due to differences in sampling efficiency between the one- and two-stage samplers.

We conducted tape-strip sampling immediately following each paint task. It was not uncommon during paint tasks for painters to apply more than one coat with a time-lapse in between coats. Therefore, if polyisocyanates rapidly absorbed into the lower layers of the stratum corneum and/or reacted in the skin, then our measurements may have underestimated exposures and these underestimations may have been larger for longer paint tasks. In this study, paint time represented the cumulative time spent painting an object, while total time represented the operating time of the sampling pumps. Thus, total time was most indicative of the time-lapse between painting initiation and completion. Total time was not significant in the dermal exposure model for isocyanurate (Table 4.6), however, which may indicate that absorption and/or reaction of polyisocyanates did not bias the results.

Typically, the duration of time between paint tasks was greater than one hour. It is possible that unreacted polyisocyanates remained in the upper layers of the stratum corneum after completion of one task to the start of the next. However, there was no evidence for this carry-over. Tape-strips collected approximately one hour after paint tasks where measurable polyisocyanates were recovered did not contain polyisocyanates. Therefore, it is probable that polyisocyanates reacted or penetrated into the lower layers of the stratum corneum within a one-hour time period.

After each paint task, we collected tape-strips from six different sites of the skin. The sites we measured depended on whether or not protective clothing was worn as well as on our professional judgment concerning which areas were most exposed. The arms were the most commonly sampled region of the skin, followed by the hands, neck, wrist, face, and legs (Table 4.2). I calculated whole-body dermal exposures based on the assumption that unsampled regions received no exposure. Thus, because different sites were sampled for different painters and different tasks, the whole-body dermal exposure estimates may not be directly comparable among painters.

Despite these limitations, this longitudinal exposure-assessment study is one of the most comprehensive studies ever conducted to date on dermal and inhalation exposures to polyisocyanates. The quantitative exposure data collected is not without limitations but, nevertheless, provides a useful picture of individual polyisocyanate exposures in automotive spray-painters.

5.6. BREATHING-ZONE CONCENTRATION MODELING

Rather than relying on PPE for protection, control interventions and technologies should be implemented wherever possible to reduce exposures in the atmosphere. The mixed models I developed identified several factors that could be used to better protect painters. Reducing polyisocyanate concentrations in paint and increasing airflow in the booth were particularly influential in the reduction of BZCs. The primary determinants were unique to each model and were able to describe greater than 20% of the variability in BZCs of HDI and isocyanurate and greater than 50% of the variability in BZCs of uretidone and biuret.

Much of the unexplained variability in the models may be due to factors affecting within-worker variability that could not be characterized in this study, such as worker orientation relative to the airflow, height of the gun during spraying, and distance of the nozzle of the gun to the surface being painted. The exposure distributions for HDI and isocyanurate had greater within-worker variability than between-worker variability (Table 3.3) which could explain why their respective models produced smaller R^2 values than the uretidone and biuret models. In addition to factors affecting within-worker variability, precise measurements on the volume and viscosity of paint sprayed, nozzle pressure of the gun, and velocity and direction of airflow at the chest of the painter would almost certainly improve the model fit by providing more accurate estimates of the overspray generation, capture, and transport (Flynn *et al.* 1999).

The goal of modeling, however, should be to produce the most parsimonious model. The more complicated a model is, the less likely it is to be used in future studies. Aside from paint concentration measurements, the models I developed used variables that can be estimate or easily obtained from painters and their work environments. The argument could even be made that paint concentrations could be estimated using data from MSDSs, although rarely do MSDSs list the individual species of polymeric HDI. Still, these models may serve useful in exposure reconstruction studies. Further validation, however, is necessary to confirm their usefulness.

5.7. DERMAL EXPOSURE MODELING

Exposure pathways leading to dermal exposure are not well understood. These pathways are further complicated by the reactivity of polyisocyanates. Using LMM, I was able to link BZC to dermal exposure estimates. In fact, the product of BZC and paint time was the most significant fixed effect in all the models. Because BZC is related to dermal exposure, control interventions identified in the BZC models may also work to reduce dermal exposure to polyisocyanates. As expected, use of protective clothing (*i.e.*, coveralls and gloves) was a significant factor responsible for an estimated 93% reduction in dermal exposure to isocyanurate. Still, isocyanurate was detected under protective clothing, indicating breakthrough. This underscores the importance of reducing airborne concentrations in addition to wearing protective clothing.

The mixed models I developed were able to describe greater than 40% of the variability in dermal concentrations of HDI, uretidone, biuret, and isocyanurate in painters who did not wear protective clothing and greater than 50% of the variability in dermal concentrations of isocyanurate in all painters. Much of the unexplained variability for HDI, uretidone, and biuret in painters without protective clothing may be due to the high percentage of non-detects (> 80% of the paint tasks). Isocyanurate, however, was detectable in greater than 95% of the paint tasks. Unexplained variability in dermal exposures to isocyanurate may be due to a number of factors, including differences in aerosol deposition onto the skin, polymerization of polyisocyanates during and/or after deposition, absorption of polyisocyanates into the skin and reactivity of polyisocyanates with macromolecules in the skin. Deposition of aerosols may depend on the surface area of the painter in relation to the reflected cone of overspray and also droplet momentum (*i.e.*, size and velocity of the droplet)

(Brouwer *et al.* 2001). Inter-individual reactivity of polyisocyanates may depend on the temperature in the paint booth, efficacy of the catalyst, volatility of the solvents, and presence and reactivity of other polyisocyanates in the paint (Randall and Lee 2002). The ability of skin to absorb polyisocyanates may depend on lipophilicity, molecular weight, and reactivity of the polyisocyanates and concomitant exposures, as well as inter-individual differences in the physiological make-up of skin. Physiological and immunological differences in skin may also affect the reaction of polyisocyanates with macromolecules in the skin.

Clearly, further research is needed to understand the fate and transport of polyisocyanates once they contact the skin. The exposure assessments and mixed models I developed in this study may provide investigators with a tool to investigate the fate and transport of polyisocyanates in workers by comparing exposure profile data with various exposure biomarkers in the skin, blood, and urine. The culmination of this and future work investigating exposure-biomarker relationships may provide a detailed understanding of the exposure pathways from the source (paint concentration gradient from source) to the breathing-zone concentrations (BZCs) to the stratum corneum (dermal concentrations from aerosol deposition) and finally to the blood and urine (exposure biomarkers) for each of the measured polyisocyanates. The exposure-assessment methods and models developed in this research will enable us to obtain detailed information on the individual absorbed doses of specific polyisocyanates and to investigate the roles of both monomeric and polymeric HDI. The role of both dermal and inhalation exposure routes in the development of respiratory sensitization and occupational asthma may thus be examined. The knowledge gained from this research will be a great asset for the advancement of the exposure and risk assessment,

and most importantly, for the protection of automotive spray-painters and other workers who are occupationally exposed to monomeric and polymeric HDI.

APPENDIX

EXPLANATION OF SAS PROGRAMS

- Filename: Import_HDI_data.sas
  - Imports paint, air, dermal, and covariate datasets

- Filename: Merge_air.sas
  - Calculates air concentrations ($\mu g/m^3$)

- Filename: Merge_paint.sas
  - Calculates paint concentrations (g/l)

- Filename: Merge_dermal_info.sas
  - Calculates dermal exposure ($ng/10\ cm^2$)
  - Merges dermal and covariate datasets

- Filename: Data_prep.sas
  - Merges all datasets (*i.e.*, air, paint, dermal, and info)
  - Calculates whole-body dermal exposure (ng/m^2)
  - Converts paint concentration from g/l to mg/l
  - Establishes identification variables, dichotomous variables, and character variables
  - Exports task-specific exposure and covariate dataset to Excel

- Filename: Air_model.sas
  - Provides REML variance estimates for BZCs
  - Provides summary statistics for air sampling and related covariates
  - Performs LMM and diagnostics
  - Performs *t*-tests (*e.g.*, one- *vs.* two-stage, NC *vs.* WA, etc.)
  - Generates dataset for calculating marginal R^2 statistics

- Filename: Site_data.sas
  - Provides sample site (*i.e.*, skin site) specific exposure data (ng/cm^2)
  - Provides summary statistics for exposures to the different body parts
  - Calculates total NCO (ng/cm^2) for comparison to measurements by Bello *et al.* (2008)
  - Exports site-specific exposure and covariate dataset to Excel

- Filename: Dermal_model.sas
  - Calculates whole-body dermal concentration (ng/mm^3)
  - Provides REML variance estimates for dermal concentrations
  - Provides summary statistics for dermal sampling and related covariates
  - Performs LMM and diagnostics
  - Performs *t*-tests (*e.g.*, NC *vs.* WA)
  - Generates dataset for calculating marginal R^2 statistics

- Filename: Dermal_model_restricted.sas
  - Calculates summary statistics for protected (gloves and coveralls worn) and unprotected (gloves and coveralls not worn) painters
  - Performs regression analysis of exposures in unprotected painters
  - Performs LMM of exposures in unprotected painters

- Filename: Pseudorsq_csdav4.sas
  - Macro for calculating marginal R^2 statistics for the full models
  - Developed by Orelien and Edwards (2008)

- Filename: Air_iso_full.sas
  - Program for calculating the marginal R^2 statistic for the LMM developed for predicting BZCs of isocyanurate
  - Because the programming languages are similar, programs developed for calculating R^2 statistics for models specific to the other analytes, booth type, and/or dermal exposure are not provided

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